Epidemiological and biochemical aspects of progression in multiple sclerosis

Marcus Koch

Abbreviations:

BMS	benign multiple sclerosis
CES-D	Center for Epidemiological Studies Depression Scale
CI	condifence interval
CNS	central nervous system
EDSS	Expanded Disability Status Scale
FSS	Fatigue Severity Scale
IMD	immuno-modulatory drugs
IMM	immuun-modulerende middelen
IQR	interquartile range
MRI	magnetic resonance imaging
MSSS	Multiple Sclerosis Severity Score
NSE	neuron-specific enolase
PFLPP	plasma fluorescent lipid peroxidation products
PPMS	primary progressive multiple sclerosis
RRMS	relapsing-remitting multiple sclerosis
SD	standard deviation
SPMS	secondary progressive multiple sclerosis

Marcus Koch is currently working at the Department of Neurology, University Medical Center Groningen the Netherlands and can best be contacted by E-mail via:

m.w.koch@ neuro.umcg.nl

Patients with a progressive form of multiple sclerosis have the worst prognosis. They can expect that their symptoms will steadily worsen, and there is currently no treatment that has a proven effect on progressive multiple sclerosis.

The underlying pathophysiology of the progressive forms of multiple sclerosis appears to be distinct from that of the relapsing-remitting form. Research on multiple sclerosis has largely been focussed on the relapsing-remitting form of the disease, while there is relatively little knowlegde of the progressive forms of MS.

In this thesis, we aimed to investigate epidemiological and biochemical factors associated with progression in MS.

Epidemiological and biochemical aspects of progression in multiple sclerosis

Stellingen behorende bij het proefschrift

"Epidemiological and biochemical aspects of progression in multiple sclerosis"

Groningen, 16 januari 2008

1. Progressie van MS is een van de leeftijd afhankelijk fenomeen. (dit proefschrift)
2. Primaire en secundaire progressie beginnen op een gelijke leeftijd. (dit proefschrift)
3. Het begin van de progressieve fase van MS is waarschijnlijk de beste epidemiologische maat voor progressief axonaal verval en dient daarom als eindpunt in epidemiologische studies te worden gebruikt. (dit proefschrift)
4. Het effect van immuunmodulerende middelen op het begin van secundaire progressie bij MS dient onderzocht te worden in een bij voorkeur publiekelijk gefinancierde gerandomiseerde gecontroleerde studie. (dit proefschrift)
5. Familiaire primair progressieve MS is een interessante beloopsvorm voor genetisch onderzoek bij MS. (dit proefschrift)
6. Vermoeidheid en depressie zijn niet geassocieerd met het beloop van MS. (dit proefschrift)
7. Sigarettenroken heeft geen invloed op progressie bij MS. (dit proefschrift)
8. Het tijdstip van geboorte heeft geen invloed op progressie bij MS. (dit proefschrift)
9. Oligoclonale banden in de liquor en perifere oxidatieve stress zijn niet geassocieerd met progressie bij MS. (dit proefschrift)
10. De concentratie van neuron specifieke enolase in het perifeer bloed is mogelijk bruikbaar als marker voor progressie bij MS. (dit proefschrift)
11. Het grondbeginsel van medisch handelen *primum non nocere* ("ten eerste: doe geen schade") komt tegenwoordig in gedrang door weinig werkzame, mogelijk schadelijke en slecht onderzochte, maar zeer goed geadverteerde behandelingen.
12. Klinische studies die door hun opzet geen klinisch nuttige informatie kunnen opleveren zijn onethisch, en artsen die patiënten in zulke studies includeren, brengen deze patiënten schade toe.
13. Het is zeer belangrijk om naar de patiënt te luisteren, want de patiënt verraadt u de diagnose. (Sir William Osler)
14. Wat artsen doen is de patiënt vermaken, terwijl de natuur de kwaal geneest. (Voltaire)
15. Als niemand u ooit tegenspreekt, betekent dat niet zondermeer dat u altijd gelijk hebt. Het kan ook betekenen dat niemand graag met u praat.
16. Holland is de enige door de mens gemaakte structuur die vanaf de maa[illegible] zichtbaar is.

The work described in this thesis was performed at the Department of Neurology, University Medical Center Groningen, The Netherlands.

The research presented in this study was financially supported by:

ZonMW, The Netherlands Organisation for Health Research and Development (The Hague, The Netherlands) and MS Anders (Amsterdam, The Netherlands).

Cover illustration:
Hokusai Katsushika: "Under the wave, off Kanagawa".

ISBN: 978-90-367-3287-1

RIJKSUNIVERSITEIT GRONINGEN

Epidemiological and biochemical aspects of progression in multiple sclerosis

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
woensdag 16 januari 2008
om 16.15 uur

door

Marcus Werner Koch
geboren op 21 februari 1977
te Lübeck, Duitsland

Promotor:	Prof. dr. J.H.A. De Keyser
Beoordelingscommissie:	Prof. dr. P. P. De Deyn Prof. dr. R. A. Dierckx Prof. dr. J. B. M. Kuks

Paranimfen: Jeroen de Vries
Harold Weitenberg

Contents

Part I

General Introduction

Chapter 1

General Introduction

Multiple sclerosis (MS) is a disease of unknown aetiology.[1] In patients with MS, neurons of the central nervous system (CNS) are lost due to two main pathophysiological mechanisms. Circumscript inflammatory lesions in the CNS, the so-called plaques, are the most obvious pathologic change in MS. The clinical correlate of such lesions is the relapse, a subacute worsening in neurological function with at least partial recovery. Many works have emphasised the importance of these lesions and investigated them with pathological, biochemical and imaging techniques. Research in MS has been dominated by the interest in inflammatory lesions. Current treatments for MS are designed to influence inflammation and one of the more important endpoints in randomised controlled trials in MS is the 'number of new T2 lesions on cranial MRI'.

The other pathophysiological mechanism that is currently believed to contribute to tissue damage in MS is a poorly understood neuronal degeneration, termed 'axonal degeneration'. Axonal degeneration is a diffuse process that appears to begin in the earliest disease stages. It encompasses inflammatory lesions, but also -and more importantly- the normal appearing white matter outside of plaques.[2] The clinical correlate of axonal degeneration appears to be a progressive worsening of neurological function independent of relapses, called the progressive disease course. While the functional impairment sustained in a relapse is at least partially reversible, the accumulation of disability in the progressive disease course is steady and unremitting.

The progressive disease course may follow an initial phase with relapses and remissions, and is then called secondary progression. Fewer patients have a progressive disease course from onset. Such patients are said to have primary progressive MS. Current treatments, which are designed to suppress inflammatory tissue damage, have no clinically meaningful effect on primary or secondary progressive MS.

Given the lack of knowlegde about the progressive phase of MS, we aimed to investigate epidemiological and biochemical factors associated with progression in MS. The results of several epidemiological studies are summarised in **Part II** of this thesis.

Most previous studies on the epidemiology and natural history of MS used the time from disease onset to certain landmark scores on the Expanded

Disability Status Scale (EDSS) (e.g. 6.0: 'requires a cane for walking' or 8.0: 'is restricted to a wheelchair'). This approach does not account for the pathophysiological differences between relapsing-remitting MS on the one hand and the progressive forms of MS on the other hand. In order to learn more about the natural history of the progressive forms of MS, we selected all patients with primary or secondary progressive MS from our natural history database and and investigated the influence of possible risk factors on the timing of progression. Our findings are summarised in **Chapter 2**.

While the approach used in the Chapter 2 is useful for comparing primary and secondary progression, it cannot be used to advise patients with newly diagnosed relapsing-remitting MS about their prognosis. In order to investigate secondary progression more closely, we selected all patients with a relapsing-remitting disease onset and investigated the influence of several factors on the risk of secondary progression (**Chapter 3**).

Chapters 4 through 6 deal with the importance of other possible risk factors for the development of primary and secondary progression: a positive family history of MS (**Chapter 4**), fatigue and depression (**Chapter 5**) and cigarette smoking (**Chapter 6**).

Chapter 7 is special in so far as it is a collaboration with our colleagues in Vancouver, Canada. The importance of the timing of birth for the risk of developing MS was recently discovered in a very large epidemiological study.[3] In our study we used the two natural history databases from British Columbia, Canada and Groningen to investigate whether the timing of birth also influences progression in MS. Using two independent databases enabled us to verify potential effects found in one patient cohort in the other cohort.

