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BRAIN VOLUME REDUCTION OVER FOUR DECADES IN MULTIPLE SCLEROSIS

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To

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ABSTRACT

Introduction: Multiple sclerosis (MS) is a common neurological disease causing intermittent or cumulative disability, predominantly among women of child-bearing age. MS is a chronic inflammatory disease of the central nervous system (CNS) characterized by early axonal damage and early atrophy progression. The disease has certain typical features, but disease progression is highly variable and has many atypical forms. In follow-up, the poor correlation between radiological findings and disability has sometimes been referred to as "the clinicoradiolological paradox". The widespread effect on the whole CNS is a challenge to radiological evaluation.

Atrophy of the brain and spinal cord that can be detected 6 months after symptom onset has been proposed as a better marker of disability. Longitudinal studies of MS-associated atrophy (over a decade) are few. Atrophy studies were often performed in association with clinical trials and technically difficult to compare.

Three-dimensional atrophy measurements demand time, advanced software, and trained persons, making them less suitable for the routine radiological evaluation of MS. Volumetric changes can be indirectly evaluated by performing serial one-dimensional and two-dimensional measurements on a routine PACS workstation using a distance tool.

Aim: Our aim was to evaluate the atrophy rate (reduction of brain volume and compensatory supratentorial ventricular enlargement) and its correlation to disability. We chose a cohort with widespread disease duration at baseline in order to study whether atrophy was accelerating or declining over time. The observed individual atrophy rates over one decade represented four decades of disease. We compared the 1D and 2D atrophy measurements to sophisticated 3D measurements to determine which of the measurements best correlate with the three-dimensional measurements.

Materials and Methods: Thirty-seven MS patients aged 24-65 years with a disease duration of 1-33 years were consecutively selected at baseline in 1995-1996 and evaluated by MRI over a mean period of 9.25 years (range 7.3-10 years) from 1995-2005. Serial brain atrophy measurements (1D and 2D) were analyzed by one radiologist on a routine PACS workstation, and these results were compared to semi-automated 3D measurements (HERMES, neuroradiological rater).

Results: Volumetric differences were found over shorter periods of time (1-7 months) but vanished at the end of the follow-up. At the end of the study, a uniform longitudinal decrease in brain volume and increase in ventricle volume was found. The 1D, 2D, and 3D measurements intercorrelated well. Frontal horn width (1D) correlated strongest with the 3D measurements. Atrophy progression was independent of the clinical course of MS. Atrophy measurements were associated with disability, and this association persisted during follow-up.

Conclusions: Despite variable clinical courses, the destructive and degenerative effects of MS seem to progress uniformly over long periods of time. The volumetric changes can be detected using 1D and 2D measurements performed on a routine PACS workstation.

LIST OF PUBLICATIONS

I. Martola J, Stawiarz L, Fredrikson S, et al. Progression of non-age-related callosal brain atrophy in multiple sclerosis: A 9-year longitudinal MRI study representing four decades of disease development. J Neurol Neurosurg Psychiatry 2007;78:375-80.

II. Martola J, Stawiarz L, Fredrikson S, et al. Rate of ventricular enlargement in multiple sclerosis: A nine-year magnetic resonance imaging follow-up study. Acta Radiologica 2008;49:570-9.

III. Martola J, Stawiarz L, Fredrikson S, et al. One-dimensional ratio measures of atrophy progression in multiple sclerosis as evaluated by longitudinal magnetic resonance imaging. Acta Radiologica 2009;50:924-32.

IV. Martola J, Bergström J, Fredrikson S, et al. A longitudinal observational study of brain atrophy rate reflecting four decades of multiple sclerosis: A comparison of serial 1D, 2D, and volumetric measurements from MRI images. Neuroradiology 2010;52:109-17. (Epub 2009 Sep 23).

CONTENTS

LIST OF ABBREVIATIONS

3rdVW, third ventricle width
ANOVA, analysis of variance
BCR, bicaudate ratio
BFR, bifrontal ratio
BPF, brain parenchymal fraction
BPV, brain parenchymal volume
BUR, biuncal ratio
CC, corpus callosum
CCA, corpus callosum area
CDMS, clinically definite multiple sclerosis
CIS, clinically isolated syndromes
CNS, central nervous system
CR, creatine
CSF, cerebrospinal fluid
EDSS, expanded disability status score
ER, Evans ratio
FA, flip angle
FHW, frontal horn width
FOV, field of view
GMV, grey matter volume
ICC, intra-class correlation
ICD, intercaudate distance
IFN-β1, interferon-β1
IgG, immunoglobulin G
MISS, midsagittal internal scull surface area
MRI, magnetic resonance imaging
MRS, magnetic resonance spectroscopy
MS, multiple sclerosis
MSSS, multiple sclerosis severity score
MTR, magnetization transfer ratio
NAA, N-acetylaspartate
NAGM, normal appearing grey matter
NAWM, normal appearing white matter
NEX, number of excitations
OCB, oligoclonal banding
PBVC, percentage of brain volume change
PCA, principal component analysis
PPMS, primary progressive multiple sclerosis
PRMS, primary relapsing multiple sclerosis
RRMS, relapsing remitting multiple sclerosis
SIENA, structural image evaluation using normalisation of atrophy
SPMS, secondary progressive multiple sclerosis
TE, time of echo
TR, time of repetition
VEP, visual evoked potential
WMV, white matter volume

1 GENERAL BACKGROUND

1.1 HISTORY OF MULTIPLE SCLEROSIS

The earliest case of retrospectively identifiable multiple sclerosis (MS) was Augustus d`Este (1794-1848), who had 26 years of progressive symptoms similar to MS, including visual impairment, recurrent motor symptoms, and leg weakness. At that time, d'Este's illness was described as follows: "paraplegia, functional in type and not yet organic" (Fredrikson et al. 1989; Landtblom et al. 2009).

Robert Carswell (1793-1857) was the first, in Pathological Anatomy 1838, to describe disseminated plaques as "a peculiar disease state of the cord and pons Varolii" accompanied by atrophy of the "discoloured portions" in the nervous system (Compston 1988). Jean Cruveilhier (1829-1842) made a similar finding in autopsies in the 1830s and clinically described a patient with progressive symptoms typical of MS (Compston 1988). Similarly, Friedrich von Frerich (Germany) provided an exhaustive description of the pathological and clinical findings of MS. In 1849, von Frerich named this affliction brain sclerosis, "hirnsklerose" (Frerich 1849) and was the first to diagnose it in a living patient, which was confirmed by autopsy years later. Jean-Martin Charcot named the disease "sclerose en plaques" (Charcot 1868). The name "multiple sclerosis" is based on multiple focal areas of scarring in the brain. Jean-Martin Charcot framed Charcot's triad, which consists of dysarthia (speech problems), ataxia (coordination), and tremor.

Brain atrophy was first described by Charcot (1877), and the compensatory brain ventricle widening due to atrophy was first documented by Friedman and Davidson (Friedman et al. 1945).

1.2 PREVALENCE TODAY

Multiple sclerosis is a common neurological disease among young adults, the mean age of disease onset is 30 years (Weinshenker et al. 1991). MS is more common among females (Debouverie et al. 2008). Genetic factors appear to contribute to the disease (Sadovnick et al. 2004). There is also a geographic variation; the northern EU is a high prevalence area (120 per 100 000) (Cooper et al. 2009).

1.3 PATHOPHYSIOLOGY

Multiple sclerosis is a common inflammatory demyelinating disease of the central nervous system. The etiology of MS is still unclear (Murray 2009). Pierre Marie, one of Charcot´s pupils, proposed in 1884 that MS could be an infectious disease. Infection would have been ideal for explaining the epidemiological variation. These hypotheses have been investigated since the time of Charcot but all investigations have failed to prove any true infectious agents. However, Epstein-Barr virus was recently proposed to have a role in triggering MS (Lunemann et al. 2007; Ascherio 2008). Genetic factors

appear to contribute to the disease; MS is more common among monozygotic twins than heterozygotic twins. Environmental factors are also considered to be important (De Jager et al. 2009).

MS is a chronic neurodegenerative disease that results in extensive demyelination, oligodendrocyte loss, and axonal degeneration (Pittock et al. 2007). The present view is that MS is a disorder mediated by autoreactive lymphocytes (Pittock et al. 2007). Later phases of the disease process are dominated by microglial activation and chronic neurodegeneration.

The inflammatory process affecting myelin is triggered by activated T lymphocytes, which recognize myelin as a foreign element and attacks it (Pittock et al. 2007). This process increases the production of antibodies and cytokines (Pittock et al. 2007). Damage to the blood-brain barrier causes leaks and activates macrophages, which stimulate matrix metalloproteinases (destructive proteins) and further cytokine production (Pittock et al. 2007). The repair process - remyelination - is an early phenomenon, but the oligodendrocytes, which formed the original myelin sheath cannot completely rebuild damaged myelin sheaths (Pittock et al. 2007).

1.4 DIAGNOSIS OF MS

In its early stages, MS may be difficult to diagnose (Trojano et al. 2001). The diagnosis of MS is clinical and supported by magnetic resonance imaging (MRI) and abnormal cerebrospinal fluid (CSF) findings (Link et al. 2006). Typical findings are bright ovoid-shaped lesions in the periventricular white matter on T2-weighted images (McDonald et al. 2001). Gadolinium contrast is used to demonstrate active inflammatory plaques with blood-brain barrier damage in T1-weighted images (Rashid et al. 2008).

The Poser criteria (Box 1) have been used since the early 1980s (Poser et al. 1983). These criteria were primarily developed to ensure that only MS patients were included in research studies and consist of clinical characteristics and a number of laboratory findings, such as evoked potential, CSF findings (positive in approximately 95% of patients), and neuroimaging. According to these findings, patients were categorized in the range from clinically definite MS to laboratory supported probable MS.

Box 1. Poser Criteria for MS Diagnosis (Poser et al. 1983)

Clinically definite multiple sclerosis 1. Two attacks plus two separate clinical lesions (detected on history and physical examination) **Or** 2. Two attacks plus one clinical lesion plus another separate paraclinical lesion (detected by evoked response testing, MRI, or urodynamic studies)
Laboratory supported definite multiple sclerosis 1. Two attacks plus one clinical lesion plus CSF, OCB, or IgG **Or** 2. Two attacks plus one paraclinical lesion plus CSF, OCB, or IgG **Or** 3. One attack plus two clinical lesions plus CSF, OCB, or IgG **Or** 4. One attack plus one clinical lesion plus one paraclinical lesion plus CSF, OCB, or IgG
Clinically probable multiple sclerosis **1.** Two attacks plus one clinical lesion **Or** **2.** One attack plus two clinical lesions **Or** **3.** One attack plus one clinical lesion plus one paraclinical lesion
Laboratory supported probable multiple sclerosis 1. Two attacks plus CSF, OCB, or IgG

Currently, the revised (2005) McDonald criteria are the basis for diagnosis (Polman et al. 2005). In 2005, the revision was performed to include new evidence, simplify primary progressive MS (PPMS) diagnosis, and clarify the role of spinal cord lesions (clearly delineated, size, signal intensity, cord swelling, and location in cross-section).

Dissemination over time and consistent abnormalities upon physical examination are enough for establishing a diagnosis of MS.

Box 2. Revised McDonald Criteria for MS Diagnosis (Polman et al. 2005)

Dissemination <u>in both:</u>	**Three of the following MRI findings:**
Space	At least one gadolinium-enhanced lesion or nine T2 lesions
Time	At least one infratentorial lesion (including focal spinal cord lesions)
	At least one juxtacortical lesion
	At least three periventricular lesions

Box 3. Diagnostic Confidence According to McDonald Criteria (Polman et al. 2005)

The diagnosis of "MS" is made if criteria fulfilled.
The diagnosis of "possible MS" is made if the criteria are not completely fulfilled.
The diagnosis of "not MS" is made if criteria are not met.

1.5 CLINICAL PRESENTATION

MS is commonly characterized by relapses, which are acute/subacute attacks lasting a minimum of 24 hours (Lublin et al. 1996). A relapse reaches its peak within days to several weeks, followed by a remission of symptoms and signs to a variable extent (Lublin et al. 1996). Among patients who have MS for more than 10 years, 17% have minimal or no disability (Pittock et al. 2004). Ninety percent of the patients are still able to walk 10 years after onset, and 75% are still able to walk at 15 years (Myhr et al. 2001).

There are no clinical findings unique to MS. Sensory symptoms are common initial features present in almost all patients during the course of the disease and include impaired vibration, proprioception, pain, and touch. Motor symptoms include nystagmus (internuclear ophthalmoplegia), weakness, and impaired co-ordination. Bowel, bladder, and sexual dysfunction are general findings usually appearing later during the disease course (Paty et al. 1993). Unilateral and often acute or subacute optic neuritis is a highly characteristic finding. Forty-eight percent of patients with optic neuritis convert to MS (Swanton et al. 2009).

Box 4. Other Symptoms of MS

Pain
Paroxysmal symptoms
Fatigue
Depression
Coordination
Heat sensitivity
Cognitive dysfunction
Epilepsy
Cognitive dysfunction
Vertigo

Box 5. Four Different MS Courses (Lublin et al. 1996)

RRMS	**Relapsing-remitting MS** Relapses with full recovery or with sequelae and residual upon recovery. No disease progression between relapses.
SPMS	**Secondary progressive MS** Initial RR course followed by progression with or without occasional relapses, minor remissions, and plateaus.
PPMS	**Primary progressive MS** Disease progression after onset with occasional plateaus and temporary minor improvements.
PRMS	**Progressive relapsing MS** Progressive disease after onset with acute relapses and partial recovery. Progression continues during the periods between relapses.

Defining the type of MS course is important not only for prognosis, but also for therapeutic decisions. The past course of the disease is used when trying to predict the future course. The initial course is most often relapsing-remitting MS (RRMS). RRMS is characterized by relapses followed by months to years of remission. Most secondary progressive MS (SPMS) patients initially have RRMS but then began to have deficits remaining between relapses without any definite periods of remission. SPMS is the most common MS course and causes the greatest amount of disability. PPMS represents 10% of MS patients who never experience remission after their initial symptoms, and disability worsening occurs without clear attacks. PPMS patients tend to be older at disease onset. Progressive relapsing MS (PRMS) is the most uncommon MS course. PRMS patients have continuous neurological decline.

1.6 CLINICAL EVALUATION OF DISABILITY

Kurtzke Disability Status Scale Expanded Disability Status Score (EDSS) (Kurtzke et al. 1983)

Box 6. The EDSS quantifies disability in eight functional systems (FS), allowing neurologists to assign a functional system score (FSS) in each FS. These functional systems are:

Pyramidal
Cerebellar
Brainstem
Sensory
Bowel and bladder
Visual
Cerebral
Other

Box 7. The EDSS is still a golden standard of disability evaluation and is defined as:

0.0	Normal neurological examination
1.0	No disability, minimal signs in one FS
1.5	No disability, minimal signs in more than one FS
2.0	Minimal disability in one FS (e.g., slight weakness or stiffness, mild gait, or visual disturbance)
2.5	Mild disability in one FS or minimal disability in two FS
3.0	Moderate disability in one FS or mild disability in three or four FS. Fully ambulatory (e.g., monoparesis, mild hemiparesis, moderate ataxia, disturbing sensory loss, prominent urinary or eye symptom, or combinations of lesser dysfunction)
3.5	Fully ambulatory but with moderate disability in one FS and more than minimal disability in several others
4.0	Fully ambulatory without aid, self-sufficient, up and about roughly 12 hours a day despite relatively severe disability; able to walk without aid or rest for 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; characterized by relatively severe disability; able to walk without aid or rest for 300 meters.
5.0	Ambulatory without aid or rest for roughly 200 meters; disability severe enough to impair full daily activities (work a full day without special provisions)
5.5	Ambulatory without aid or rest for roughly 100 meters; disability severe enough to preclude full daily activities
6.0	Intermittent or unilateral constant assistance (cane, crutches, braces) required to walk roughly 100 meters without resting
6.5	Constant bilateral assistance (canes, crutches, braces) required to walk roughly 20 meters without resting
7.0	Unable to walk more than approximately 5 meters, even with aid, essentially restricted to a wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair roughly 12 hours a day
7.5	Unable to take more than a few steps; restricted to a wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; may require motorized wheelchair
8.0	Essentially restricted to bed or chair or perambulated in wheelchair but may be out of bed by self much of the day; retains many self-care functions, generally has effective use of arms
8.5	Essentially restricted to bed much of day; has some effective use of arms, retains some self care functions
9.0	Confined to bed; can still communicate and eat.
9.5	Totally helpless in-bed patient; unable to communicate effectively or eat/swallow

Box 8. A simpler overview of the disability scale would be:

0	No deficits
3	Moderate motor deficits
9	Confined to bed

1.7 MEASURING CLINICAL DISEASE PROGRESSION OVER TIME (DISEASE DURATION)

The EDSS has several limitations (Hobart et al. 2000); it is insensitive to clinical changes and overemphasizes motor deficits. On the other hand, cognitive function is difficult to measure. The scale is ordinal and not nominal (for example linear), which causes problems in the evaluation. The disability (EDSS) may vary between time points. Symptom progression over time cannot be evaluated using EDSS because disease duration is not included in the Kurtzke Disability Status Scale.