In the studies contained in **Part III** of this thesis, we related baseline measurements of several laboratory measures with progression in the course of the following years. **Chapter 8** investigates the effect of cerebrospinal fluid oligoclonal bands on progression of disability and the development of secondary progression during the following five years. **Chapter 9** contains a study on the influence of plasma fluorescent lipid peroxidation products, a measure of oxidative stress, on progression of disability during the following five years, and **Chapter 10** investigates the relation of S100beta

and neuron-specific enolase, two markers of glial and neuronal cells, with progression in the subsequent five years.

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Part II

Epidemiological aspects of progression in multiple sclerosis

Chapter 2

Progression in multiple sclerosis: further evidence of an age dependent process

Marcus Koch, Jop Mostert, Dorothea Heersema, Jacques De Keyser

J Neurol Sci 2007;**255**:35-41.

Abstract

Background: The relapsing-remitting phase and the progressive phase of multiple sclerosis (MS) seem to be the result of distinct pathophysiological processes. Previous research on the natural history of MS was largely focussed on relapses and disability scores. Recent research suggests that progression in MS may be an age dependent process independent of relapses.

Objective: To investigate the influence of several potential risk factors on the timing of secondary and primary progression in MS.

Methods: In this study we evaluated 438 patients with progressive MS. The influence of the variables gender, initial disease course, onset manifestation and age at disease onset on age at progression and time to progression were evaluated with Kaplan-Meier survival analyses and Cox regression models.

Results: The analysis of these data showed that the initial disease course (relapsing-remitting or primary progressive) had no influence on the age at progression. Gender had no influence on age at progression in patients with primary progressive MS (PPMS) and secondary progressive MS (SPMS) nor on time to progression in SPMS patients. PPMS patients with visual or brainstem/cerebellar onset had a significantly younger age at progression. SPMS patients with motor onset had a significantly higher age at progression and longer time to progression. Time to progression was significantly shorter in SPMS patients with higher age at disease onset.

Conclusion: Our data give further support to the notion that progression in MS is an age dependent process independent of relapses.

Introduction

Both inflammatory demyelination and axonal degeneration have been recognised as characteristic pathological features of multiple sclerosis (MS) from the early days of histopathological research in MS.[1] Nevertheless, most research efforts have been focussed on inflammatory demyelination; and this element of MS pathophysiology was thought to be the main contributor to accumulating disability. The interest in axonal degeneration as a pathophysiological mechanism grew when studies using magnetic resonance spectroscopy showed that axonal loss correlated with increasing disability in patients with MS.[2;3] Axonal degeneration in MS appears to start early in the disease course[4] and encompasses plaques as well as the normal appearing white matter.[5]

In our current concept of MS pathophysiology both focal inflammatory demyelination and diffuse neurodegeneration coexist and play distinctive roles in the disease course. The clinical phenomenon of the relapse is the result of a focal inflammatory demyelinating lesion, whereas neurodegeneration is a diffuse process most prevalent in patients with a progressive disease course.[6] Axonal degeneration is probably the most important contributor to secondary progression.[7]

Natural history studies have provided some insight into the time course of relapses and progression in MS. Relapses due to focal lesions define the clinical picture early in the disease, but previous research showed that they do not influence the time course of disability accumulation.[8] After the onset of progression, the disease follows a uniform course, no matter whether the progression is primary or secondary.[9]

Confavreux and coworkers reported that the age at onset of progression in their patient cohort is very similar in primary and secondary progressive MS,[10] corroborating earlier observations that relapses appear to have little or no influence on the timing of progression.[11;12] These findings suggest that the development of a progressive disease course may be an age dependent phenomenon mediated by diffuse neurodegeneration in the CNS, which is largely independent of focal inflammations.

Most natural history studies define disease progression as the arrival at cer-

tain landmark disability scores (e.g. EDSS 4, 6, 8). This approach does not distinguish between the clinical effects of focal demyelinating lesions and progressive axonal degeneration since disability may be caused by residual symptoms following relapses as well as progressive axonal degeneration.

Considering the distinct pathophysiology of progression in MS we evaluated possible predictive variables affecting the age at onset of progression and the time from disease onset to onset of progression in 438 patients with progressive MS prospectively followed at the Groningen MS clinic.

Methods

Groningen MS Database

The Groningen MS Database is a prospective database of all MS patients attending the University Medical Center Groningen (UMCG) MS Clinic, where patients are followed at 3 to 12-monthly intervals with intercurrent visits if necessary. The UMCG is the main secondary and tertiary referral centre for MS in the province of Groningen (population approximately 575,000). Data acquisition started in 1985, and the diagnosis of MS was established according to the Poser criteria.[13] Currently, the database contains clinical data of 672 patients.

Patients

There were 438 patients with a progressive disease course. Progression was defined as the continuous worsening of neurologic symptoms unrelated to relapses for at least one year.[9] The year of the first relevant symptom was taken as the year of disease onset. Both the year of disease onset and the year of progression were established from the database and confirmed by the patient records.

Of the progressive patients, 228 had secondary progressive MS (SPMS), and 210 patients were progressive from disease onset (primary progressive MS, PPMS). Patients who where progressive from disease onset with an occasional superimposed relapse ('relapsing-progressive MS') were classified as PPMS, and patients experiencing a single relapse followed by progression were classified as SPMS.

63 (28%) of the SPMS patients and 13 (6%) of the PPMS patients were using immunomodulatory drugs: 62 SPMS and 12 PPMS patients were using interferon beta. Glatiramer acetate was used by one SPMS and one PPMS patient. No other immunomodulatory or immunosuppressive drugs were used, except for corticosteroids (intravenous methylprednisolone or oral dexamethasone) for the treament of exacerbations.

The endpoints in our analysis were age at onset of progression ('age at progression') for both patient groups, and time from disease onset to onset of progression ('time to progression') in patients with SPMS.

The investigated variables were gender, manifestation at disease onset (labeled 'visual' in patients with optic neuritis, 'motor' in patients with arm and or leg weakness, 'sensory' in patients with sensory symptoms, 'brainstem/cerebellar' in patients with oculomotor signs, vertigo, intention tremor or nystagmus, and as 'other' in patients with muscular cramp, fatigue, sphincter or sexual symptoms), and initial disease course (PPMS vs. SPMS). In SPMS patients, we also recorded age at disease onset (grouped as 'younger than 20 years', '20-29 years', '30-39 years' and 'above 40 years').

Statistical analyses

Group differences in demographic and clinical variables between SPMS and PPMS patients were assessed with Fisher's exact test, Pearson's chi square test, and the Mann-Whitney U test where appropriate.

The influence of the variables on age at progression (PPMS and SPMS patients) and time to progression (SPMS patients) were investigated with Kaplan-Meier survival analyses. Group differences were assessed with the log-rank test and variables with significant group differences were included in a Cox multivariable regression model with gender as a covariate. In the analyses of SPMS patients we felt that age at disease onset should not be used as a covariate in the model for age at progression as the latter must necessarily be greater than the former in every case. We did therefore not include age at disease onset into the regression model for age at progression.

Statistical significance was taken to be at the two-tailed 0.05 level. All statistical analyses were carried out with the SPSS statistical software package version 12.

Results

An overview and comparison of the variables between SPMS and PPMS patients is given in Table 2.1.

Table 2.1: Comparison of patient characteristics between secondary and primary progressive MS patients.

	All patients	SPMS	PPMS	p
Number of patients	438	228	210	—
Gender:				
women	278	155 (68%)	123 (58.6%)	0.047*
men	160	73 (32%)	87 (41.4%)	
Age at disease onset				
mean, SD	34.37, 11.3	30.00, 9.73	39.12, 10.98	<0.0005‡
median, range	33, 9-68	29, 10-60	39, 9-68	
Age at disease onset, groups: n				
<20 years	34	30 (13.2%)	4 (1.9%)	
20-29 years	124	92 (40.4%)	32 (15.2%)	<0.0005†
30-39 years	136	66 (28.9%)	70 (33.3%)	
40 years and above	144	40 (17.5%)	104 (49.5%)	
onset manifestation: n				
visual	101	84 (36.8%)	17 (8.1%)	
motor	111	24 (10.5%)	87 (41.4%)	
sensory	148	79 (34.6%)	79 (37.6%)	<0.0005†
Brainstem/cerebellar	48	35 (15.4%)	13 (6.2%)	
other	20	6 (2.6%)	14 (6.7%)	

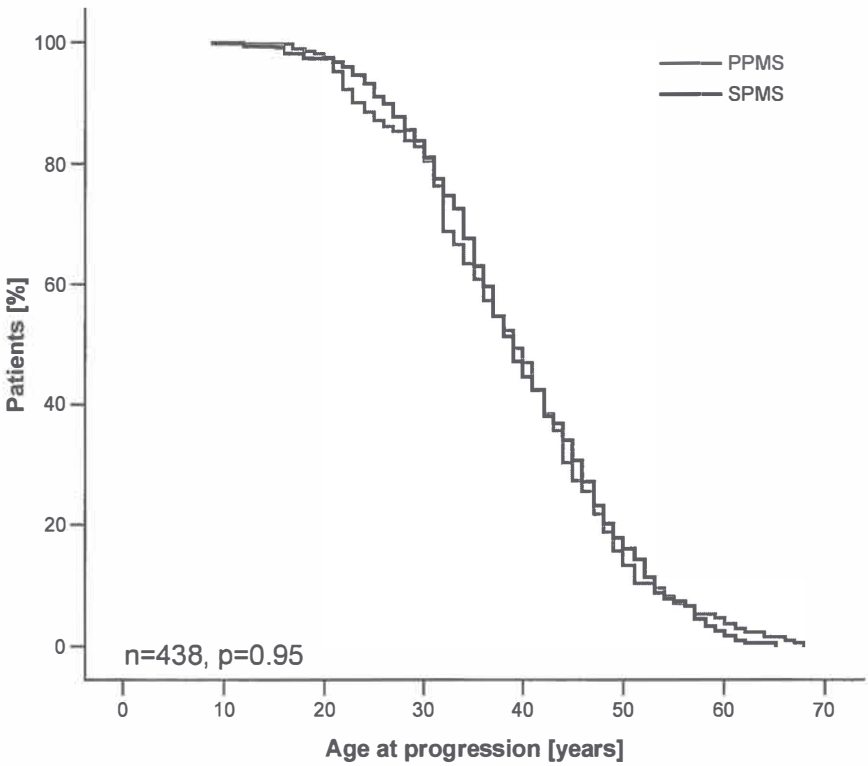
*: Fisher's exact test, †: Pearson's chi square test, ‡: Mann-Whitney U test

There were significantly more men in the PPMS group, and the age at disease onset was significantly lower in SPMS patients. The symptoms at disease onset were significantly different between PPMS and SPMS patients with a predominance of visual onset symptoms in SPMS patients and motor symptoms in the PPMS group.

Initial disease course

The initial disease course had no significant influence on the age at progression. The mean age at progression in PPMS was 39.1 years (95%CI 37.6-40.6) versus 39.5 years (95% CI 38.2-40.9) in SPMS (p=0.95, Figure 2.1).

Figure 2.1: Influence of the initial disease course on age at progression in all patients.



Age at progression

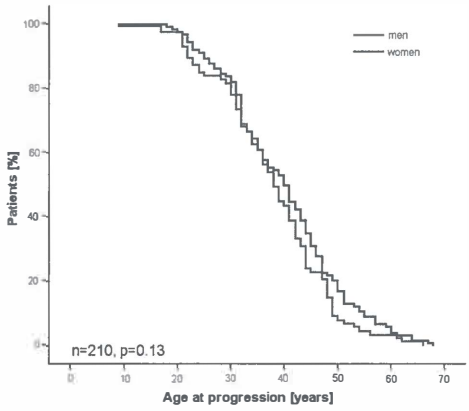
Gender had no significant influence on age at progression in either PPMS or SPMS patients (Tables 2.2 and 2.3; Figures 2.2 and 2.3). In PPMS patients, visual onset and brainstem/cerebellar onset were associated with a younger age at progression. Mean age at progression was 32.4 years in patients with visual onset versus 39.7 in patients with non-visual onset. Mean age at progression was 29.5 years in brainstem/cerebellar onset versus 39.8 years in patients with non-brainstem/cerebellar onset (Table 2.2; Figure 2.2).

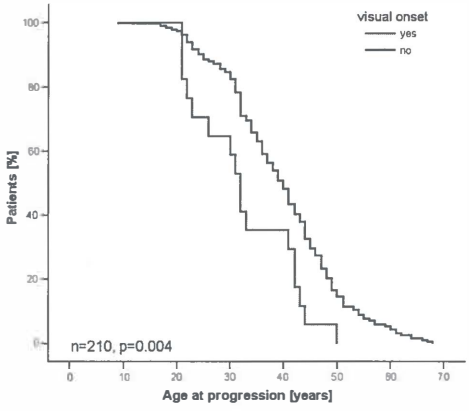
Table 2.2: Potential risk factors affecting age at primary progression

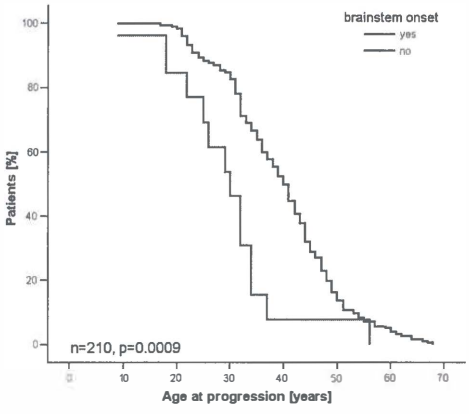
Factor	n	mean age at progression (95% CI)	p*
All patients	210	39.12 (37.63-40.61)	—
Gender			
Women	123	39.96 (37.98-41.94)	0.13
Men	87	37.93 (35.69-40.17)	
Onset manifestation			
Visual			
No	193	39.69 (38.15-41.24)	0.004
Yes	17	32.38 (28.06-37.11)	
Motor			
No	123	37.61 (35.61-39.58)	0.073
Yes	87	41.24 (39.04-43.44)	
Sensory			
No	131	38.54 (36.61-40.42)	0.47
Yes	79	40.08 (37.75-42.4)	
Brainstem/cerebellar			
No	197	39.75 (38.25-41.24)	0.0009
Yes	13	29.54 (23.56-35.52)	
Other			
No	197	39.25 (37.71-40.78)	0.61
Yes	13	37.35 (31.28-43.43)	

*: log-rank test

Figure 2.2: Influence of gender, visual onset and brainstem/cerebellar onset on age at primary progression







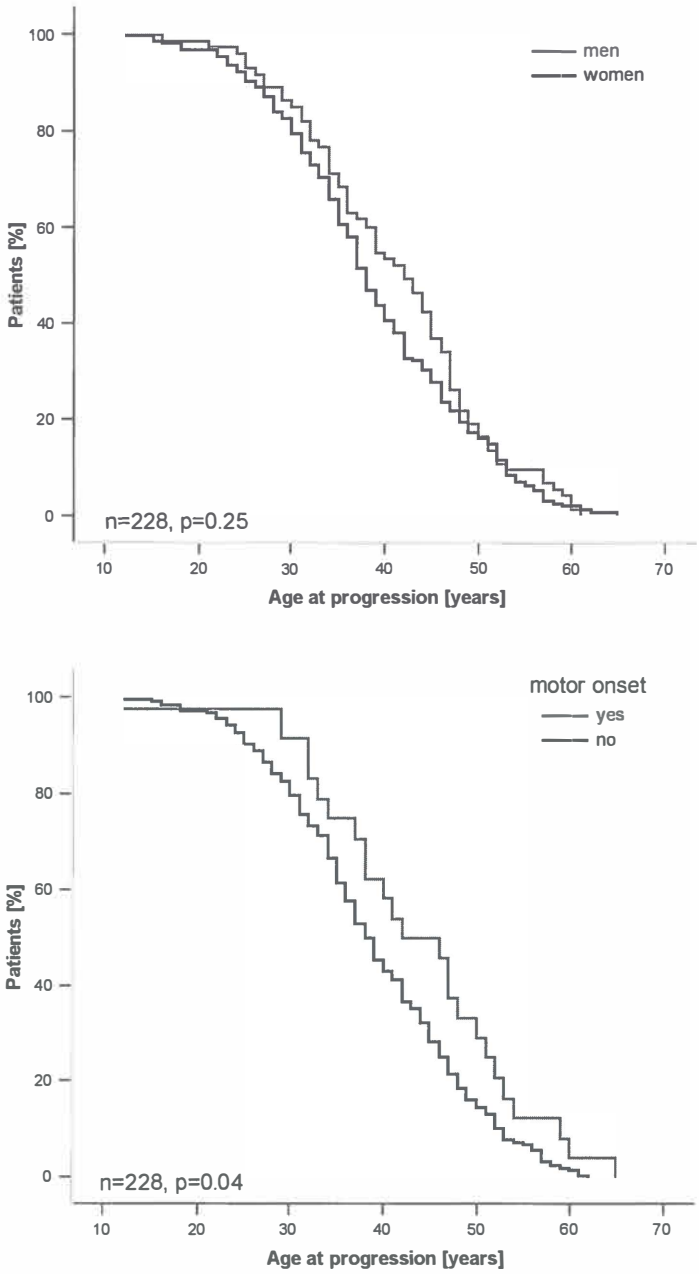
SPMS patients with motor onset were significantly older at the onset of the progressive phase (mean age at progression in years: 43.3 vs. 39.1 in patients with non-motor onset symptoms, p=0.04) (Table 2.3, Figure 2.3).