Global multiple sclerosis severity scores (MSSS) was generated from 98.992 European patients' EDSS data (Roxburgh et al. 2005). However, the data were based on individual EDSS values at one time point and did not represent individual EDSS follow-ups. The patients that died due to MS were not included. The MSSS provides an opportunity to evaluate a patients' EDSS at a particular moment against "global mean development".

According to a 25-year follow-up study of 308 patients, 80% of the patients reached the progressive phase by the endpoint, 15% had died, 65% had reached an EDSS of 6 (walking-aids), and 50% reached an EDSS of 6 within 16 years of disease onset (Runmarker et al. 1993).

1.8 THERAPIES FOR MULTIPLE SCLEROSIS

A lot of therapies have been tested for the treatment of MS. Some examples from the 1870s (W. Moxom) include faradic stimulation, strychnine, and arsenic. Because infectious genesis was suspected to cause MS, various antibiotic, antiviral, and antifungal drugs have been tested since the 1940s (Murray et al. 2009). Corticosteroids (methylprednisolone) have been used for 50 years to treat acute attacks and reduce inflammation (Murray et al. 2009). The treatments currently registered include interferon (IFN)-β1a, IFN-β1b, glatiramer acetate, and natalizumab. Several new drugs, including oral regimens and different monoclonal antibodies, are presently being investigated in phase II-III studies (Bermel et al. 2008).

1.9 RADIOLOGICAL FINDINGS IN MS

As previously mentioned, T2 high signalling multifocal oval periventricular white matter changes (Figure 1) are the most characteristic MRI findings in MS (McDonald et al. 2001). MRI findings have added to the understanding of the macroscopic pathophysiology. However, MRI findings lack specificity at the microscopic level (Neema et al. 2007) and are often temporary. Since the earliest MRI studies, atrophy has been recognized as a feature of later stages of the disease. Modern high quality MR images allow the radiologist to detect small brain volume changes in early stages of MS. Recently, brain atrophy and cortical lesions have been recognized as pathological markers of disease progression. Short term atrophy progression seems to be more pronounced in grey matter than white matter. Grey matter atrophy seems to be an early

phenomenon of disease progression (Chard et al. 2002). Grey matter atrophy is reported to correlate with disability and cognitive worsening (Amato 2004).

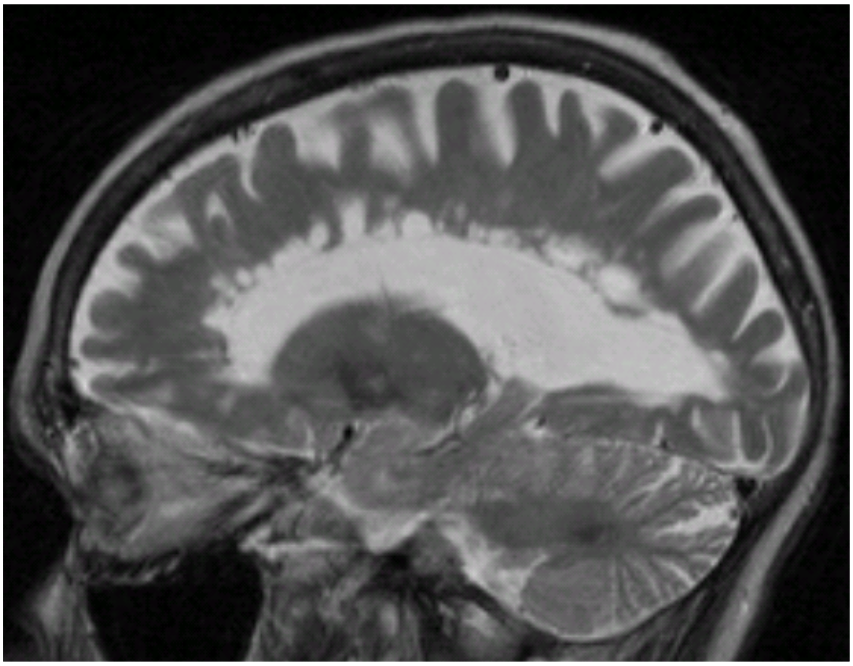


Figure 1. T2 lesions in periventricular white matter.

Other findings are T1 hypointense lesions, termed "black holes", T1 gadolinium enhanced lesions, optic neuritis, myelitis (Figure 2) and grey matter lesions (Traboulsee 2004). The grey matter lesions are difficult to detect on MRI, and many lesions are too small to be identified by conventional MRI. Signs of atrophy are an enlargement of intra- and extracerebral liquor spaces, compensatory ventriculomegaly, and widening of the cerebral sulci (Bermel 2006). Atrophy reflects diffuse degenerative damage in the brain (Traboulsee 2004) and spinal cord (Stankiewicz 2009).

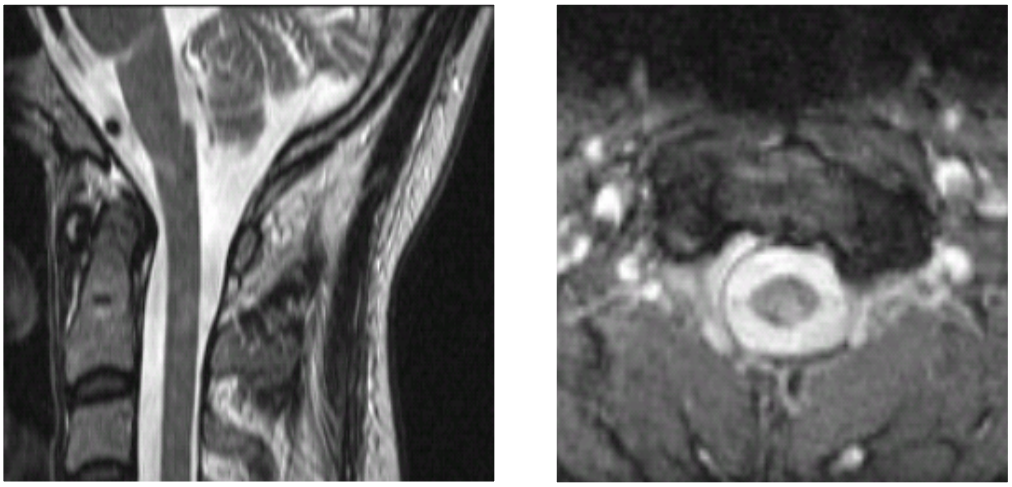


Figure 2. T2 hyperintense lesions in both the medulla oblongata and spinal cord, representing myelitis.

The magnetization transfer ratio (MTR) has been increasingly used to evaluate myelin content and axonal count. Magnetic resonance spectroscopy (MRS), diffusion tensor MRI, and diffusion-weighted imaging may provide insight into diffuse abnormal changes in normal appearing white matter (NAWM), normal appearing grey matter (NAGM), and axonal loss (Rovaris et al. 2009). Diffusion tensor imaging is sensitive to microscopic changes in the tissue appearing normal in conventional images, but it is unable to clearly distinguish between the various physiopathological processes involved (Cassol et al. 2004).

Functional MRI can be used to evaluate specific functional compensatory mechanisms of neural pathway damage in MS (Krause et al. 2009). MRI perfusion has revealed decreased blood perfusion in the grey matter of MS patients, particularly patients with a progressive disease course. Several pathological mechanisms have been postulated for explaining these changes in NAWM (Law et al. 2004). High field MRI has been increasingly used for the clinical evaluation of MS (Kangarlu et al. 2009). The advantages are higher magnetic field strength, chemical shift, and increased signal-to-noise ratio (SNR), which contributes to increased lesion detection ability. The new generation of ultra-high field beyond 3T has submillimetre resolution, and 3T can detect lesions missed by conventional 1.5T sequences (Stankiewcz et al. 2009).

Proton magnetic resonance spectroscopy (^{1}HMRS) measures the N-acetylaspartate (NAA) concentration (neuronal metabolite). The concentration of NAA is measured most commonly as a ratio of NAA to creatine (Cr) and decreases when neuronal dysfunction, damage, or axonal or neuronal loss occurs (Bjartmar et al. 2002). A decreased NAA/Cr ratio is found within MS lesions (Matthews et al. 1996).

Positron emission tomography has revealed glucose metabolism abnormalities in both the grey and white matter, and it may provide additional information on these diffuse tissue changes (Blinkenberg et al. 2000).

1.10 PROBLEMS IN CLINICAL AND MRI FOLLOW-UPS

Multiple sclerosis usually involves multiple parts of the CNS, which may have a complex affect on patient disability. Disease variability effects on different components of the CNS, therapy response, and disease course challenges the evaluation of MS. The “clinico-radiological paradox” was established by Frederik Barkhof to describe the poor correlation between the disability of MS patients and MRI findings (Barkhof 2002).

Less than 10% of MRI lesions are associated with changes in disability (Traboulsee 2004). MRI lesions are mostly non-permanent, and MRI has not yet been able to aid in defining the underlying microscopic disease (Bermel et al. 2008). Patients can be clinically silent with many new MRI lesions or the patient can be radiologically “silent” while having new symptoms (Traboulsee 2004). Also, there might not be a direct

temporal relationship between clinical changes and MRI findings (Giorgio et al. 2008). T2 high signalling lesions and T1 black holes may disappear. T1 contrast enhanced lesions disappear over time. Different inflammatory processes reduce or increase brain volume. The temporary nature of these findings challenges the radiological evaluation and its predictive value. However, the accumulation of T2 lesions has been reported to correlate with atrophy (Chard et al. 2003). Treatments can reduce brain volume (Anderson et al. 2006).

Box 9. Monitoring Treatment Effects in Clinical MRI Follow-ups (Bermel et al. 2008).

New T2 lesions
Enlargement/reduction of T2 lesions
T2 lesion load
T1-hypotense lesions (“blackholes”)
T1 gadolinium enhanced lesions

Box 10. Future options for detecting more subtle MS-related parenchymal abnormalities.

Magnetization transfer MRI
Diffusion-weighted MRI
Perfusion imaging
Proton magnetic resonance spectroscopy

1.11 EVALUATION OF ATROPHY IN MS

Several manual, semi-automated, and automated methods are available for evaluating regional and whole brain atrophy (Giorgio et al. 2008). When choosing the most suitable method, several aspects should be considered, including accuracy, speed, practicality, sensitivity, and reproducibility. The most optimal method for research use might not be the most practical for routine radiological use.

Thin volumetric (1 mm) sequences are preferred because anatomy is easier to define and partial volume effects are less disturbing. A multi-centre volumetric study using different scanners and sequences found them to intercorrelate (Jasperse et al. 2007).

Regional linear (1D) and area (2D) measurements are the oldest and most often used in routine radiology (Evans 1942; Barkhof 1998). However, the measurements are

affected by slice selection (slice positioning), slice thickness, interindividual normal variation, and performance.

Volumetric methods can be performed manually but are time consuming. Semi-automated and automated methods are faster and have better reproducibility (Giorgio et al. 2008). These methods can be used for both global (whole brain volume) and focal (caudate nuclei) measurements (Pagani et al. 2005). Voxel-to-voxel analyses are used for grey matter volume evaluations of the whole brain (no region of interest) (Giorgio et al. 2008). Serial evaluations are preferred in order to better evaluate the progression of atrophy. One automated method is structural image evaluation using normalization of atrophy (SIENA), which determines brain displacement between two scans and calculates the percentage of brain volume change (PBVC) (Giorgio et al. 2008).

Nowadays, automated measurements are preferred due to their reproducibility and efficacy. When a specific region, such as the thalamus, is measured, the semi-automated method may improve the validity due to its interactive control during segmentation steps. However, both methods demand skilled persons and software and, thus, are used less in routine radiology (Giorgio et al. 2008).

2 AIMS OF THE THESIS

The aim of this thesis was to study:

1. Atrophy progression (1D, 2D, 3D) reflecting four decades of disease;
2. The relationship between atrophy and disability;
3. The relationship between the rate of atrophy and MS courses;
4. The intercorrelation between two methods (1D and 2D) on a routine workstation versus the semiautomated (3D) Hermes method;
5. The best routine (1D or 2D) method for evaluating atrophy progression.

3 MATERIALS AND METHODS

3.1 SETTINGS

The original study based on data from 1995-1996 was orientated to neuroimmunology and contrast enhancement. No normal controls were included. Our study was performed from 1995-1996 to 2003-2005 at the Department of Neurology, Karolinska University Hospital. All patients provided informed consent. The study was approved by the ethical committee at Karolinska Institutet.

3.2 PATIENTS (PAPER I, II, III, AND IV)

Forty-five consecutively referred ambulatory patients were included in the study. All patients had clinically definitive MS (CDMS) according to the Poser criteria (Poser et al. 1983) and MS according to the revised McDonald criteria (Polman et al. 2005). The clinical diagnosis of MS was made by experienced neurologists.

Gender*	Female	70.27% (n=26)
	Male	29.73% (n=11)
Interferon*	Treatment	64.86% (n=24)
	Non-treatment	35.14% (n=13)
MS course*	RRMS	43.2% (n=16)
	SPMS	46.0% (n=17)
	PPMS	10.8% (n=4)
Age (years)**		42 (10.29, n=37)

* Percentage of total (sample size)

** Mean (1 SD, sample size)

Table 1. Cohort characteristics at baseline. Most patients were female, and 24 of the 37 follow-up patients had received interferon treatment during the follow-up period. There was neither any randomized selection nor information available on compliance. Eight patients were lost to follow-up: one emigrated, one had postoperative contraindicating metal clips, two died, and the remaining four refused to participate or had no contact with the MS clinic.

The remaining 37 patients had a broad range of disease duration at baseline in 1995-1996 (1-33 years). The 37 patients had a mean age of 42.0±10.3 years (range 24-65 years) and represented three MS courses: relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS). Six of the 37 patients converted from RRMS to SPMS during follow-up and were included in the SPMS group for statistical analyses.