Table 2.3: Potential risk factors influencing the age at secondary progression

Factor	n	mean age at progression (95% CI)	p*
All patients	228	39.58 (38.24-40.93)	—
Gender			
Women	155	38.87 (37.22-40.50)	0.25
Men	73	41.11 (38.78-43.44)	
Onset manifestation			
Visual			
No	144	39.89 (38.20-41.58)	0.65
Yes	84	39.06 (36.84-41.28)	
Motor			
No	205	39.14 (37.75-40.53)	0.04
Yes	23	43.33 (38.62-48.04)	
Sensory			
No	149	39.47 (37.73-41.21)	0.76
Yes	79	39.80 (37.72-41.88)	
Brainstem/cerebellar			
No	193	39.74 (38.31-41.17)	0.66
Yes	35	38.71 (34.89-42.54)	
Other			
No	220	39.73 (38.36- 41.10)	0.41
Yes	6	34.17 (29.54-38.79)	

*: log-rank test

Figure 2.3: Influence of gender and motor onset on age at secondary progression



Multivariable Cox regression analysis of the PPMS patients showed visual and brainstem/cerebellar onset to be an independent predictive factor of a younger age at progression (hazard ratios: visual onset 2.06, 95%CI 1.23-3.43; brainstem/cerebellar onset 2.53, 95%CI 1.43-4.49) (Table 2.4).

Table 2.4: Multivariable analysis of potential risk factors affecting age at primary progression

Factor	n	age at progression hazard ratio (95% CI)
All patients	210	—
Gender		
Women	123	1.0 (reference)
Men	87	1.14 (0.86-1.51)
Onset manifestation		
Visual		
No	193	1.0 (reference)
Yes	17	2.06 (1.23-3.43)
Brainstem/cerebellar		
No	197	1.0 (reference)
Yes	13	2.53 (1.43-4.49)

Multivariable analysis of the SPMS patients showed that motor onset was no predictive factor for age at progression (Table 2.5).

Table 2.5: Multivariable analysis of potential risk factors influencing the age at secondary progression

Factor	n	age at progression multivariable hazard ratio (95% CI)
All patients	228	—
Gender		
Women	155	1.0 (reference)
Men	73	1.15 (0.87-1.52)
Onset manifestation		
Motor		
No	204	1.0 (reference)
Yes	24	0.65 (0.42-1.01)

Time to progression

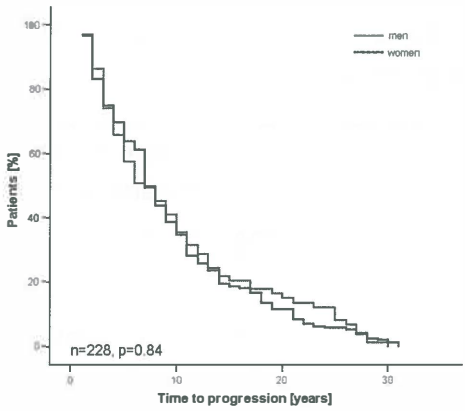
In SPMS patients, time to progression was similar for men and women, and strongly influenced by the age at disease onset. The older the patients were at disease onset, the shorter the time to secondary progression (Table 2.6, Figure 2.4). SPMS patients with motor onset symptoms had a significantly longer time to progression (mean time to progression in years: 12.9 versus 9.2 in patients with non-motor onset symptoms; p=0.03) (Table 2.6; Figure 2.4).

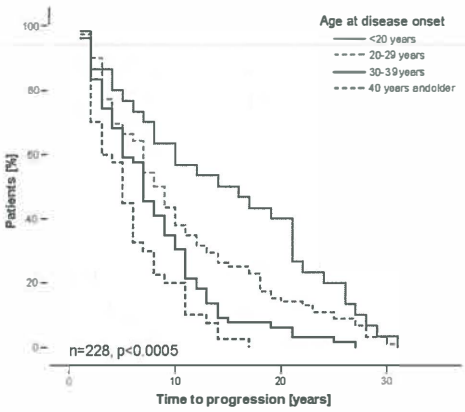
Table 2.6: Potential risk factors influencing the time to secondary progression

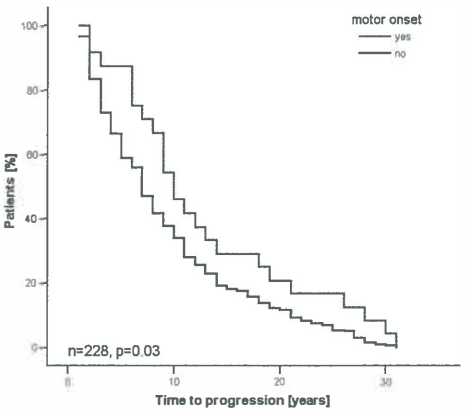
Factor	n	Mean time to progression [years] (95% CI)	p*
All patients	228	9.62 (8.63 - 10.62)	—
Gender			
Women	155	9.50 (8.33 - 10.67)	0.84
Men	73	9.88 (7.99 - 11.76)	
Age at onset, years			
younger than 20	30	14.80 (11.38-18.22)	<0.0005
20-29	92	10.70 (9.01 - 12.38	
30-39	66	8.06 (6.66 - 9.46)	
40 and above	40	5.85 (4.55 - 7.14)	
Onset manifestation			
Visual			
No	144	9.53 (8.21 - 10.84)	0.91
Yes	84	9.79 (8.29 - 11.28)	
Motor			
No	205	9.23 (8.21 - 10.26)	0.03
Yes	23	12.92 (9.42 - 16.41)	
Sensory			
No	149	10.29 (9.05 - 11.52)	0.1
Yes	79	8.37 (6.71 - 10.02)	
Brainstem/cerebellar			
No	193	9.64 (8.58 - 10.71)	0.99
Yes	35	9.51 (6.71 - 12.32)	
Other			
No	220	9.57 (8.57 - 10.59)	0.77
Yes	6	11.33 (5.42 - 17.25)	

*: log-rank test

Figure 2.4: Influence of gender, age at disease onset and motor onset on time to secondary progression







In the Cox regression model, we found that age at disease onset and motor onset were predictive factors for the time to progression (Table 2.7).

Table 2.7: Multivariable analysis of potential risk factors influencing the time to secondary progression

Factor	n	Time to progression hazard ratio (95% CI)
All patients	228	—
Gender		
Women	155	1.0 (reference)
Men	73	1.09 (0.82-1.44)
Age at onset, years		
younger than 20	30	1.0 (reference)
20-29	92	1.50 (0.99-2.28)
30-39	66	2.25 (1.43-3.53)
40 and above	40	3.47 (2.10-5.73)
Onset manifestation		
Motor		
No	204	1.0 (reference)
Yes	24	0.59 (0.39-0.92)

Discussion

Our finding that the age at progression is independent of the initial disease course is in keeping with the findings reported previously.[10–12] This confirms the idea that relapses do not influence the development of a progressive disease course in MS.

Male sex has long been viewed as a factor predicting worse outcome in MS. This idea was based on studies using the EDSS scale and the time from disease onset as endpoints.[14;15] Newer studies which use age rather than time from disease onset as endpoints show conflicting results. Tremlett and colleagues found no difference between men and women for the time to reach EDSS 6.0 in their cohort of 2,837 patients,[16] whereas Confavreux and coworkers reported a longer time to reach EDSS 6.0 in women in their cohort of 595 patients.[17] In our study we found no significant gender differences in the age at progression or in the time to progression.

Visual and brainstem/cerebellar manifestation at disease onset were associated with a younger age at progression in PPMS patients. Both findings are based on small patient numbers, but it is interesting to note that Tremlett and colleagues reported cerebellar or brainstem/cerebellar symptoms to be associated with a shorter time to EDSS 6.0 in their study on 299 PPMS patients, whereas onset with optic neuritis had no significant influence on time to EDSS 6.0.[18]

Disease onset with motor symptoms has previously been reported to be associated with a shorter time to landmark EDSS-scores.[19;20] One possible explanation for this finding may lie in the use of the EDSS scale in the named studies. The EDSS scale emphasises ambulation over other functional measures. One would expect that patients with motor onset are likely to worsen quicker in ambulation function than patients with other onset manifestations, simply because their motor system is subject to damage for a longer period of time. The higher EDSS scores in patients with motor onset would then not represent the disease course but rather reflect the peculiarities of the used endpoint. Somewhat surprisingly, we found SPMS patients with motor onset symptoms to have a significantly higher age at progression and a significantly longer time to progression in our study, and multivariable regression analysis showed motor onset

symptoms to be a predictive factor for a longer time to progression in SPMS patients. This finding is difficult to explain and we believe it may be artificial. Patients with motor onset are likely to be more disabled than patients with other onset manifestations. Establishing the time of onset of the progressive phase in patients who are already disabled can be difficult.

The fact that time to progression in SPMS patients is highly associated with the age at disease onset is in keeping with earlier studies, in which the time to landmark disability scores was found to be associated with age at disease onset.[15;20] These data suggest that the development of a progressive disease course is an age dependent process.

In conclusion, our data show that the initial disease course (relapsing-remitting or primary progressive) has no important influence on the age at progression. Gender had no influence on age at progression in PPMS and SPMS, nor on time to progression in SPMS. This supports the hypothesis that the development of a progressive disease course in MS is an age dependent process unrelated to relapses. In our opinion, age at progression and time to progression are more important endpoints in natural history studies than landmark disability. These two endpoints should be used to evaluate other established patient cohorts. The finding that visual and brainstem/cerebellar onset may carry a worse prognosis in PPMS should be evaluated in other patient cohorts. The finding that motor onset symptoms in SPMS patients are associated with a higher age at progression and a longer time to progression may be artificial.

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Chapter 3

Factors associated with the risk of secondary progression in multiple sclerosis

Marcus Koch, Maarten Uyttenboogaart, Annemarie van Harten, Jacques De Keyser

under peer review

Abstract

Objective: To investigate factors associated with the risk of secondary progression in relapsing-remitting onset multiple sclerosis (MS).

Methods: We used Kaplan-Meier survival analyses and a multivariable Cox regression model to estimate the influence of the factors gender, age at disease onset, use of immunomodulatory drugs (IMD), and clinical manifestation at disease onset on the time to secondary progression in a hospital-based cohort of 571 MS patients with a relapsing-remitting disease onset.

Results: Gender and onset manifestation had no significant influence on the timing of secondary progression. A higher age at disease onset was associated with a shorter time to secondary progression (multivariable hazard ratio per year increase: 1.02, 95% CI: 1.01-1.03). The use of IMD was associated with a longer time to secondary progression (multivariable hazard ratio: 0.30, 95% CI: 0.15-0.61).

Conclusions: The inverse relationship between age at disease onset and onset of secondary progression is in keeping with previous natural history studies. The beneficial effect of IMD treatment on the time to secondary progression should be taken as hypothesis-generating rather than as proof of a treatment effect, and needs to be further evaluated in well-designed randomised controlled trials.

Introduction

In the majority of patients with multiple sclerosis (MS), the beginning of the disease is characterised by a relapsing-remitting disease course. The further evolution of the disease is highly variable; while some patients have a "benign" disease course and remain free of disability for more than 10 to 15 years, others quickly accumulate significant disability and convert to secondary progressive MS (SPMS).

Previous studies examining risk factors associated with the timing of secondary progression limited the analyses to patients who had already developed SPMS.[1;2] This approach excludes patients with a prolonged relapsing-remitting phase. The results of such studies are therefore not representative of the whole population of patients with a relapsing-remitting disease onset, and are consequently not useful for predicting prognosis in the early stages of the disease.

Including all patients with a relapsing-remitting disease onset into the analyses and censoring those who do not develop SPMS is a straightforward method to estimate the overall risk of secondary progression.

In this study, we use this approach to identify predictive factors associated with the risk of secondary progression in our hospital-based MS cohort.

Patients and methods

Groningen MS database

The Groningen MS database is a prospective computerised database containing the clinical data of all patients attending the MS Clinic at the University Medical Center Groningen (UMCG) outpatient department. Patients are seen at the clinic at 3- to 12 month intervals with intercurrent visits if necessary. The UMCG is the main secondary and tertiary referral centre for MS in the province of Groningen (population approximately 575,000). Data acquisition started in 1985. Currently, the database contains data on 809 patients.

Patients

For this study, we selected all patients with a relapsing-remitting disease onset. All patients included in this study had a diagnosis of definite MS according to the Poser diagnostic criteria.[3] Secondary progression was defined as the continuous worsening of neurological symptoms unrelated to relapse for at least one year.[4] Patients with a progressive disease course from onset (primary progressive MS) were excluded. Patients who were progressive from disease onset with an occasional superimposed relapse ('relapsing-progressive MS') were classified as primary progressive and excluded. The year of the first relevant symptom was taken as the year of disease onset.

Statistical analyses

The endpoint in our analyses was the time from disease onset to the onset of secondary progression. Patients who did not develop secondary progression were included in the analyses with their data censored at the time of loss to follow-up at the clinic, the time of death or the time of inclusion in the analyses of this study, whichever came first.

The investigated variables were gender, manifestation at disease onset (labelled 'visual' in patients with optic neuritis, 'motor' in patients with arm or leg weakness, 'sensory' in patients presenting with pain or sensory symptoms, 'brainstem/cerebellar' in patients with oculomotor signs, vertigo, intention tremor or nystagmus, and 'other' in patients with other onset manifestations such as sphincter dysfunction or sexual symptoms), use of immunomodulatory drugs (IMD) (for at least one year before the onset of progression or censoring) and the age at disease onset.

In a univariate analysis, the influence of these variables on time to secondary progression was assessed with Kaplan-Meier survival analyses. In these analyses, age at disease onset was divided into four categories ('20 years or younger', '21 to 30 years', '31 to 40 years' and 'above 40 years'). Variables with differences in the univariate survival analyses with p-values of less than 0.1 were included in a multivariable Cox regression model with gender as a mandatory covariate. In the Cox regression model, age at disease onset was entered as a continuous variable.

Statistical significance was taken to be at the two-tailed 0.05 level. All statistical analyses were performed with the SPSS statistical software package version 14 (SPSS Inc., Chicago, USA).

Results

Patients

An overview of the baseline characteristics of the study participants is given in Table 3.1. Of the 571 patients with a relapsing-remitting disease onset, 268 (47%) had developed secondary progression. Of the remaining 303 patients, 270 (89%) were censored at the time of inclusion in this study, 30 (10%) at the time when lost to follow-up and 3 (1%) at the time of their death.

Sixty-three patients were using IMD: 52 (83%) interferon beta, and 11 (17%) glatiramer acetate. No other immunomodulatory or immunosuppresive treatments were used, except for corticosteroids (intravenous methylprednisolone or oral dexamethasone) for the treatment of exacerbations.

Table 3.1: Baseline characteristics.