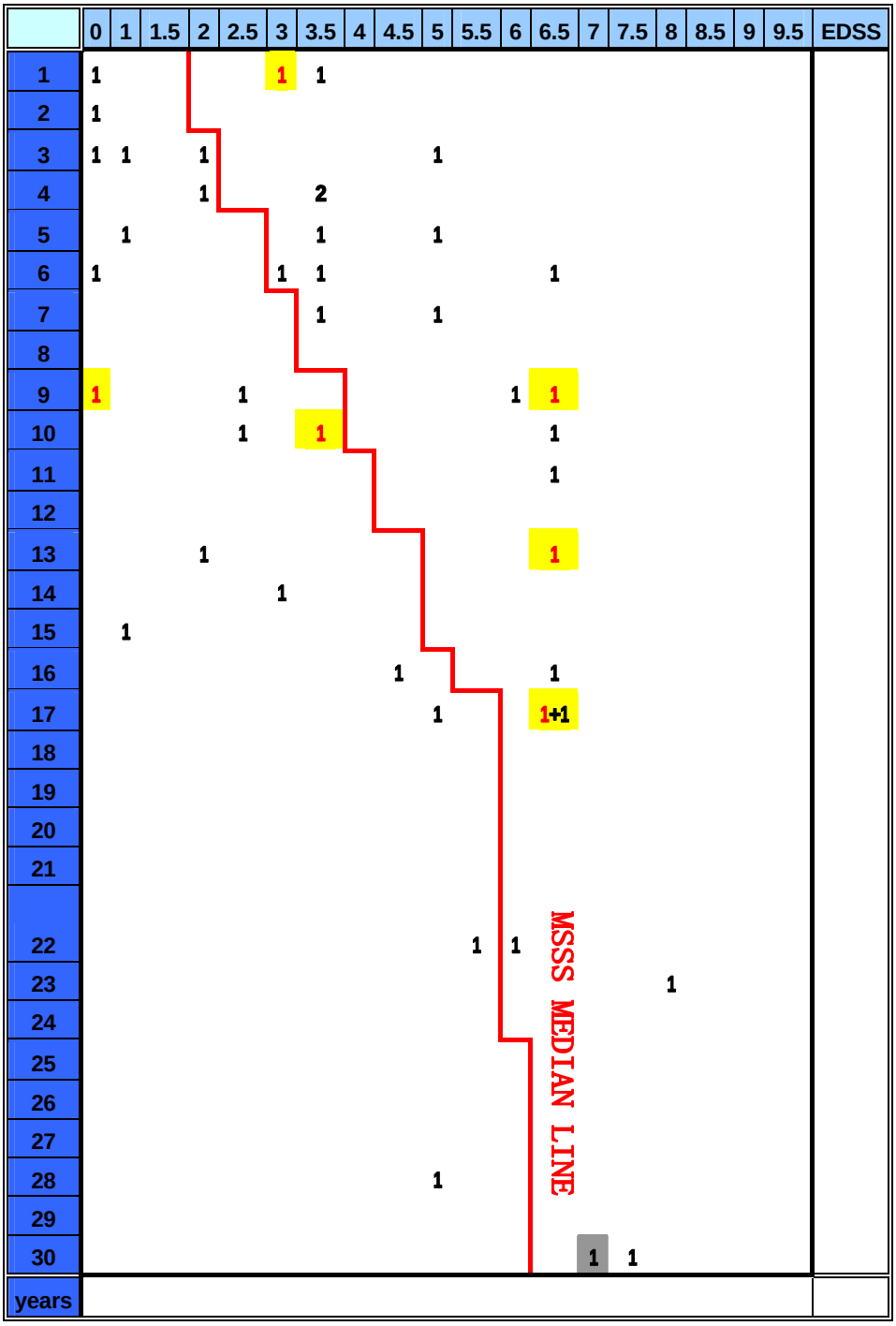


Figure 3. Clinical disability was monitored using the expanded disability status score (EDSS) (Polman et al. 2005). EDSS scores were adjusted to disease duration by converting to MS severity scores (MSSS) (Roxburgh et al. 2005). At study entry in 1995-1996, 43 of the original 45 patients (including losses to follow-up, yellow background) were distributed on both sides of the median MSSS (no signs of disability-related selection bias, confounding by indication). One patient with 33-year disease duration at baseline (grey background) was considered as 30-year duration. For two patients (losses to follow-up), data concerning MS onset and follow-up were lacking and could not be included.

3.2.1 Disability development reflects four decades

Individual EDSS development over 9-10 years (1995-2005), including follow-up, for 37 patients was evaluated (Figure 4). Twenty-five patients had an increased EDSS, six were stable, and six patients had a decreased EDSS.

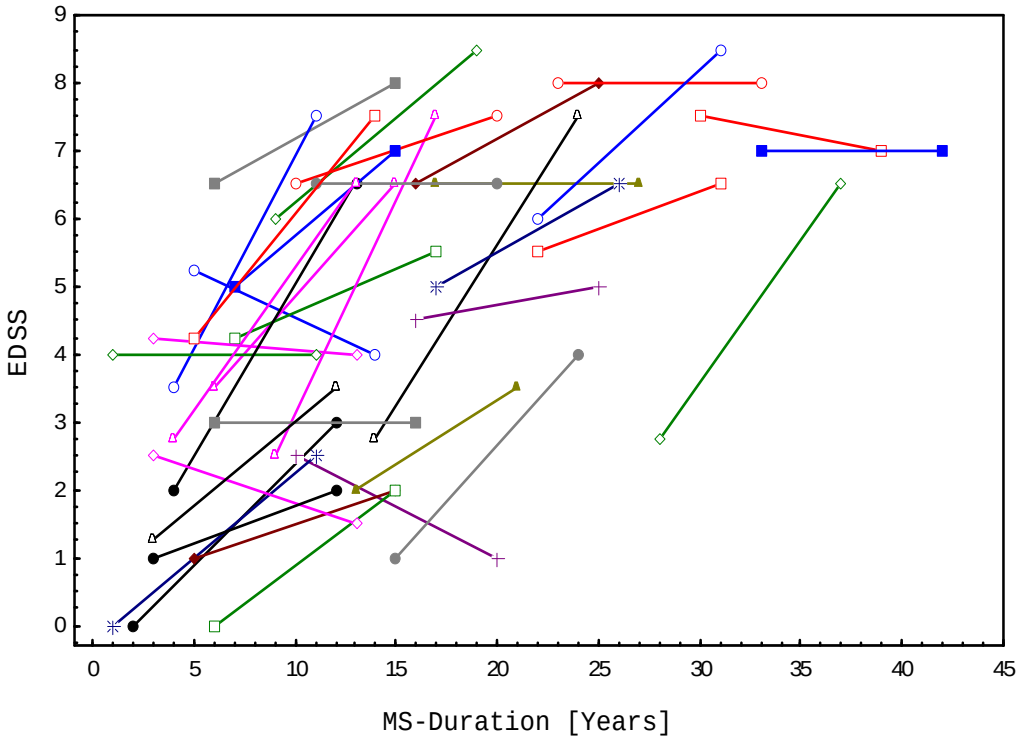


Figure 4. Disability improved in six patients with a change in the mean EDSS of 0.8 (range 0.25-1.25) during the 9-year follow-up. Four of these patients had fluctuating EDSS scores between the first and second measurement in 1995-1996. There was not a second EDSS for two patients in 1996. Therefore, we calculated a mean EDSS using both the first and second EDSS in 1995 and 1996.

3.3 MAGNETIC RESONANCE IMAGING

3.3.1 Time points for all investigations performed (1995-2005)

Measurements in Paper I were based on two time points: the first in 1995-1996 and a second at the end of the study in 2003-2005.

Measurements in Papers II and III were based on three to five time points, as well as all available clinical MRI investigations from 1996 to 2003, which were included in order to improve the analyses. The first two time points were in 1995-1996 (range 1-7 months), and the third was at the end of the study in 2003-2005.

Measurements in Paper IV were based on three time points: the first two in 1995-1996 (range 1-7 months) and at the end of the study in 2003-2005.

3.3.2 Imaging protocol (Paper I)

The MRI examinations were performed with sagittal T2-weighted MR images obtained at baseline in 1995-1996 and at the end of the study in 2003-2005 using 1.5T scanners with a standard head coil (1995-1996 GE Signa and 2003-2005 Siemens Magnetom Vision). The field of view (25 cm) and matrix (256 x 256) were the same for both examinations. In 1995-1996, scans were performed using fast spin echo (TR/TE/FA/NEX = 4000/76/90/1). Slice thickness was 5 mm with no gaps. In 2003-2005, scans were performed using turbo spin echo (TR/TE/FA/NEX = 3500/96/180/2), with 4-mm-thick slices and a 0.4 mm distance.

3.3.3 Imaging protocol (Papers II, III, and IV)

The MRI examinations were performed on 1.5-Tesla MR systems: a Signa Advantage scanner (General Electric Medical Systems, Milwaukee, WI) at baseline in 1995-1996, and a Magnetom Vision scanner (Siemens, Erlangen, Germany) at the end of the study in 2003-2005. Both systems obtained T1-weighted 5-mm axial images using a standard T/R birdcage head coil.

In 1995-1996, MRI examinations were performed twice (range of 1-7 months) using a spin echo pulse sequence (TR/TE/FA/NEX = 640/13/90/2). The field of view was 250 x 188 with a matrix size of 256 x 256 and no gaps between slices. In 2003-2005, MRI scans were performed using a turbo spin echo pulse sequence (TR/TE/FA/NEX = 570/14/90/2). The field of view was 220 x 220, matrix size 256 x 192, and the distance between slices 1.5 mm.

We included 24 MRI scans of 16 patients (same cohort of 37 patients) examined between 1996 and 2003. Twenty-one of the scans were performed on the same MRI scanner (Siemens Magnetom Vision) using the same protocol as mentioned above. The remaining three MRI examinations were performed on a 1.5T Siemens Symphony scanner with T1-weighted, 5-mm, axial images (TR/TE/FA/NEX = 525/15/90/2) and a 1.5-mm distance between slices. The field of view was 220 x 220 and the matrix size 256 x 192.

3.4 IMAGE ANALYSIS

3.4.1 Paper I (2D; in absolute values, mm^2)

The MR data was analyzed by a radiologist blinded to the clinical data using a PACS workstation (Sectra, Sweden). The midsagittal callosum area (Figure 5) and total intracranial area were measured on T2-weighted sagittal images in the midline (Barkhof et al. 1998). For orientation, local anatomy, including CSF filled interhemispheric fissure (IF), anterior commissure (AC), posterior commissure (PC), and the vein of Galen, were used as landmarks and reference points. The midline internal skull surface area (MISS) was delineated by the bony margins of the skull and reflected the total

intracranial volume. Intracranial volume (3D) is age independent in adulthood (Sullivan et al. 2001) and therefore MISS (2D) is constant over time.

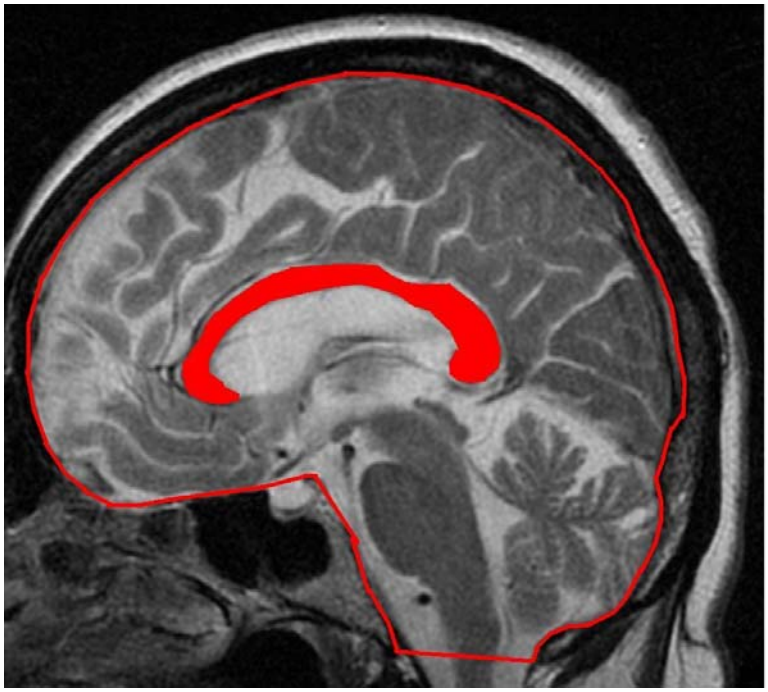


Figure 5. The corpus callosum midsagittal area is shown in red, and the midsagittal internal scull surface area (MISS) is within the red line.

Corpus callosum measurements were repeated three times and a mean value calculated. The MISS was measured once for each MR scan (Figure 5). To assure the comparability of serial callosal measurements, a normalized ratio was applied by dividing the mean corpus callosum area by the MISS. This CCA/MISS ratio was used as an internal calibration control because of the long interval (9 years) between the two MRI examinations, the use of different scanners at baseline and the end of the study, and slightly different imaging parameters. The normalized ratio was based on the fact that skull volume is known to be stable in adulthood (Sullivan et al. 2001).

3.4.2 Paper II (1D; in absolute values, mm)

Three serial linear axial distances (Figure 6) of the third and lateral ventricles were measured on an axial 2D single-image slice using a distance tool after visual selection of the corresponding slice position for each examination. Measures were defined as frontal horn width (FHW), intercaudate nucleus distance (ICD), and third ventricle width (3^{rd} VW).

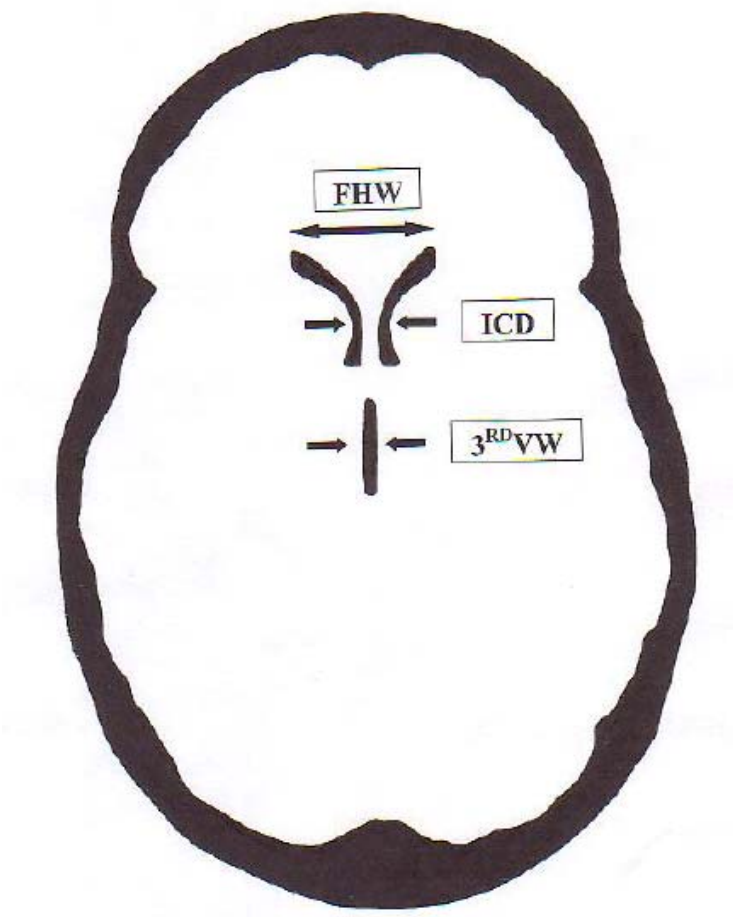


Figure 6. The three linear measurements of the third and fourth ventricles.

3.4.3 Paper III (1D; in normalized relative values)

After parallel visual selection of the identical slice position for each examination, four serial linear ratios (Figure 7) were measured on an axial section image using a distance tool. Measures were defined as the biuncal ratio (BUR), bicaudate ratio (BCR), bifrontal ratio (BFR), and Evans ratio (ER) (Bermel et al. 2002; Frisoni 1996; Laakso et al. 1995).

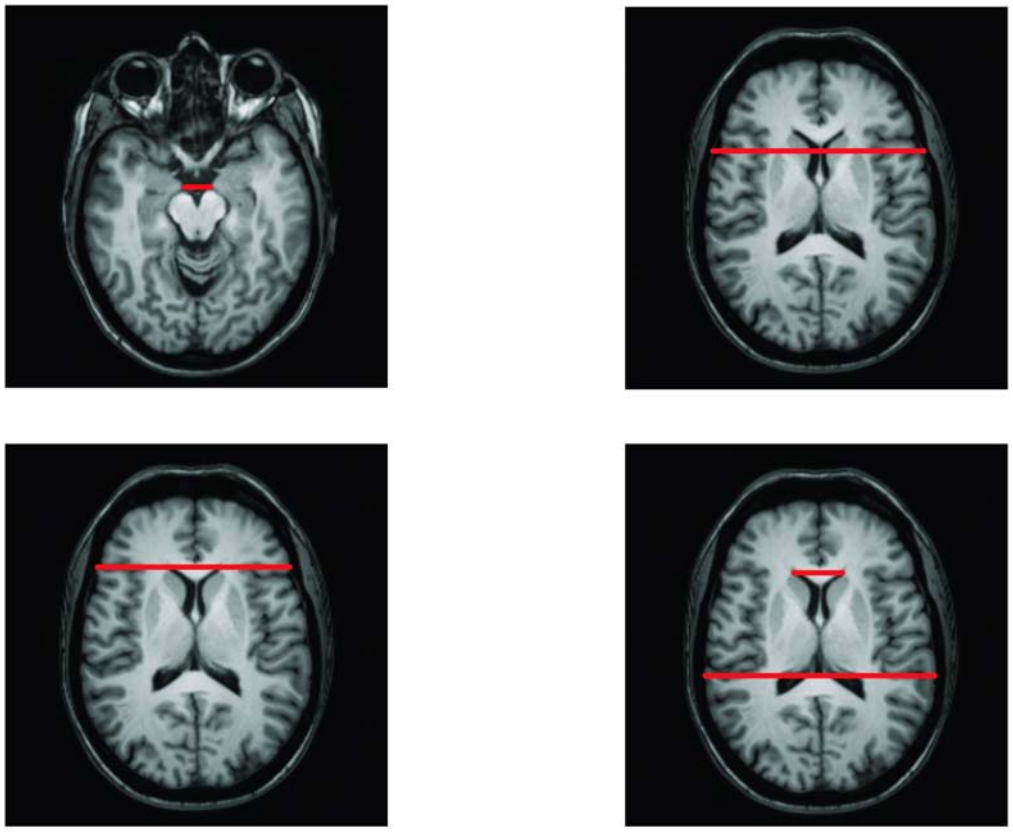


Figure 7. The four linear ratio measures**:**

Top left: Biuncal ratio (BUR) was the minimal distance between hippocampal uncus divided by the maximum intracranial width (same as Evans ratio).

Top right: Bicaudate ratio (BCR) was the minimal distance between the caudate nuclei divided by the intracranial width at caudate level.

Bottom left: Bifrontal ratio (BFR) was the maximum width between the frontal horns divided by the intracranial width at the frontal level, measured on the same vector.

Bottom right: The ER was the maximum width between frontal horns divided by the maximum intracranial width.

3.4.4 Paper IV (3D; in absolute values, cm3)

Images were transferred to a HERMES workstation (Nuclear Diagnostics, Stockholm, Sweden) on which volumetric measurements were performed by a neuroradiological rater (Y.Z) who was blinded to all clinical information. A semi-automatic tool in HERMES (MultiModality, Region Growing) was directly used in volumetry of the lateral ventricles, the third ventricle, and the whole brain volume with original axial T1-weighted images.

According to the individual variations in signal intensity distribution, a test of the growing effect by visual evaluation was done to set specific individual parameters

before each final growing volume was obtained. When leakage of the region of interest occurred after growing, manual masking before growing was used.

Intraclass correlation (ICC) was calculated from 10 randomly selected subjects with double measurements. The ICC of semi-automatic volumetry of the lateral ventricles, the third ventricle, and brain parenchymal volume (BPV) was 0.93.

3.5 STATISTICAL ANALYSIS

3.5.1 Data characteristics

A distinct feature of the collected data was an unequal spacing between measures in the patients. The unequal spacing was due to the fact that MS duration was different in the patients. Simpler statistical methods would fail to incorporate this characteristic, for example, the classical ANOVA for repeated measures. In classical ANOVA, time has to be modelled as a categorical variable. The solution in Papers I-IV was to use statistical methods that can treat time as a continuous variable.

3.5.2 Introduction

A wide range of statistical methods and tools were used throughout Papers I-IV. Regression-like analyses, such as random coefficient models, were used to evaluate longitudinal changes in brain atrophy. To investigate the effects on disability measures EDSS and MSSS, exact binary logistic regression was performed.

In Papers II-IV, principal component analysis (PCA) was used to reveal patterns and associations among the different brain atrophy measures in regards to their change over the duration of the study. Also, Spearman correlations were used as an explanatory tool to examine the association between brain atrophy and disability measures at baseline and the last follow-up. Profile line plots were produced to illustrate the longitudinal change in brain atrophy. The graphs included each patient's brain atrophy measure at each time point and the estimated brain atrophy change.

Results were presented in tables with a measure of location (e.g., regression slope, odds ratio), standard error (if applicable), 95% confidence intervals, and *P*-values. All regression analyses were multivariable and included important predictors that are described in detail below.

3.5.3 Paper I

Measurements of the corpus callosum area (CCA) were made at two different time points, 1995-1996 and 2005, which posed a problem for using more sophisticated statistical methods as they require more than two observations per subject to estimate a within-subject variance. The solution was to use a summary statistics approach (Davis 2002) to evaluate effects on CCA changes. Using summary statistics means reducing multivariate measures to one meaningful univariate measure. Examples of summary

statistics include individual slopes, the average response of single subjects, and the difference between the last and first longitudinal measurement (Weiss 2005).

The disability measures EDSS and MSSS are obtained through a self-validated questionnaire; as such, the data is categorically ordinal. Ascribing a numeric values to ordered categorical data would be wrong (Cambell 2007); thus, EDSS and MSSS were categorized into two clearly defined classes: 1, if an increase occurred at follow-up; 0, if a decrease occurred at follow-up or if the patient was unchanged.

3.5.4 The National Institutes of Health (NIH) method

This is a two stage method often referred to as the National Institutes of Health method (Davis 2002). At the first stage, an ordinary least square regression is performed on each subject, estimating the new outcome variable change per time unit,

$$Y_t = \alpha + \beta * time_t$$

where Yt is the predicted outcome at time t, α is Y when t=0, and β is the linear change per time unit of the outcome.

At the second stage, simple parametric or nonparametric statistics can be applied to evaluate effects on the linear change.

3.5.5 Exact logistic regression

When performing a logistic regression, there are different techniques to estimate odds ratios, *P*-values, and confidence limits. The most common approach is to assume that the null distribution is chi-square. This assumption is valid if the sample size is large enough and if cases and non-cases are fairly represented in the contingency table. When sample size or representation is sparse, there is a risk of an increased type I error rate, and sometimes an increased risk of a type II error rate. A more conservative approach is to use exact methods instead (LogXact 7 2005).

3.5.6 Spearman rank correlation coefficient

The Spearman rank correlation, r_s, is a simple nonparametric measure of the relationship between two variables. The relationship does not need to be linear, and the Spearman rank correlation can be used to assess general relationships (Altman 1999).

$$r_s = 1 - \frac{6\sum_{i=1}^{n} d_i^2}{N^3 - N}$$

where d_i is the difference in the rank for subject *i* and N is the sample size.

3.5.7 Analysis of longitudinal change

The NIH method was used to analyse effects on changes in the CCA. First, a linear CCA change per month was calculated for each MS patient as described above. Second, an ordinary multivariable regression analysis was performed to investigate the influence of disease duration, age at MS onset, gender, type of MS course, and IFN usage on the linear changes in CCA. Age can be entered in a number of ways in a model: age at follow-up, age when entering the study, age at MS course debut, and as disease duration. The different age variables might have a high intra-correlation; thus, age at MS onset and disease duration was included in the models, representing cross-sectional and longitudinal effects, respectively.

3.5.8 Analysis of disability measures

Exact logistic regression was performed to evaluate the effects on changes in the EDSS and MSSS. The type of MS course, MS duration, age at MS onset, gender, and linear change in CCA per month were included in the model as effect variables. The redundant variables were eliminated from the model using a backward selection procedure. The outcome was binary, and odds ratios were used as an estimated effect measure.

3.5.9 CCA versus disability measures

Spearman correlations between CCA and the disability measures EDSS and MSSS were calculated separately at baseline and follow-up. This part of the study should be viewed as exploratory because no model was set up. The Spearman correlations were calculated using the original ordinal scale of EDSS and MSSS.

3.5.10 Papers II-IV

More than two MRI measurements (time points) were available for each patient, and a within-patient variation could be estimated and more sophisticated statistical methods used in Papers II-IV. Because data were collected on different occasions for each patient, there was a need to treat time (duration) as a continuous variable. A random coefficient model was the method of choice because time effects can be modelled such as a covariate than in an ordinary regression analysis. In each of these studies, multiple outcome variables were present and the question of how they interacted needed to be addressed, which was done using PCA. Principal component analysis can detect clustering among a set of variables.

3.5.11 Random coefficient models

Random coefficient models (Figure 8) permit time to be modelled as a continuous variable and, thus, are suitable when data is collected on different occasions for each subject (Fitzmaurice et al. 2004). The method has some similarity with the NIH method because regression lines are fitted for each subject. However, random coefficient

models estimate the covariance between pairs of repeated measures, making it a more flexible method. Random coefficient models are a combination of fixed and random components. The fixed effects are estimates shared by all subjects in the population; the random effects are specific for each subject. The random effects are modelled by fitting regression lines to each subject, and these can vary with regard to intercepts and slopes. In a random intercept model, the intercept (between-subject variation) varies, but the model predicts a common slope that is shared by all subjects. Thus, all subjects have the same estimated change over time. In a random intercept and slope model, different intercepts and slopes are estimated for each subject, and the subjects have a subject-specific estimated change over time.

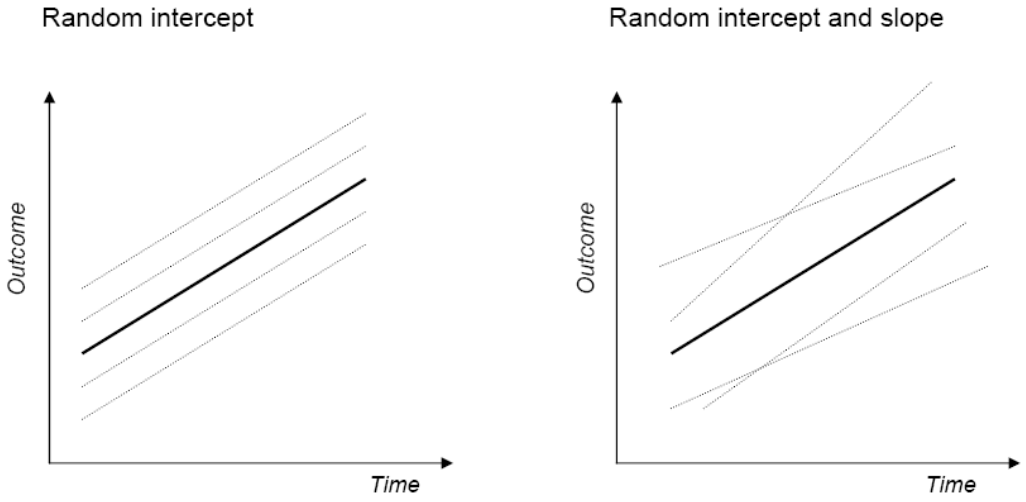


Figure 8. Random coefficient models. The fixed effect is indicated by the solid line. Four different subjects are represented by dashed lines.

The intercept and slope can be assumed to correlate. For example, an estimated positive correlation between intercept and slope would indicate that the subjects at a high outcome level (e.g., at baseline) have a larger increase over time, and vice versa. Due to the flexibility of this modelling approach, being parsimonious in the number of estimated parameters is vital. Akaike's Information Criterion (AIC) can be used to find an appropriate model among candidate models (Fitzmaurice et al. 2004).

There are other information criteria that can be used, such as the Bayesian Information Criterion (BIC). The BIC is usually more conservative then AIC and will favour models with less estimated parameters.

The random coefficient model is a large sample method and can lead to an increased type I error rate when analysing small data sets. To avoid liberal *P*-values, a correction

of the tests performed is advisable. The correction can be achieved using the method suggested by Satterthwaith or Kenward and Roger (Fitzmaurice et al. 2004).

3.5.12 Principal components

Principal component analysis is a multivariate statistical method that can be used to identify patterns among variables. If variables correlate highly, they may be a measure of the same latent construct. The latent construct is not directly observable (e.g., satisfaction, IQ, general health) but can be indirectly observed through measurable variables. Principal components are parameterised so that the first component accounts for the maximum variance of the data, the second component accounts for the maximum of variance not accounted for by the first component, and so on (Armitage 2007). The relationship between a variable and a component is measured by a pattern coefficient, which is also called loading. The pattern coefficient is interpreted as a correlation, and variables can be regarded as belonging to a component if this coefficient is high (e.g., $r>0.7$). For example, five variables are measured, A-E. If A, B, and C display a pattern coefficient greater than 0.7 with a component, then A-C measures the same latent structure (e.g., satisfaction, IQ, general health).

3.5.13 Analysis of longitudinal change

Throughout Papers II, III, and IV, random coefficient models were used to investigate the longitudinal changes in brain atrophy. The method could be used because more than two measures were available for each individual. Disease duration, age at MS onset, gender, type of MS course, and IFN usage was entered into each model as effect variables. Because the rate of change was quite different for each patient, a model with a random intercept and random slope was used in each analysis. Stepwise backward selection was used to eliminate each model of redundant variables. Variables were included regardless of *P*-value if suggested by previous findings or if they had a particular clinical importance.

3.5.14 Overall patterns of longitudinal change

The outcomes measured are a reflection of the same disease and may be highly intra-correlated. To evaluate the correlation between longitudinal changes of the outcome measures, a PCA was performed. The longitudinal change was estimated for each patient in the mixed linear models. Principal component analyses were carried out using the outcomes of interest in each paper, with the exception of Paper I. The longitudinal change of every outcome in Papers II-IV was used in that PCA.

3.5.15 Disability measure analysis

The probability of increased disability, as shown by an increased EDSS and MSSS, was analysed using exact logistic regression. The type of MS course, MS duration, age at MS onset, gender, and change in the outcome of interest per month were included in the model as effect variables. Stepwise backward selection was used to eliminate each

model of redundant variables. Because the outcome was binary, odds ratios were used to estimate effect.

3.5.16 Atrophy measures versus disability measures

Spearman correlations between the atrophy measures and the EDSS and MSSS were calculated at baseline and follow-up. This part of the study should be viewed as exploratory because no model was set up. The EDSS and MSSS were analysed using their original ordinal scale.

4 RESULTS AND COMMENTS

4.1 PAPER I

4.1.1 Aims

The corpus callosum (CC) forms the roof of the third and lateral ventricles. The size of the CC (measured as CCA) in a midsagittal image is age-independent in the normal adult population up to the 7th decade; therefore, it can be used as a marker of non-age-related, pathological, brain atrophy. We investigated whether and how CCA decreases in size over time in MS patients.

4.1.2 Main results

Highly significant, non-age-related progression of corpus callosum atrophy (Figure 9-10) was found with a persistent association between CCA and disability status (Table 2) over a 9-year follow-up study of MS patients, representing four decades of disease development. We did not find significant variation in the atrophy rate between the three MS courses.

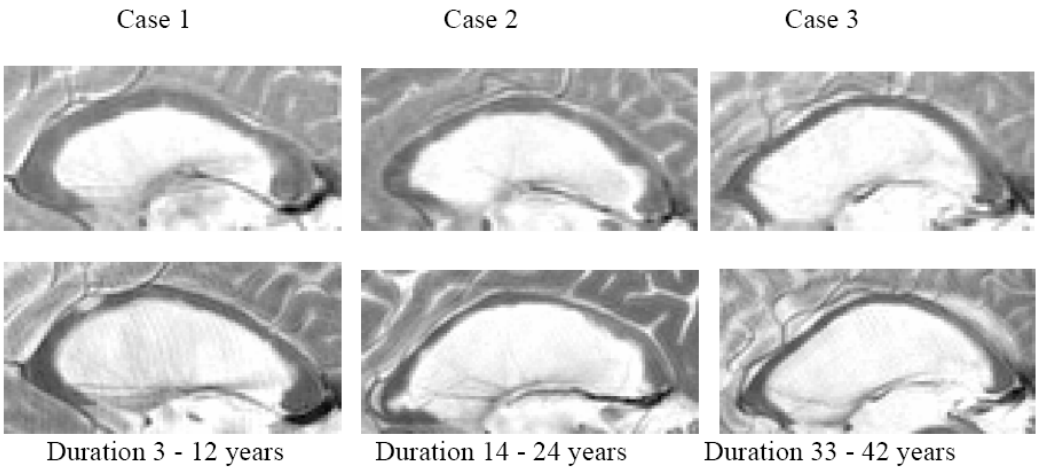


Figure 9. MRI T2-weighted sagittal images showing a thinning of the corpus callosum during follow-up in three patients between baseline (above) and the end of the study (below).