	All patients	RRMS	SPMS
n	571	303	268
Gender (n, %)			
Women	398 (70%)	216 (71%)	182 (68%)
Men	173 (30%)	87 (29%)	86 (32%)
Age at disease onset, years (median, IQR)	29, 23-37	29, 23-38	29, 23-36
Disease duration, years: (median, IQR)	16, 10-25	13, 6-19	21, 15-30
Use of IMD (n, %)	63 (11%)	55 (18%)	8 (3%)
Onset manifestation (n, %)			
Visual	176 (31%)	79 (26%)	97 (36%)
Motor	67 (12%)	34 (11%)	33 (12%)
Sensory	227 (40%)	139 (46%)	88 (33%)
Brainstem/cerebellar	80 (14%)	37 (12%)	43 (16%)
Other	21 (4%)	14 (5%)	7 (3%)

Time to secondary progression

The results of the Kaplan-Meier analyses are shown in Table 3.2. A higher age at disease onset was associated with a shorter time to secondary progression ($p=0.005$) and patients using IMD had a significantly longer time to secondary progression ($p<0.0005$). A visual onset manifestation was associated with a shorter time to secondary progression, but this was not statistically significant ($p=0.08$).

Survival plots on the influence of age at disease onset and IMD use on the time to secondary progression are shown in Figures 3.1 and 3.2.

Table 3.2: Univariate analysis.

Factor	n	patients developing secondary progression	Mean time to progression (95% CI)	p*
All patients	571	268 (47%)	23.30 (20.88-25.72)	—
Gender				
Women	398	182 (46%)	22.28 (19.96-24.60)	0.56
Men	173	86 (50%)	23.59 (19.63-27.55)	
Age at disease onset:				
20 years and younger	74	41 (55%)	24.35 (19.49-29.21)	0.005
21 to 30 years	242	111 (46%)	22.81 (20.22-25.41)	
31 to 40 years	163	73 (45%)	21.17 (18.16-24.20)	
above 40 years	92	43 (47%)	15.77 (12.37-19.17)	
IMD use				
Yes	63	8 (13%)	25.58 (21.33-29.83)	<0.0005
No	508	260 (51%)	22.32 (19.94-24.70)	
Onset manifestation:				
Visual				
Yes	176	97 (55%)	18.79 (16.37-21.21)	0.08
No	395	171 (43%)	24.91 (21.92-27.90)	
Motor				
Yes	67	33 (49%)	26.17 (20.25-32.09)	0.24
No	504	235 (47%)	21.17 (19.26-23.08)	
Sensory				
Yes	227	88 (39%)	21.13 (18.83-23.42)	0.25
No	344	180 (52%)	22.35 (19.65-25.05)	
Brainstem/cerebellar				
Yes	80	43 (54%)	20.07 (15.90-24.24)	0.31
No	491	225 (46%)	23.50 (20.80-26.20)	
Other				
Yes	21	7 (33%)	21.43 (16.28-26.58)	0.26
No	550	261 (48%)	23.11 (20.67-25.54)	

*: log-rank test
CI: confidence interval

Figure 3.1: Influence of age at disease onset on time to secondary progression.

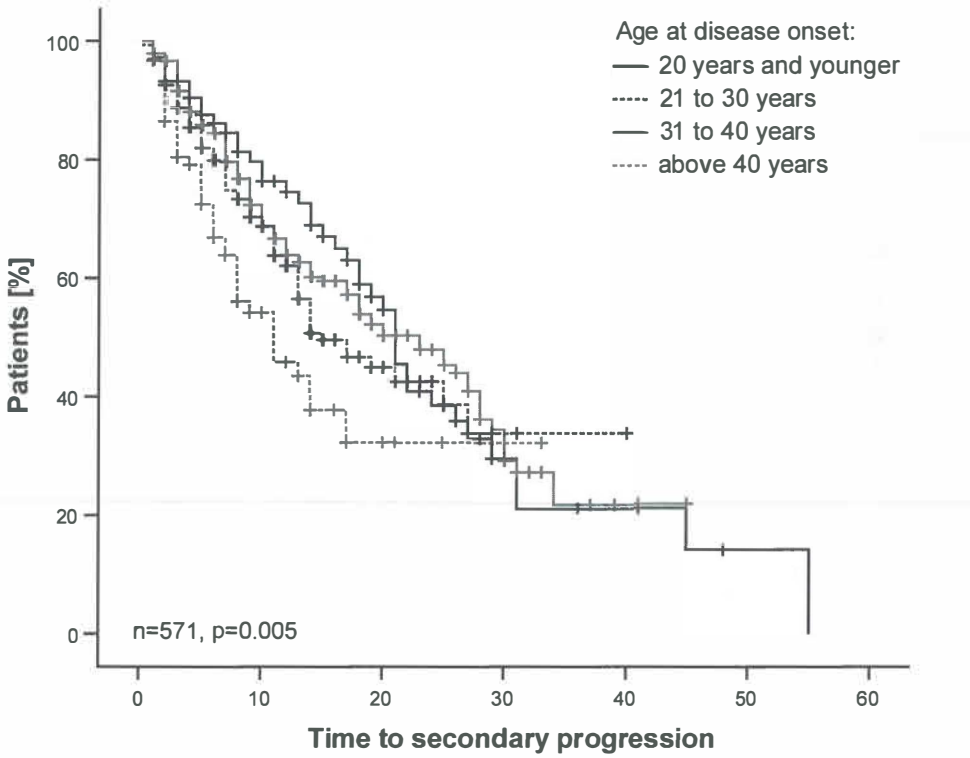
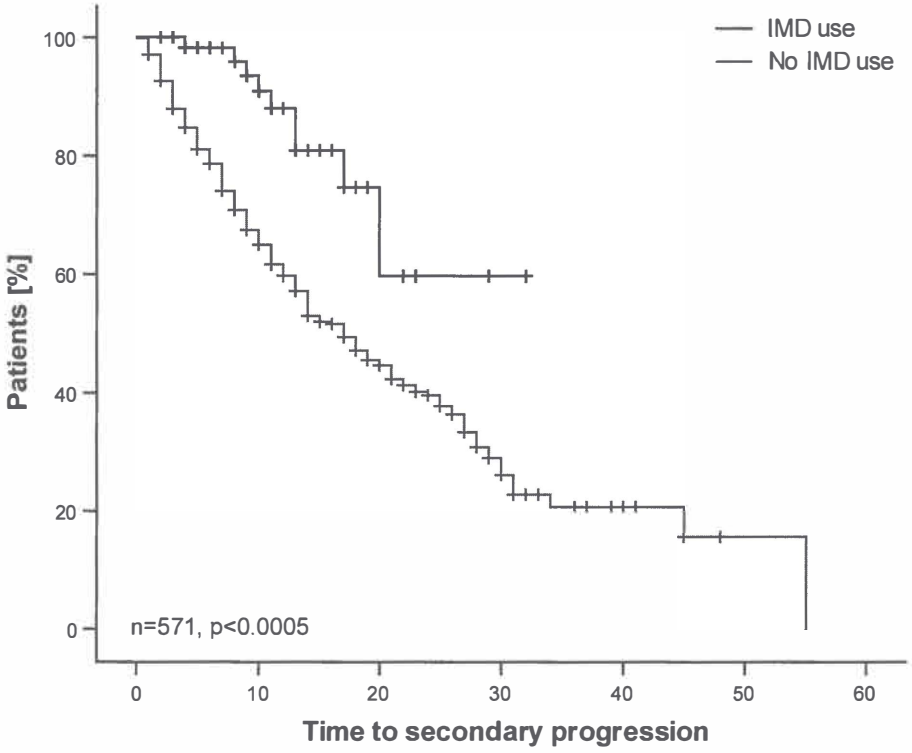


Figure 3.2: Influence of IMD use on time to secondary progression.



Age at disease onset, IMD use, visual onset manifestation and gender were included as covariates in a Cox regression model. The results of this regression analysis are listed in Table 3.3. Age at disease onset (hazard ratio per year increase 1.02, 95% CI: 1.01-1.03, p=0.005) and IMD use (hazard ratio 0.30, 95% CI: 0.15-0.61, p<0.0005) were the predictive variables in this model.

Table 3.3: Multivariable analysis

Factor	n	Cox regression hazard ratio (95% CI)	p
Gender			
Women	398	1.0 (reference)	0.54
Men	173	1.09 (0.84-1.40)	
Age at disease onset	571	1.02* (1.01*-1.03*)	0.005
IMD use			
No	508	1.0 (reference)	<0.0005
Yes	63	.30 (0.15-0.61)	
Visual onset			
No	395	1.0 (reference)	0.09
Yes	176	1.24 (0.97-1.60)	

CI: confidence interval
*: per year increase

Discussion

In this study, we found that age at disease onset and IMD treatment influenced the risk of secondary progression in patients with a relapsing-remitting disease onset, whereas gender and onset manifestation had no influence.