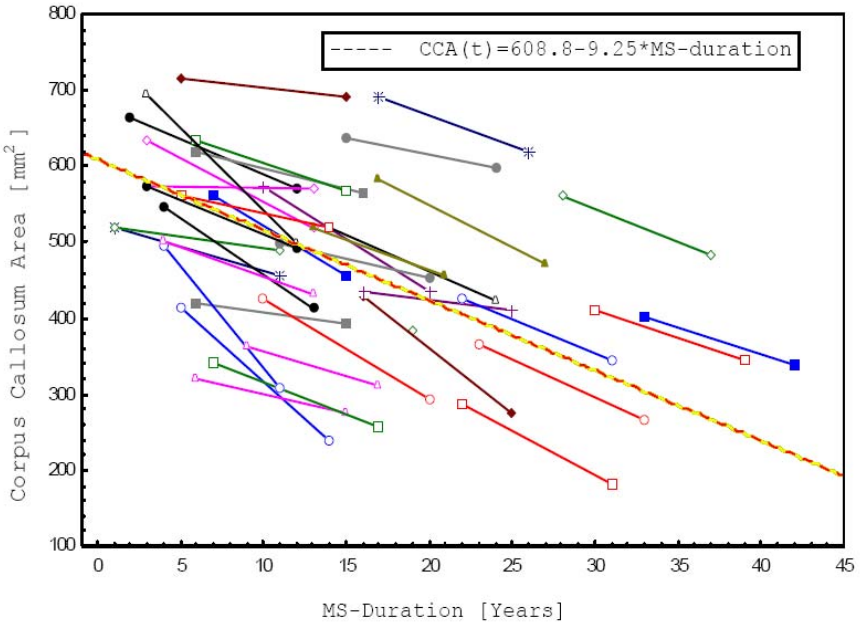


Figure10. Individual evolution of the corpus callosum area, measured at baseline and the end of the study (1.80% annual CCA decrease).

	CCA * ($mm^{2)}$	Individual EDSS Prob(increase)**	Individual MSSS Prob(increase)**	MRI follow-up (years) *
Gender				
Female	-9.21/7.60/25	0.65/17/26	0.61/6/23	9.31/0.83/26
Male	-9.26/4.7/11	0.72/8/11	0.75/14/8	9.09/0.68/11
Interferon				
Treatment	-8.44/5.72/23	0.79/19/24	0.81/17/21	9.13/0.80/24
Non-treatment	-9.71/5.65/13	0.46/6/13	0.3/3/10	9.46/0.52/13
MS course				
RRMS	-8.52/5.84/16	0.63/10/16	0.60/9/15	9.43/0.63/16
SPMS	-10.90/6.42/16	0.71/12/17	0.62/8/13	9.11/0.88/17
PPMS	-5.61/2.34/4	0.75/3/4	1.00/3/3	9.0/0/4
Overall	-9.25/5.62/36	0.67/25/37	0.65/20/31	9.24/0.72/37

*Mean gradient/SD/number of patients
**Probability/number of patients with increase/number of patients

Table 2. The MSSS scale ends at 30 years of disease duration. We could only monitor 31 of the 37 patients to the end of the study.

We concluded that serial evaluations of the CCA might be a robust method for monitoring a non-age-related decrease in the CCA, reflecting the progression of irreversible destructive changes in MS.

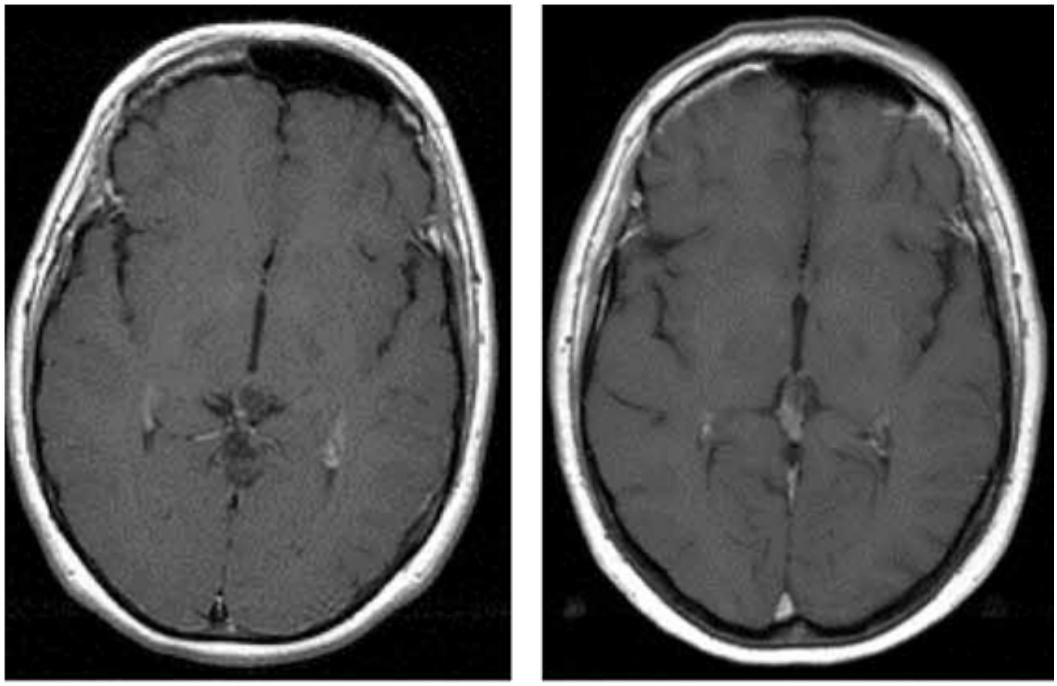
4.2 PAPER II

4.2.1 Aims

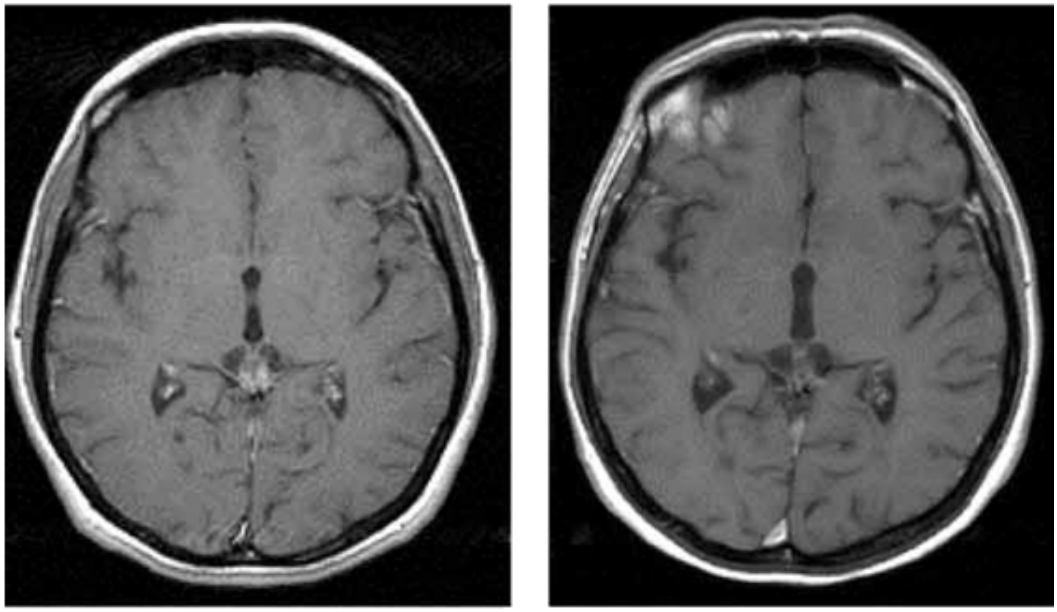
The assessment of brain atrophy by linear measures of ventricular width (Figure 6) has been reported to correlate well with 3D measures. Therefore, serial linear measures with no need for advanced 3D evaluation may prove to be robust markers of irreversible, destructive changes. The aim of Paper II was to evaluate the rates of supratentorial ventricular enlargement, representing four decades of disease.

4.2.2 Main results

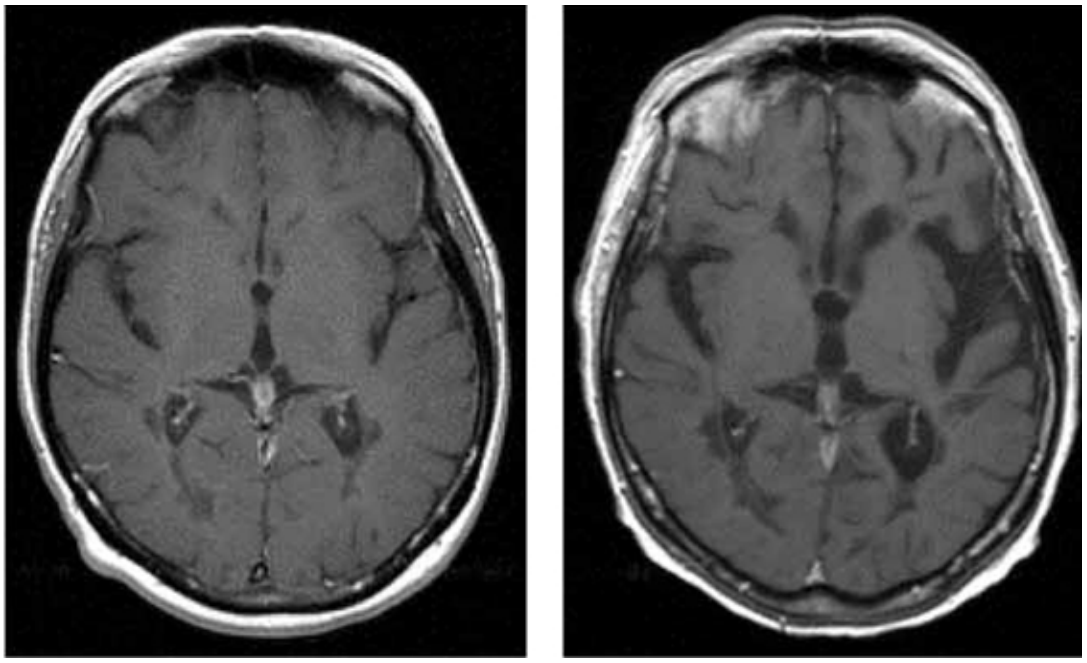
A uniform, significant progression of ventricular widening, representing four decades of disease, was found during a 10-year follow-up (Figures 11-14). Our results suggest that the annual progression of the 3rdVW, ICD, and FHW behave in the same manner. We found a correlation between the progression of supratentorial ventricular enlargement and disease duration. This measure of cerebral atrophy was independent of MS course.



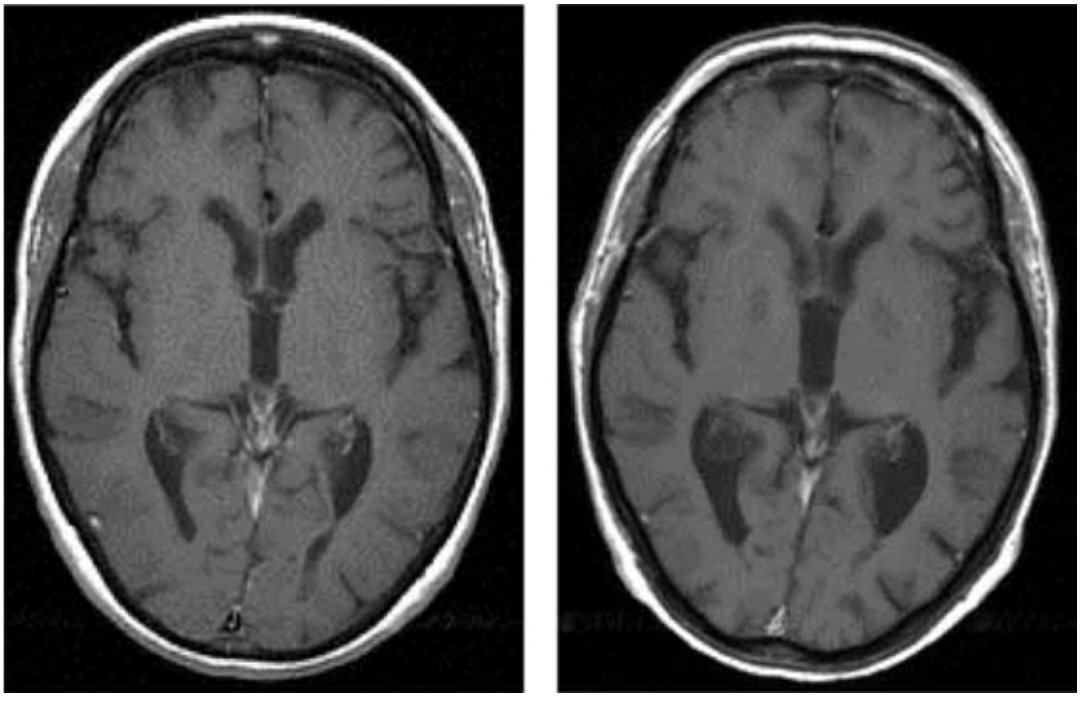
Case 1: **width of the 3rd ventricle 2 -> 3.4 mm**



Case 2: **width of the 3rd ventricle 3.9 -> 6.0 mm**



<u>Case 3:</u> width of the 3rd ventricle 5.9 -> 8.6 mm



<u>Case 4:</u> width of the 3rd ventricle 7.8 -> 10.3 mm

Figure 11. Atrophy progression reflecting four decades of disease. Note the widening of both the intra- and extracerebral cerebrospinal fluid spaces.

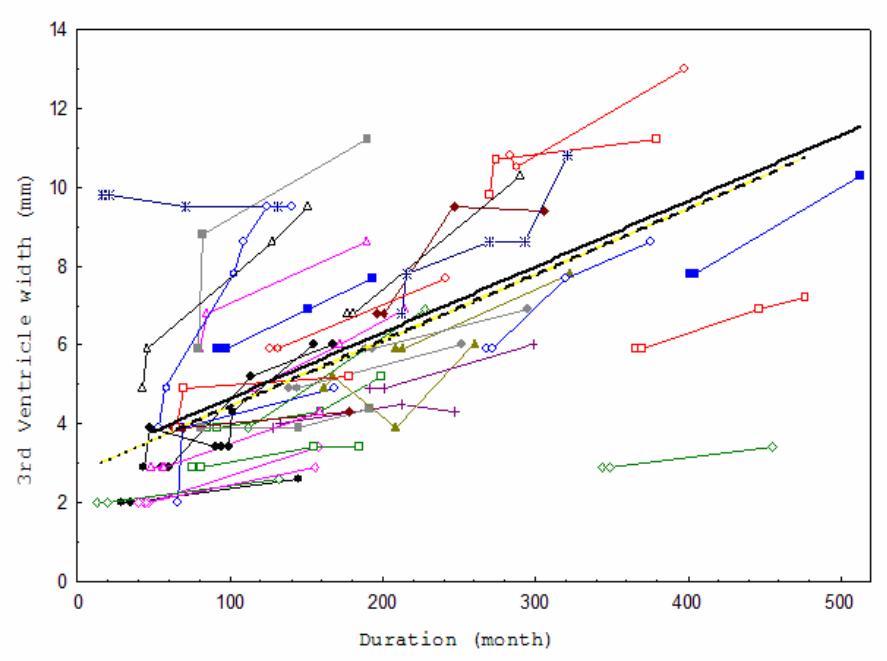


Figure 12. Mean predicted progression of the third ventricle width by MS group. The lines represent the model predicted 3rdVW of RRMS (bold dashed line), PPMS (dashed line), and SPMS (solid line).

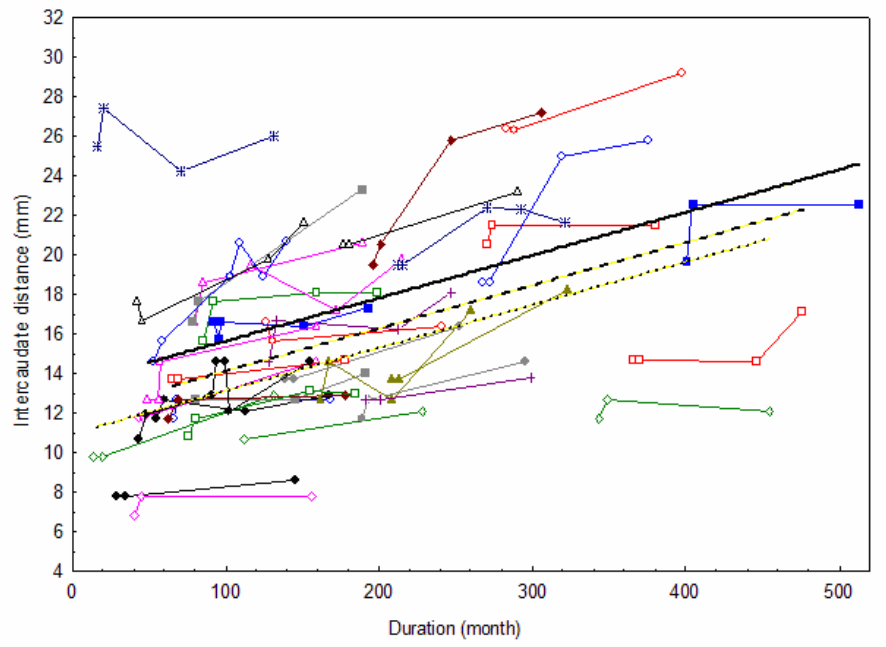


Figure 13. Mean predicted progression of intercaudate distance (ICD) with individual follow-up values. The lines represent the model predicted ICD of RRMS (bold dashed line), PPMS (dashed line), and SPMS (solid line).

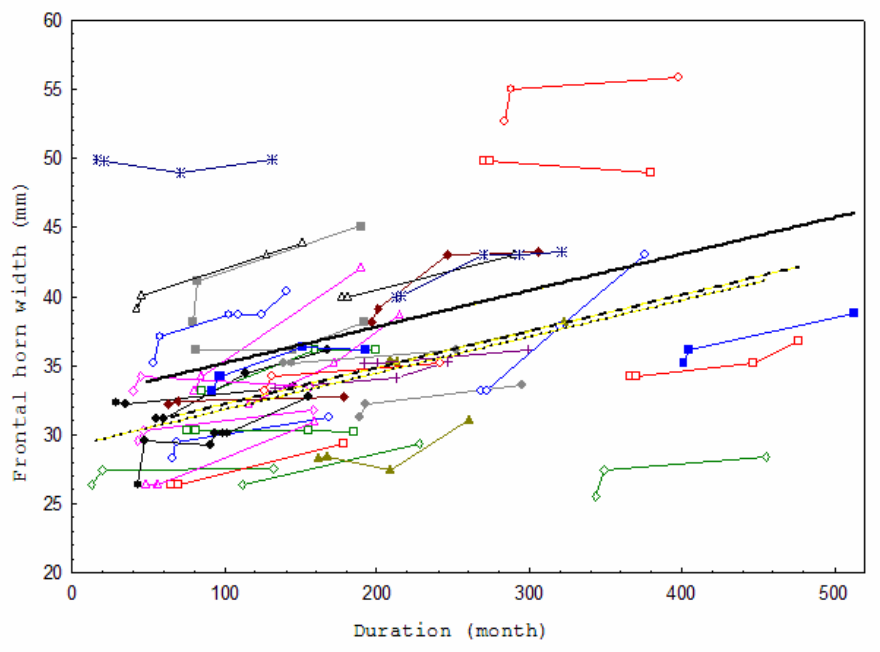


Figure 14. Mean predicted progression of frontal horn width (FHW)with individual follow-up values. The lines represent the model predicted FHW of RRMS (bold dashed line), PPMS (dashed line), and SPMS (solid line).

The rates of both third ventricle widening and frontal horn widening were associated with clinical deterioration. The significant association between the three ventricle enlargement measures and disability status persisted during follow-up (Table 3).

Ventricular size positively correlated with the EDSS and MSSS. A positive correlation was found between the atrophy rate measured over 10 years and the EDSS and MSSS. Spearman rank correlation revealed a low to moderate association between the ventricular measurements and EDSS/MSSS at baseline and the end of the study (Table 3).

	EDSS		MSSS	
	Baseline	**End**	**Baseline**	**End**
Third ventricle width	0.45	0.46	0.031	0.52
Front horn width	0.38	0.34	0.078	0.22
Intercaudate distance	0.42	0.36	0.14	0.36

Table 3. Spearman correlation between ventricle measures and disability (expressed by EDSS/MSSS) at baseline and at the end of the study.

Subjects with a 1-mm annual increase of 3rdVW had 1.83 and 2.43-times higher odds of an increased EDSS and MSSS, respectively. An annual increase in the FHW of 1 mm yielded 1.50 and 1.95-times higher odds of an increased EDSS and MSSS, respectively.

There was no notable effect of ICD progression on the odds of an increased EDSS or MSSS. There was no significant effect of baseline disease duration on EDSS or MSSS changes.

Taken together, in Paper II we found uniform progression of ventricular enlargement over four decades of disease, suggesting similar brain atrophy progression over time.

4.3 PAPER III

4.3.1 Aims

Normalised 1D measures adjusted for individual head size have been used for decades to evaluate brain atrophy. The aim of Paper III was to evaluate atrophy progression over four decades of disease using normalised ratios (Figure 7).

4.3.2 Main results

There were significant changes in all 1D ratios during follow-up. The four measured ratios, bicaudate, bifrontal, Evans, and biuncal, showed a linear increase over four decades of disease (Figure 15 A-D).

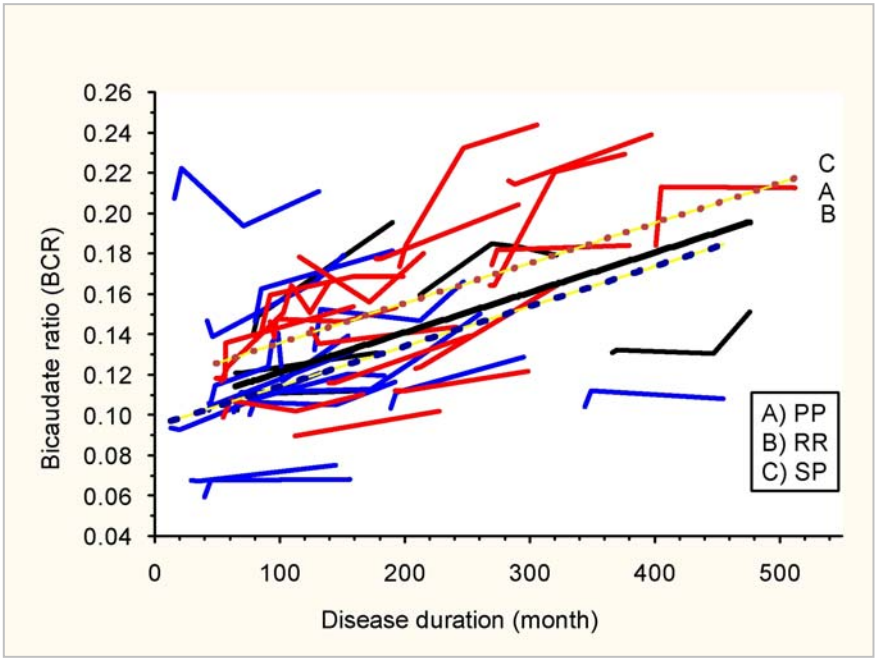


Figure 15 A. Observed and predicted progression of the bicaudate ratio (BCR). The lines represent model-predicted BCR for RRMS (dashed line), PPMS (solid line), and SPMS (bold dashed line).

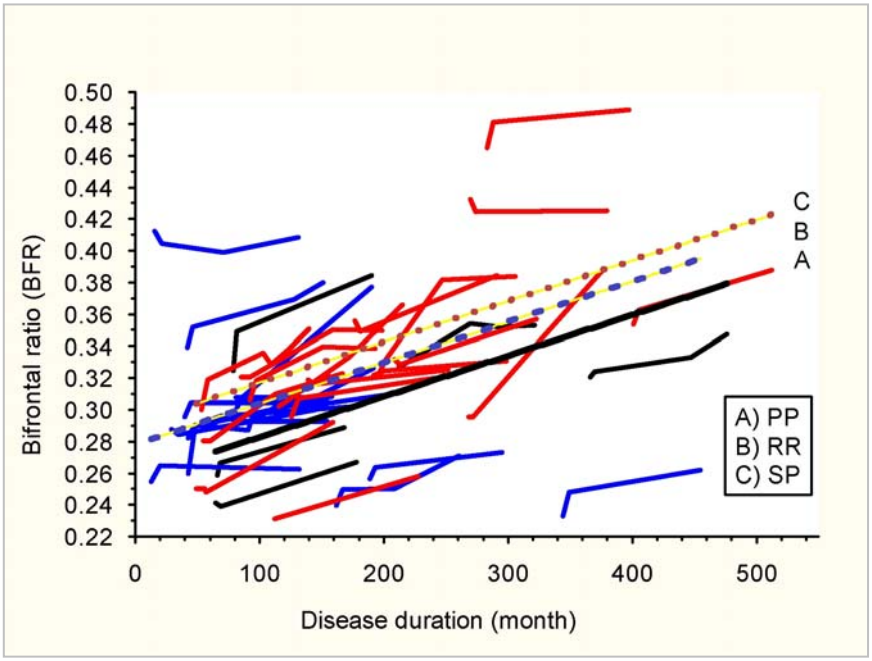


Figure 15 B. Observed and predicted progression of the bifrontal ratio (BFR). The lines represent model-predicted BFR for RRMS (dashed line), PPMS (solid line), and SPMS (bold dashed line).

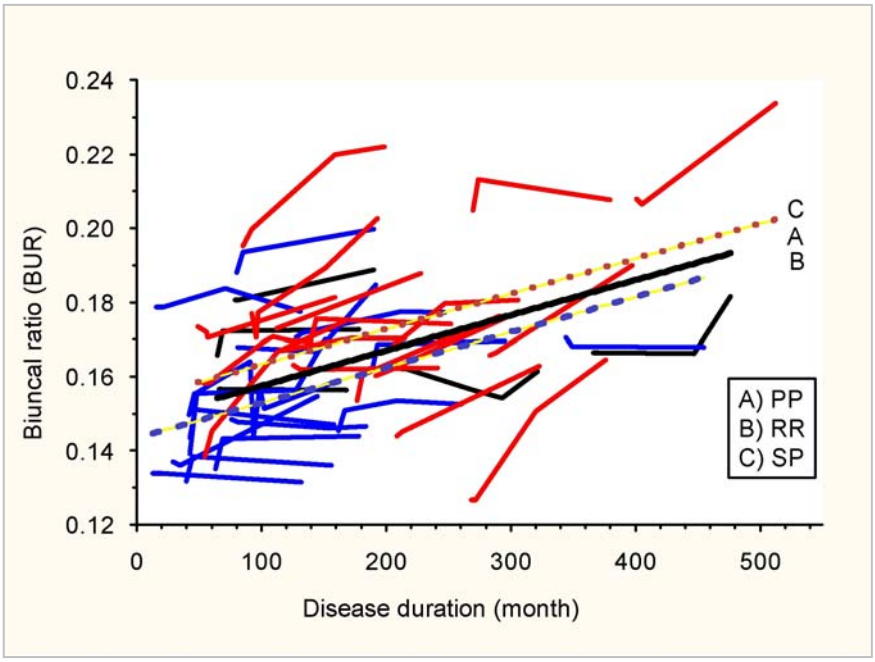


Figure 15 C. Observed and predicted progression of the Evans ratio (ER). The lines represent model-predicted ER for RRMS (dashed line), PPMS (solid line), and SPMS (bold dashed line).

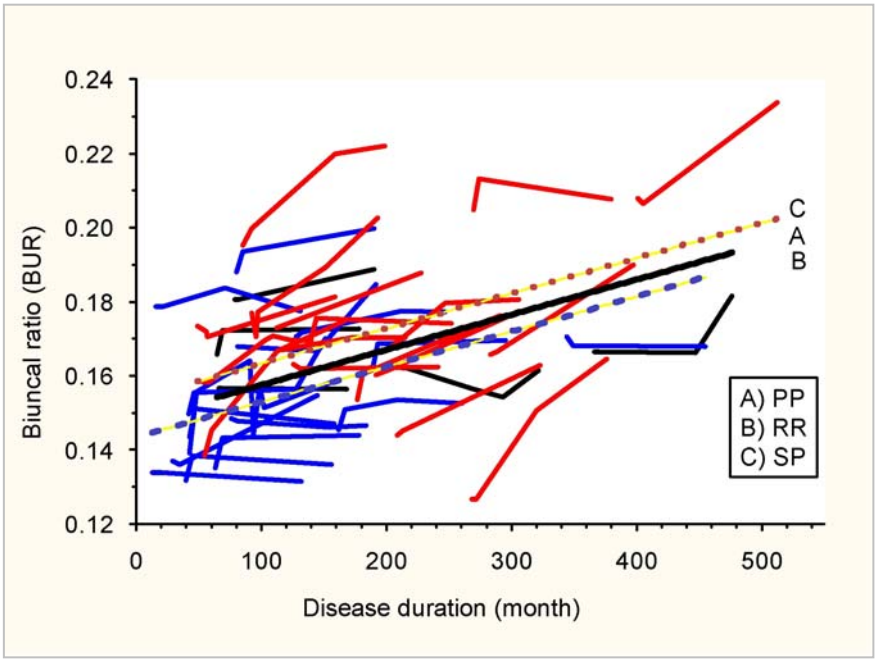


Figure 15 D. Observed and predicted progression of the biuncal ratio (BUR). The lines represent model-predicted BUR for RRMS (dashed line), PPMS (solid line), and SPMS (bold dashed line).

The statistical analysis did not show accelerated or declining progression (duration by duration, $P>0.05$, not shown). There were no significant differences between the atrophy rates of the different MS courses (Table 4**).**

Effect	Estimate*	Standard error	95% CI Lower	95% CI Upper	*P*-value
Bicaudate ratio					
Disease duration (month)*	0.0024	0.0004	0.0012	0.0036	<0.001
Age at onset (year)*	0.0021	0.0006	0.0008	0.0033	0.002
MS group: PP vs. RR**	0.0067	0.0154	-0.0247	0.0381	0.666
MS group: PP vs. SP**	-0.0146	0.0156	-0.0462	0.0170	0.355
MS group: RR vs. SP**	-0.0213	0.0107	-0.0430	0.0004	0.055
Bifrontal ratio					
Disease duration (month)*	0.0031	0.0004	0.0024	0.0036	<0.001
Age at onset (year)*	0.0030	0.0010	0.0010	0.0049	0.005
MS group: PP vs. RR**	-0.0012	0.0246	-0.0512	0.0488	0.961
MS group: PP vs. SP**	-0.0343	0.0245	-0.0841	0.0156	0.171
MS group: RR vs. SP**	-0.0331	0.0170	-0.0678	0.0016	0.061
Evans ratio					
Disease duration (month)*	0.0025	0.0004	0.0012	0.0036	<0.001
Age at onset (year)*	0.0024	0.0009	0.0005	0.0042	0.014
MS group: PP vs. RR**	-0.0049	0.0229	-0.0516	0.0418	0.831
MS group: PP vs. SP**	-0.0253	0.0229	-0.0718	0.0212	0.277
MS group: RR vs. SP**	-0.0204	0.0159	-0.0528	0.0120	0.209
Biuncal ratio					
Disease duration (month)*	0.0011	0.0002	0.0008	0.0015	<0.001
Age at onset (year)	0.0009	0.0004	0.0000	0.0018	0.052
MS group: PP vs. RR**	0.0037	0.0112	-0.0192	0.0265	0.745
MS group: PP vs. SP**	-0.0094	0.0112	-0.0322	0.0134	0.406
MS group: RR vs. SP**	-0.0131	0.0077	-0.0289	0.0027	0.101

*Annual linear change

**Post-hoc comparisons between means derived from the fitted mixed linear models

Table 4. Annual changes in normalised measurements versus MS course.