Our finding that a higher age at disease onset is predictive of a greater risk of secondary progression is in keeping with previous studies which limited their analyses to patients with SPMS.[1;2] These studies showed that the time to secondary progression decreased with advancing age at disease onset, which was defined as the first relapse. The pathophysiological mechanism underlying this effect is not known, and there are no studies in humans on the effect of age on the pathologic changes in MS. Because the disease may be ongoing for many years before the first relapse occurs, a later symptomatic onset may give the false impression of a reduced time to secondary progression. On the other hand, the effect could be related to an age dependent dysfunction of repair processes after demyelination. A study in rats showed that CNS remyelination is less efficient in older animals, due to impaired recruitment and differentiation of oligodendrocyte precursor cells.[5] A similar mechanism could contribute to the age dependence of the onset of secondary progression, based on the idea that remyelination can rescue axons from degeneration. Kornek and colleagues compared axonal degeneration in MS lesions at different stages of lesion evolution. Besides marked axonal degeneration in actively demyelinating plaques, they also describe a "slow-burning" axonal degeneration that is present even in inactive demyelinated plaques. This "slow-burning" axonal degeneration was absent in fully remyelinated shadow plaques,[6] which suggests that a more efficient remyelination could reduce the amount of axonal degeneration in MS, and consequently prolong the time to secondary progression.

Our other main finding is that IMD use reduced the risk of secondary progression. The relatively low number of patients using IMD in our cohort precludes further analyses by type of IMD used and by duration of IMD use, and calls for an explanation. In our hospital, we do not regard IMD therapy as mandatory in every patient with RRMS. We offer IMD treatment to every patient with a definite diagnosis of RRMS, but the decision whether

or not to start such treatment is made individually with every patient, after discussion of the possibility of a benign disease course and the possible benefits and side effects of IMD use. A significant number of patients decide against immediate treatment. We also never start IMD treatment in patients with clinically isolated syndromes suggestive of MS. In view of this policy, a selection bias of this study in favour of IMD use, in the sense that patients with a more benign disease course were more likely to receive IMD, is unlikely.

Most previous epidemiological studies have not investigated the influence of IMD treatment on the timing of progression or the time to landmark disability. One exception is the recent study by Trojano and coworkers, who attempted to assess the influence of interferon beta treatment on the time to secondary progression and the time to landmark disability scores. They used propensity score weighting to create groups of treated and untreated patients from two hospital-based patient cohorts, and compared the time to secondary progression, the time to EDSS 4.0 and the time to EDSS 6.0 between these groups. They report a significant beneficial effect of interferon beta treatment on all of these measures, but several serious baseline imbalances between treated and untreated patients in this study make the interpretation of their findings difficult.[7] Other natural history studies were often intentionally limited to untreated patients.

IMDs reduce the number of relapses and lesions on brain MRI.[8;9] It has been known since the first serial MRI studies, that the majority of new lesions visible on brain MRI scans are clinically silent,[10;11] and a recent examination of 821 patients with RRMS or SPMS enrolled in the Silvia Lawry Center for MS Research database found no correlation of baseline T2-lesion burden on MRI and relapse rate in the following year.[12] The clinical phenomenon of the relapse is a poor reflection of the ongoing tissue damage in MS.

We have recently found that a greater T2-lesion burden on baseline MRI is associated with a shorter time to secondary progression in patients with RRMS,[13] which suggests that a greater cerebral lesion load lowers the critical threshold after which axonal degeneration becomes clinically apparent. It would seem plausible that the ability of IMD to reduce the amount of cerebral tissue damage, as shown by their ability to reduce the number and volume of new MRI lesions,[14;15] could result in a preservation of the

functional axonal reserve capacity, and prolong the time to secondary progression.

While our study, and other observational studies, can suggest an effect of IMD use on the risk of secondary progression, our results should be taken as hypothesis-generating. A definite proof of a treatment effect of IMD should come from well-conducted randomised controlled trials (RCTs).

It has sometimes been suggested that long-term RCTs in MS were unethical or impractical, since blinding and placebo treatment would have to be continued for many years before a treatment effect on the time to secondary progression could be measured. This has been the main argument to limit the duration of IMD studies to two years or less, and to use changes in EDSS scores as surrogate markers of progression. Ebers and co-workers have convincingly shown in their analysis of patient data from 31 placebo arms of MS treatment trials that changes in EDSS scores are unreliable outcome measures.[16] In order to produce useful answers to clinical questions, RCTs should use the onset of secondary progression as the primary outcome measure.

The open-label extension of interferon beta[17] and glatiramer acetate[18] treatment trials has been proposed as an alternative to new long-term RCTs, but these open-label extensions lost more than 30% of the originally randomised patients. This and the high risk of introducing a new selection bias into the trial extension (because the patients who benefitted most from the treatment are the most likely to wish for a continuation of the trial medication) make the results of such trial extensions unreliable.

Two recent examples of well-designed, publicly funded RCTs on the pharmacologic treatment of epilepsy, the MESS and SANAD trials, could be taken as an inspiration for the design of useful trials in MS. Both trials were unblinded and used no placebo control, but nevertheless provided clinically useful information in a field that had previously been dominated by short-term trials designed to acquire drug approval rather than to answer clinical questions.

The MESS trial[19] compared policies of immediate versus deferred antiepileptic treatment after a first seizure and could serve as a template for a trial comparing immediate versus deferred treatment of clinically isolated syn-

dromes. The SANAD trials provided a much-needed head-to-head comparison of the most commonly used antiepileptic drugs.[20] A similar study design could be used to compare the effect of the most common IMDs and immunosuppressants on the timing of secondary progression in MS.

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Chapter 4

Progression in familial and non-familial multiple sclerosis

Marcus Koch, Maarten Uyttenboogaart, Marco Heerings, Dorothea Heersema, Jop Mostert, Jacques De Keyser

Mult Scler [in press]

Abstract

Objective: To investigate whether the timing of secondary or primary progression is different between patients with familial and non-familial multiple sclerosis (MS).

Methods: Information on the family history of 313 patients with MS was taken from our prospective hospital-based database. We used Kaplan-Meier analyses and Cox regression models to evaluate differences between familial and non-familial MS in several endpoints. We investigated the risk of developing secondary progression in all patients with a relapsing-remitting disease onset, the length of the relapsing-remitting phase and age at onset of progression in patients with secondary progressive MS and the age at disease onset in patients with primary progressive MS.

Results: Among the primary progressive patients, those with familial MS had a significantly younger age at disease onset than patients with non-familial MS (mean 33.04 years versus mean 37.73 years in non-familial MS, p=0.02). There were no significant differences between familial and non-familial MS patients in any other investigated measure.

Conclusion: Familial MS appears related to the time of disease onset in primary progressive MS. Patients with familial primary progressive MS may be an important patient group for future genetic research in MS.

Introduction

Multiple sclerosis (MS) is traditionally regarded as an inflammatory demyelinating disease of the central nervous system. Although axonal degeneration is known to occur in multiple sclerosis since the earliest pathologic descriptions of the disease, scientific interest in this pathophysiological mechanism has only developed in the last decade. Axonal degeneration is a diffuse process encompassing inflammatory lesions as well as the extralesional normal appearing white matter and is believed to be the main mechanism underlying the development of a progressive disease course of MS.[1;2]

Most previous studies on the epidemiology of progression in MS used the time from disease onset until certain landmark disability scores (e.g. requires a cane for walking, restricted to bed etc.) to investigate disease evolution. Tremlett and coworkers advocate comparing the age at landmark disability scores rather than time from disease onset,[3] since we cannot be certain that the disease process in MS starts when symptoms first appear. Indeed, evidence from migration studies suggests that the disease may start at an early stage in a patients life, but becomes clinically apparent at a much later stage.[4]

Axonal degeneration starts early in the disease course, but remains clinically silent because the brain has a large functional reserve capacity and initially compensates for the loss of degenerated neurons. Once the ongoing neurodegeneration has exhausted this reserve capacity, deficits become clinically apparent.[5]

It is now well established that progression in MS is an age dependent process: progression occurs at a remarkably similar age in primary progressive and secondary progressive patients.[6–8]

Twin concordance studies show that hereditary factors are associated with the risk of developing MS,[9] whereas the influence of hereditary factors on disease evolution is less certain. The latest study comparing disease evolution in familial and non-familial MS patients failed to show differences in the time to landmark disability scores between the two groups.[10]

In this study, we compared the risk of secondary progression and the age at secondary and primary progression between patients with familial and non-familial MS.