The ER and BFR were associated with disability developed over four decades of disease. Changes in the ER and BFR reflected more aggressive disease progression, which was also expressed by the MSSS (Table 5).

	EDSS		MSSS	
	Baseline	**End**	**Baseline**	**End**
Bicaudate ratio	0.37	0.46	0.12	0.33
Bifrontal ratio	0.33	0.35	0.08	0.21
Evans ratio	0.24	0.34	-0.04	0.23
Biuncal ratio	0.44	0.46	0.24	0.45

Table 5. Spearman correlation of the relationship between disability and normalised atrophy ratio measurements at baseline and the end of the study.

All four normalised ratios showed uniform atrophy progression, suggesting a similar atrophy rate over four decades of disease independent of MS course. Disability status correlated with the 1D measure; a serial evaluation of the ER and BFR might contribute to the radiological evaluation of MS patients.

4.4 PAPER IV

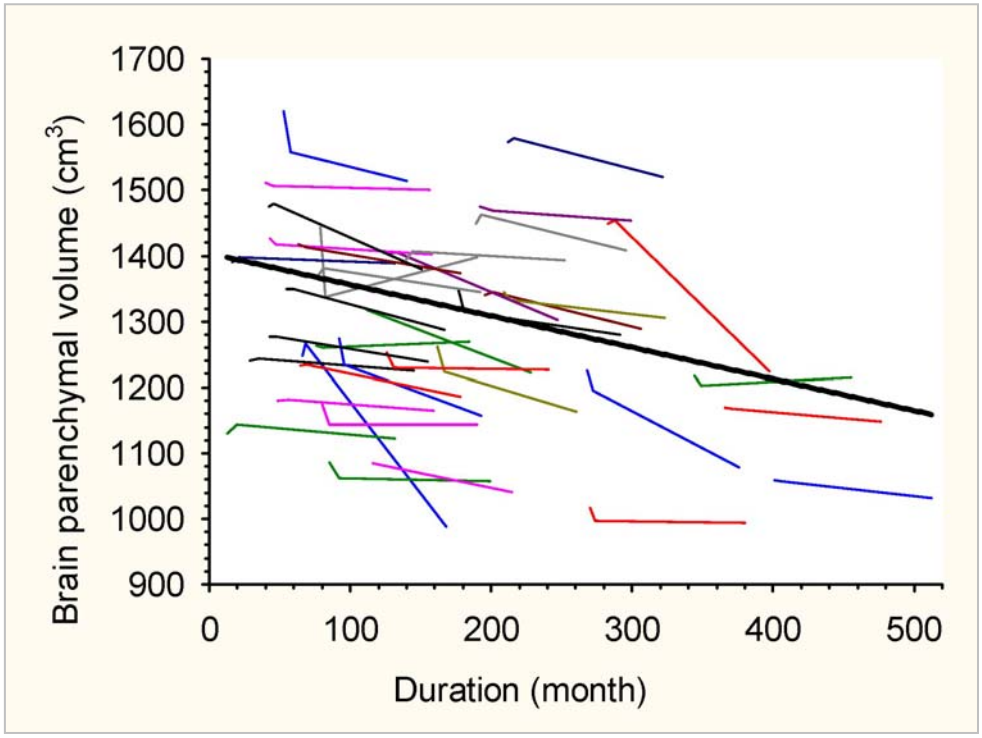
4.4.1 Aims

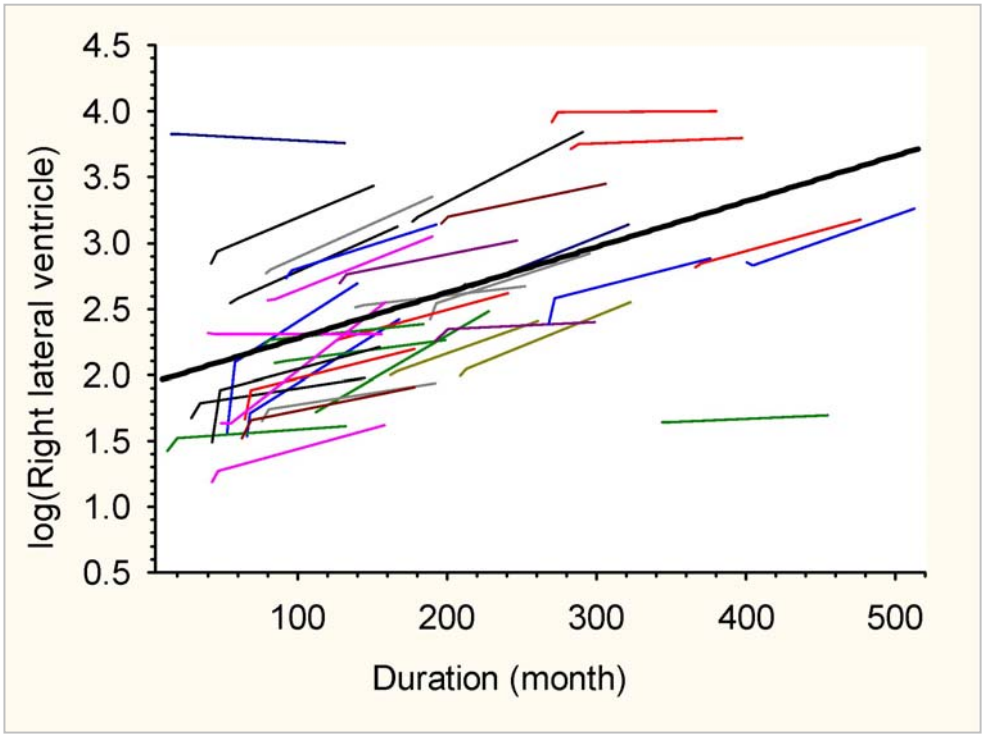
The assessment of brain atrophy by linear measures (1D) and area measurements (2D) has been reported to correlate well with 3D measures. The aim of Paper IV was to compare 1D and 2D (routine workstation) measurements with 3D (semiautomated, HERMES) measures to determine which of the routine measurements best correlates to 3D findings and, further, disability.

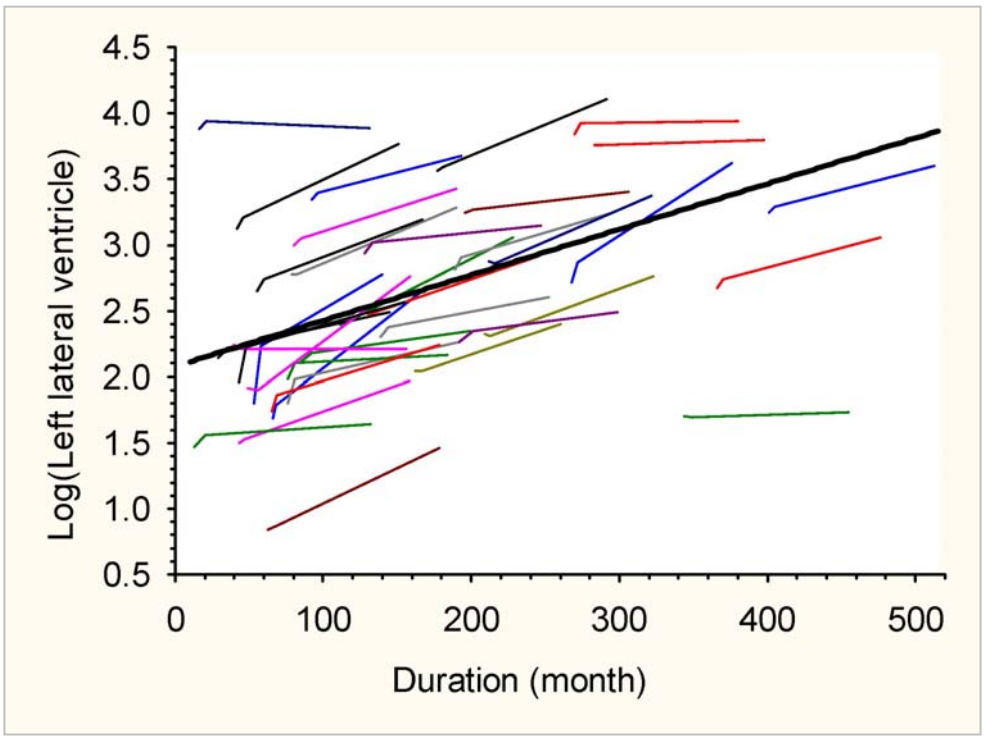
4.4.2 Main results

We found a uniform brain volume reduction with no sign of significant alternating atrophy progression over the decades (Figure 16 and Table 6). We also found a uniform rate of third lateral ventricle enlargement. The enlargement rate of the lateral ventricles was equal, indicating a symmetric progression of cerebral atrophy during follow-up.

We found that the differences in brain and supratentorial ventricular volumes over a short time (1-7 months after baseline) were similar to our previous studies (Paper II and Paper III). However, the changes seen over a short time difference vanished over longer periods of time (Figure 16).


Brain parenchymal volume (cm3)
1700
1600
1500
1400
1300
1200
1100
1000
900
0
100
200
300
400
500
Duration (month)


log(Right lateral ventricle)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
100
200
300
400
500
Duration (month)



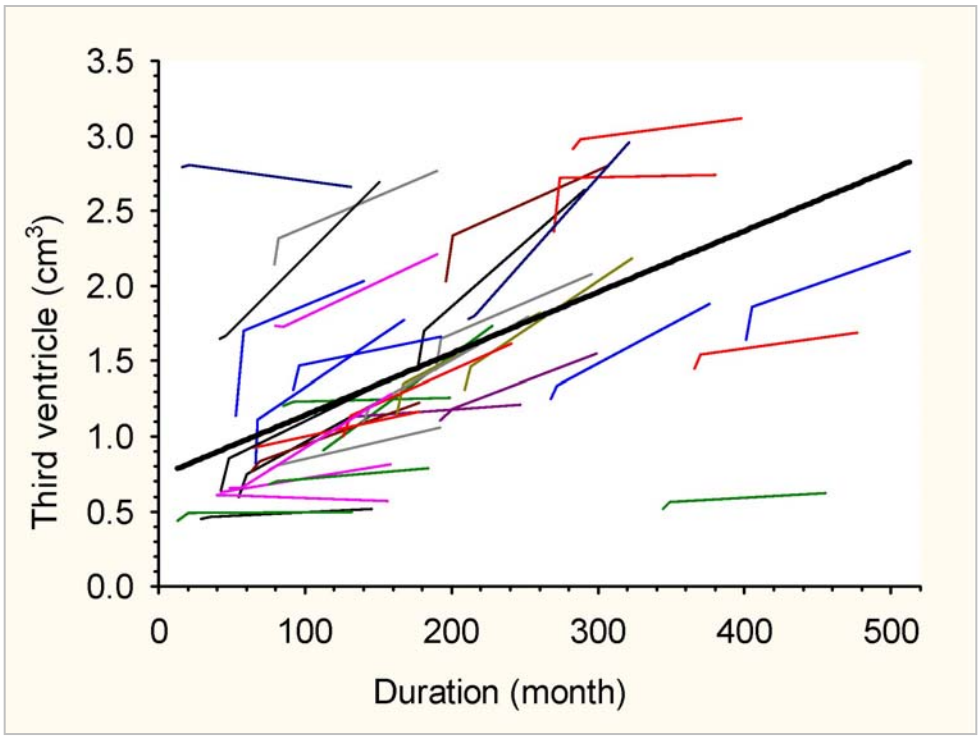


Figure 16. Volumetric changes in brain, third ventricle, and left and right lateral ventricle volume.

Effect	Estimate	SE	95% CI Lower	95% CI Upper	*P*-value
			3rd ventricle (cm^3)		
Disease duration (per year)	0.049	0.006	0.037	0.061	<0.0001
			Brain parenchymal volume (cm^3)		
Disease duration (per year)*	-5.72	1.08	-7.92	-3.54	<0.0001
			Lateral ventricle (Log-cm^3)		
Disease duration (per year)**	0.042	0.004	0.032	0.051	<0.0001

*Results of male vs. female not shown
**Results of left side vs. right side not shown

Table 6. The annual volumetric changes over four decades of disease.

The 3D measures correlated with our previous 1D and 2D results (Papers I, II, and III) except for the BUR (Table 7).

Disease rate	Component 1	2	3	4
Frontal horn width (1D)	0.86	-	-	-
Bifrontal ratio (1D)	0.85	-	-	-
3rd ventricle width (1D)	0.85	-	-	-
Evans ratio (1D)	0.81	-	-	-
Mean lateral ventricle (log, 3D)	0.79	-	-	-
Intercaudate distance (1D)	0.73	-	-0.67	-
3rd ventricle (3D)	0.72	-	-	-
Bicaudate ratio (1D)	0.71	-	-0.68	-
Biuncal ratio (1D)	-	-	-	-0.73
Brain parenchymal volume (3D)	-	0.64	-	-
Corpus callosum (2D)	-	0.62	-	-
Eigen values	5.75	1.5	1.21	0.9
Variance explained (%)	52.2	13.7	10.96	8.14

Table 7. Principal component analysis of the intercorrelation of all measurements in Papers I-IV.

The 3D measures were associated with disability (EDSS) and the MSSS (Table 8).

EDSS

		95% CI		
Effect	**Odds ratio**	**Lower**	**Upper**	***P*-value**
Brain parenchymal volume*	1.72	0.4	7.71	0.449
3rd ventricle**	1.36	0.99	1.95	0.053
Mean lateral ventricle***	1.24	1.01	1.62	0.037

MSSS

		95% CI		
Effect	**Odds ratio**	**Lower**	**Upper**	***P*-value**
Brain parenchymal volume*	0.99	0.82	1.19	0.945
3rd ventricle**	1.52	1.01	2.57	0.044
Mean lateral ventricle***	1.46	1.09	2.21	0.006

*unit 0.1, **unit 0.001, ***unit 0.01.
Results regarding duration at entry are not shown.

Table 8. The univariate results of 3D measurement analyses.

Despite variable clinical courses, the degenerative effects of MS progression expressed in brain atrophy seem to uniformly progress over longer periods of time. These volumetric changes can be detected using 1D and 2D measurements performed on a routine PACS workstation. The FHW (1D) correlated strongest with the 3D measurements.

5 GENERAL DISCUSSION

5.1 SUMMARY OF FINDINGS

Focal lesions visible on MRI are important diagnostic features of MS, but they correlate poorly with clinical progression. Neuroaxonal atrophy is an irreversible process, whereas focal lesions fluctuate over time (Figure 17). Thus, changes in the brain and spinal cord volumes have become increasingly strong candidates for better correlation with the long-term development of disability in MS. To the best of our knowledge, this is the longest follow-up study investigating the rate of atrophy in MS, demonstrating a progressive atrophy over four decades of disease. Fisher et al. (2002) reported declining atrophy in an 8-year longitudinal clinical trial. We found that individual short term brain volume differences appear to vanish over longer periods of time.

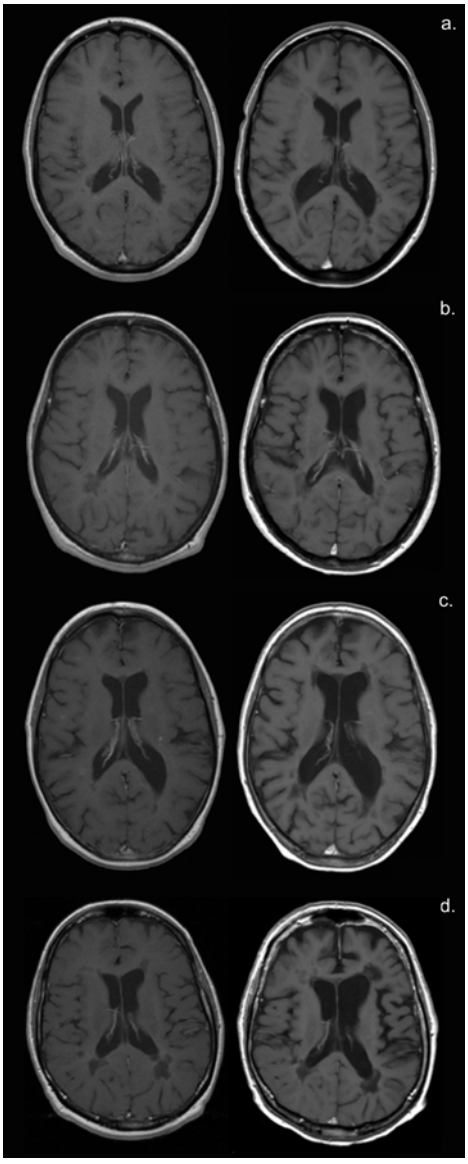


Figure 17. Atrophy progression over four decades of MS. Note the widening of both the sulci and lateral ventricles in the first (a), second (b), third (c), and fourth (d) decades of MS progression (NB images refer to different MS patients).

Several studies have proposed differences in the annual atrophy rate (0.5-1.4%) between MS courses (Bermel et al. 2003; Chardt et al. 2002; De Stefano et al. 2003; Lin et al. 2003; Turner et al. 2001; Ingle et al. 2002; Stevenson et al. 2000). Our findings do not conflict with the atrophy rates differences found with these shorter time follow-ups. In agreement with the results from two studies (Fox et al. 2000; Kalkers et al. 2002), we did not find any significant difference in the atrophy rates of three MS courses.

An association with physical disability persisted during follow-up, which agrees with other studies (Traboulsee 2004; Bermel et al. 2006; Giorgio et al. 2008). This finding may be interpreted as support for the evolving concept of MS as a steadily progressing neurodegenerative disease in which signs of inflammation, such as relapses or focal lesions, are less relevant in longitudinal follow-up observations.

5.2 COMMENT ON PAPER I

We investigated whether and how CCA decreases over time in MS patients. The CC forms the roof of the third and lateral ventricles and plays a central role in interhemispheric communication, containing a large number of axons (2 x 108) (Aboitiz et al. 1992). The CCA in a midsagittal image is age-independent in the normal adult population up to 7th decade (Mitchell et al. 2003; Sullivan et al. 2001) and, therefore, can be used as a marker of non-age-related, pathological brain atrophy. Atrophy of the CC reflects both local atrophy (lesions) and general brain atrophy (Wallerian degeneration secondary to axonal damage) (Reich et al 2009; Mesaros et al. 2009). Rudick et al, (2000) reported a correlation between whole brain atrophy and callosal atrophy. Pelletier et al. (2001) reported that callosal atrophy can be detected in early stages of MS and with mild disability in RRMS. These findings suggest that callosal atrophy may serve as an early morphological marker of CC involvement. Simon et al. (2000) reported similar results in their two-year longitudinal study of brain atrophy; significant callosal atrophy was found, as well as increased ventricular volume, after one year of MRI follow-up, and an annual CCA decrease of 4.9% using 5-mm slices was reported for RRMS patients (Simon et al. 1999). Furthermore, Simon et al. reported a small, but significant, correlation between the CCA and EDSS at baseline, but no further significant correlation between decreased CCA and EDSS change was noted at the end of the study (Simon et al. 1999). These results are in agreement with our results. Furthermore, Pelletier et al. (2001) reported a persistent association between CCA and EDSS in a 5-year longitudinal study of RRMS patients when using 5-mm MRI slices, which is in agreement with our results. Impaired auditory, motor, and sensory interhemispheric transfer were associated, as well as callosal atrophy (Pelletier 2001). Schreiber et al. (2001) reported an association of CCA with EDSS in MS patients. However, Barkhof et al. (1998) reported an insignificant correlation between CCA and both EDSS and the duration of MS. The data in our study did not show a significant correlation with a change in disability during the follow-up. The atrophy rate did not correlate with disability changes.

5.3 COMMENT ON PAPER II

Ventricular enlargement reflects a loss of volume in several structures because deep grey matter consists of thalami, basal ganglia, and the periventricular white matter, all of which form the walls of the third and lateral ventricles**.** MS-related ventricular enlargement within one year of disease duration was shown in a study of clinically isolated syndromes (CIS) (Dalton et al. 2006), but the rate of ventricle enlargement in the long term (over a decade) and its relationship with different MS courses has not been investigated. Dalton et al. (2006) reported, in their one-year longitudinal study, significant ventricular enlargement in CIS, RRMS, and SPMS, which was more pronounced in the SPMS patients than the RRMS patients. On the other hand, a 15-month longitudinal study showed that RRMS patients had significantly more progressive ventricular enlargement than SPMS and PPMS patients (Pagani et al. 2005). RRMS patients converted to SPMS within a median of 16 years (Eriksson et al. 2003; Runmarker et al. 1993). Studying whether the ventricular enlargement rates of RRMS and SPMS subgroups differ over decades of disease is of interest. Such comparisons may confirm a "dissociation" between persistent clinical deterioration and changes in brain atrophy rates.

Our data demonstrated a uniform, significant progression of ventricular widening, spread over four decades of disease, during a 10-year follow-up. The rate of ventricular widening was independent of age at onset and MS course. Our results did not indicate an interaction effect between disease duration and age at onset, suggesting an equal progression rate of ventricle enlargement between individuals of different ages at onset, but progression occurred at a higher rate level if disease onset occurred later in life. If disease onset was later in life, the patients' ventricles were already enlarged to some extent, but the rate of further ventricular enlargement rate was similar for all patients independent of age at onset.

5.4 COMMENT ON PAPER III

Normalised (adjusted for individual head size) 1D measures have been used for the evaluation of brain atrophy for decades (Butzkueven et al. 2008; Evans 1942). The ER is a classical neuroradiological measure and has been used since the 1940s (Evans 1942). Bermel et al. (2002) found that the BCR is higher in MS patients than controls, indicating subcortical atrophy (Bermel et al. 2002), and did not find any significant difference in the caudate volume between RRMS and SPMS, suggesting that destruction of the caudate occurs both early and late in disease progression. The BFR reflects a reduction of the frontal brain volume (Frisoni et al. 1996). The BUR reflects a volume reduction in the anterior parts of the medial temporal lobes (Frisoni et al. 1996; Laakso et al. 1995). The interuncal distance and BUR have been used to evaluate Alzheimer's disease, but the BUR has never been applied in MS. We found that changes in the BCR, BFR, and ER intercorrelated, reflecting similar atrophy progress,

but changes in the BUR did not significantly interact with changes in other ratios (BCR, BFR, and ER).

The ER was associated with changes in disability. The ER and BFR were associated with an increased MSSS, which reflects more aggressive disease progression. The BCR did not correlate with the EDSS. This finding was in accordance with Bermel et al. (2002), who reported that BCR does not correlate with the EDSS, but it does correlate with cognitive impairment. Bermel also found a significantly decreased caudate volume in MS patients, but no correlation with the EDSS, disease duration, whole-brain atrophy, or total T2 hyperintense or T1 hypointense lesion load (Bermel et al. 2003).

In conclusion, we found uniform regional atrophy progression rates reflecting four decades of MS duration. Changes of ER and BFR were associated with more severe disease progression expressed by MSSS. Serial, linear and normalized ratio measures can be performed on any PACS workstation and may contribute to routine clinical evaluation of MS destructive changes.

5.5 COMMENT ON PAPER IV

Most studies concerning MS brain volume atrophy are currently performed with automated or semi-automated methods, which have limited accessibility in clinical radiological use (Turner et al. 2001; Pagani et al. 2005; Sharma et al. 2004). Serial 1D or 2D measures, which can be performed on any PACS workstation, might be sufficient for revealing a progression of the net destructive inflammatory processes causing atrophy (Turner et al. 2001; Butzkueven et al. 2008).

We found uniformly progressing atrophy, representing four decades of MS duration, with 1D, 2D, and 3D measures correlating with one another. We noticed short term volumetric changes disappearing over time, indicating that the rate of atrophy might be independent of MS course. The FHW (1D), which correlated strongest with volumetric changes, was found to be the best measure for revealing atrophy progression. Supratentorial ventricular enlargement correlated with both the EDSS and MSSS. Serial 1D and 2D measures can be performed on any PACS workstation and may contribute to the routine evaluation of global irreversible tissue damage in MS.

5.6 ATROPHY IN NORMAL AGING AND OTHER CAUSES OF ATROPHY

The effect of brain volume changes during aging in healthy controls has to be taken into account and, in particular, discussed in longitudinal studies of different diseases (brain pathologies). Several studies have addressed the issue of atrophy. Scahill et al. (2003) reported that normal aging can explain 0.32% of annual whole brain atrophy and 650 mm3 of annual ventricular enlargement, accelerating after the age of 70 years.

Brain atrophy is not a specific pathology that develops exclusively in MS; it is a well known pathology associated with many diseases, including dementia, hypertension,

Alzheimer's disease, epilepsy, stroke, Huntington's disease, and schizophrenia. Moreover, brain volume shrinkage affecting the whole brain or only a limited part of a brain goes along with normal aging and has been analysed in a range of publications (Henley et al. 2006; Meyer et al. 2007; Whitwell 2008; Borgwardt et al. 2008).

5.7 BACKGROUND OF VOLUMETRIC CHANGES IN MS

There are several pathological and physiological variables that may affect brain volume evaluations in MS. Variables causing increased brain volume are oedema, inflammation, gliosis (tissue bulk), and remyelination. Variables causing decreases in brain volume are axonal loss, resolution of inflammation and oedema, gliosis (retraction scarring), demyelination, dehydration, normal aging, and anti-inflammatory treatment (Anderson et al. 2006). This complexity is likely to add to the uncertainty related to atrophy measurements in MS (Giorgio et al. 2008). For example, at what rate atrophy develops over longer periods of time is still unknown. An atrophy threshold for disability progression may be present, and a critical location of atrophy might be important for worsening disability. Differences in focal atrophy rates between different MS courses are still not adequately understood.

5.8 ATROPHY, DISEASE COURSE, AND DISABILITY IN MS

Brain atrophy starts with the first symptoms (Rocca et al. 2008) and continues through the disease course (Fisher et al. 2002). Atrophy seems to start in the frontotemporal lobes and extend to other relevant regions, such as the motor cortex, in more advanced MS (Sailer et al. 2003). As previously mentioned, some studies have reported differences between MS courses that are expressed in the rate of brain atrophy. However, in studies with a mixed population of MS courses (RRMS, SPMS, and PPMS), no significant atrophy rate differences have been found between the MS courses (Fox et al. 2000; Kalkers et al. 2002). Atrophy is reported to be correlated with disability (Fazekas et al. 2007; Bermel et al. 2006; Giorgio et al. 2008; Horakova et al. 2009). This finding is in agreement with our data.

5.9 LIMITATIONS OF THE STUDY

Longitudinal studies of atrophy in MS are few (Fisher et al. 2002; Fox et al. 2000; Kalkers et al. 2002; Bermel et al. 2003; Chardt et al. 2002; De Stefano et al. 2003; Lin et al. 2003; Turner et al. 2001; Ingle et al. 2002; Stevenson et al. 2000, Audoin et al. 2006; Richert et al. 2006; Fisher et al. 2008; Horakova et al. 2009) and difficult to perform because technical differences are unavoidable due to a continuous updating of MRI scanners. Studies are often performed in association with clinical trials (Fisher et al. 2002; Horakova et al. 2009), which challenges the comparison of atrophy rates. Multiple sclerosis affects the entire CNS, including the spinal cord, leading to problems in interpreting results (Lin et al. 2003).

Normal controls for a direct comparison would have been ideal. The lack of normal healthy controls is a common dilemma in longitudinal studies. We did not include any normal healthy controls in our MRI examinations because of a different primary design of the study in 1995-1996, which had not planned for such a long follow-up. Thus, the use of controls afterwards in 2003-2005 was meaningless. Therefore, the results had to be interpreted with the other studies of brain volume changes in normal aging serving as controls. However, in our first paper, we found that the predicted area of the corpus callosum was representative of the normal population in the same age span. Therefore, the results had to be compared with the other studies of brain volume changes in normal aging (Scahill et al. 2003; Ge et al. 2002). Our results are in agreement with reports concerning the normal aging of healthy adults representing the same age span as our cohort (Mitchell et al. 2003; Sullivan et al. 2001)These reports showed no signs of elevated age-related atrophy for the same age span (Mitchell et al. 2003; Sullivan et al. 2001). However, the lack of normal controls prevented us from defining how much MS added to the annual atrophy rate caused by normal aging.

The sparse representation of older patients limits our study. Older patients had larger supratentorial ventricles and smaller corpus callosum at entry, but their rates of regional atrophy progression did not significantly differ from the younger patients. The RRMS and SPMS groups were comparable in size. However, the small number of patients in the PPMS group challenged the comparison between MS courses.

Intracranial volume is known to be stable in adulthood (Sullivan et al. 2001). Therefore, we used the midsagittal internal scull surface area for calibration due to an update of the scanners during follow-up (Sullivan 2001).

The 3D evaluation was affected by technical differences due to the use of the 1.5-mm gaps in the MR images at the end of the study. These differences biased, to some extent, the statistical model of “the four decade overall atrophy rate”. Only MRI examinations at the end of the study (2003-2005) were performed with 1.5-mm interslice gaps. These gaps could cause some problems in interpreting the volumetric results. However, the semiautomatic volumetric software (HERMES) compensated for the gaps. HERMES measured each imaging slice as 6.5-mm-thick (5 mm + 1.5 mm). Our main aim was to compare patients with disease duration spread over four decades in order to investigate whether the atrophy rate was declining or accelerating. This conclusion was possible despite the slight uncertainty caused by gaps. The 1D and 2D measurements were not affected by gaps.

Our measurements could, to some extent, be biased for technical reasons, such as slice location and the partial volume effects of 5-mm-thick slices. However, the strong intercorrelation of the changes in 1D and 2D measurements with 3D measurements (evaluated using a different technique and by a different person) supports our conclusion that there is significant uniform longitudinal atrophy.

Some limitations related to the use of disability scales have to be considered as well. The EDSS is known to overemphasize motor function and largely lacks an input of cognitive assessment; it may assess spinal processes rather than cerebral processes. Another problem with using EDSS is its lack of linearity (Hobart et al. 2000). If we had better measures of disability, it might be possible to address the question of brain atrophy as measured by MRI with morphometric methods that actually reflect clinical disability. The MSSS was developed to classify patients or groups of patients into severity groups, taking into account the duration of the disease. Therefore, we used the MSSS to assess relative severity at baseline and after the 10-year follow-up rather than using "uncorrected" EDSS scores.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

6.1 CONCLUSIONS

Radiological evaluations of MS are challenging because the disease affects the entire CNS, including the spinal cord. The correlation of MS-related atrophy of the whole CNS can be detected within six months of symptom onset. CNS volume can be affected by opposing processes at the same time.

Differences in the atrophy rate over short periods of time disappeared over longer periods of time. Therefore, atrophy seems to be a continuous process over longer periods of time. All measures of atrophy were independent of MS course and suggested a uniform symmetric volume reduction over four decades of disease.

The rates of both third ventricle widening and frontal horn widening were associated with clinical deterioration. The significant association between the corpus callosum sagittal area, three ventricle enlargement measures, BCR, BFR, ER, brain volume, third ventricle volume, volumes of the lateral ventricles, and disability status persisted during follow-up.

Serial 1D, 2D, and 3D measures correlated, except for the BUR; measurements performed on a routine PACS workstation are capable of revealing the progression of the destructive effects of MS. Therefore, 1D and 2D measures add information to the follow-up of the patient.

6.2 FUTURE PERSPECTIVES

Serial linear 1D and 2D measurements, which do not require advanced 3D software assessment, may prove to be useful in monitoring irreversible and destructive changes in the brain of MS patients, providing additional information for monitoring the clinical course and treatment.

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