Patients and methods

Groningen MS Database

The Groningen MS database contains data on disease course and disability accumulation of the patients attending our MS clinic. Data have been collected prospectively since 1985 by means of 3- to 12-monthly follow-up at the Groningen MS clinic. The collected data include gender, age, disease subtype, time of disease onset, time of secondary progression and use of immunomodulating drugs (IMD). All patients were diagnosed with definite MS according to the Poser criteria.[11] The year of the first relevant MS symptom was taken as the year of disease onset. Progression was defined as the continuous unremitting worsening of neurological impairment unrelated to relapses for at least one year.[7]

Patients with a progressive disease course from onset were defined as primary progressive MS (PPMS). Patients who were progressive from disease onset with an occasional superimposed relapse ('relapsing progressive MS') were classified as PPMS. Patients with a single relapse followed by the development of progression were classified as secondary progressive MS (SPMS).

Family History

A detailed pedigree analysis was obtained from the patients during their visits to the MS clinic, and this information was recorded in the database. We did not routinely contact or examine affected family members. After 1997, we no longer routinely recorded family history in the database. We therefore limited our analyses to the patients registered in the database until this date.

Patients were divided into familial and non-familial cases. Patients with familial MS were further subdivided by degree of relatedness to the other affected family member(s). Following the criteria of Weinshenker and colleagues,[12] we classified patients with multiple affected family members including at least one first degree relative as '1st degree plus', patients

with one affected first degree family member as '1st degree' and patients with one or more affected second or third degree relatives as '2nd or 3rd degree'.

Statistical analyses

Included vs. not included and familial vs. non-familial cases

Gender, disease subtype, disease duration, age at disease onset and IMD use were compared with Fisher's exact test, Pearson's chi square test, and the Mann-Whitney U test where appropriate.

Familial MS and risk of secondary progression

For this analysis, the length of the relapsing-remitting phase was determined in all patients with a relapsing-remitting disease onset (RRMS and SPMS patients). Patients were followed from disease onset until onset of secondary progression or censored if secondary progression did not develop. Group differences in the length of the relapsing-remitting phase were assessed with Kaplan-Meier survival analyses. We estimated the hazard ratio for conversion to secondary progression with a Cox-regression model including gender, familial MS and age at disease onset as covariates.

Familial MS and length of the relapsing-remitting phase

Group differences in length of the relapsing-remitting phase were evaluated in all SPMS patients with Kaplan-Meier survival analyses. Gender, familial MS and age at disease onset were included as covariates in a Cox regression model.

Familial MS and age at progression

This analysis was performed for SPMS and PPMS separately. The influence of gender, familial MS and degree of relatedness on the age at secondary progression (SPMS) or age at disease onset (PPMS) was assessed using Kaplan-Meier survival analyses and Cox regression models. Gender and familial MS were entered into the regression model. In SPMS patients, age at disease onset was evaluated with the Kaplan-Meier method but not included in the regression model, since age at progression must necessarily be greater than age at disease onset in every case.

Statistical significance was taken to be at the two-tailed 0.05 level. All statistical analyses were performed with the SPSS statistical software package version 14.

Results

Patient sample

Information on family history of MS was available for 313 patients of the total 466 patients included in the database until 1997 (67%). There were 82 patients (26%) with a family history of MS, and 231 patients (74%) with non-familial MS. Included patients had a significantly shorter disease duration and younger age at disease onset than not included patients and IMD use was more common among the included patients. There were no significant differences in gender and disease subtype distribution between included and not included patients (Table 4.1).

Table 4.1: Comparison of baseline characteristics between included and not included patients

	included cases	not included cases	p
n	313	153	—
Gender (n, %)			
Women	216 (69%)	105 (69%)	1.0*
Men	97 (31%)	48 (31%)	
Disease course			
RRMS	98 (31%)	41 (26%)	
SPMS	121 (39%)	56 (37%)	0.33†
PPMS	94 (30%)	56 (37%)	
Disease duration (median, IQR)	14, 9-22	19, 12-24.5	<0.0005‡
Age at disease onset (median, IQR)	31, 24-28	32, 24-44	0.02‡
Use of IMD (n, %)	56 (18%)	15 (10%)	0.03*

*: Fisher's exact test, †: Pearson's chi square test, ‡: Mann-Whitney U test
IQR: interquartile range, SD: standard deviation

Table 4.2 shows that there were no statistically significant differences in gender, disease course, disease duration, age at disease onset and use of IMD between patients with familial vs. non-familial MS.

Table 4.2: Comparison of baseline characteristics between familial and non-familial MS cases

	non-familial cases	familial cases	p
n	231	82	—
Gender (n, %)			
Women	155 (67%)	61 (74%)	0.26*
Men	76 (33%)	21 (26%)	
Disease course			
RRMS	61 (26%)	18 (22%)	
SPMS	99 (43%)	41 (50%)	0.59†
PPMS	71 (31%)	23 (28%)	
Disease duration (median, IQR)	23, 18-30	27, 19 - 35	0.06‡
Age at disease onset (median, IQR)	30, 23-37	31.5, 26-38	0.31‡
Use of IMD (n, %)	46 (20%)	10 (12%)	0.13*

*: Fisher's exact test, †: Pearson's chi square test
‡: Mann-Whitney U test
IQR: interquartile range, SD: standard deviation

Familial MS and risk of secondary progression

The results of the survival and regression analyses are shown in Table 4.3. Seventy-nine (36%) of the 219 patients with a relapsing-remitting onset were censored: 71 at the time of inclusion into this study, 7 at the time when lost to follow-up and one patient at the time of her death. There were no significant differences in the length of the relapsing-remitting phase for any variable. A survival plot for familial MS and risk of secondary progression is shown in Figure 4.1.

Figure 4.1: Influence of familial MS on the risk of secondary progression in patients with a relapsing-remitting disease onset.

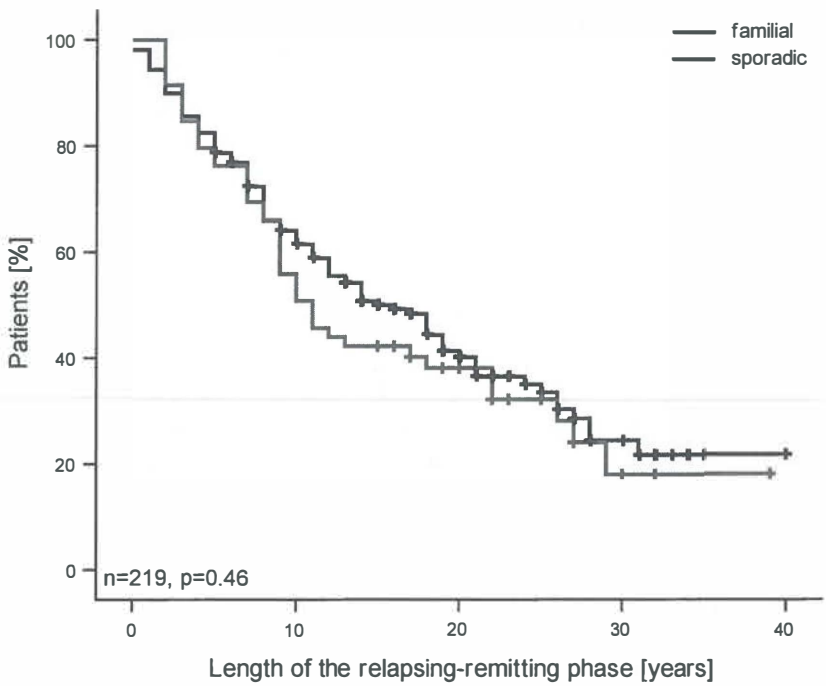


Table 4.3: Potential factors influencing the risk of secondary progression in all patients with relapsing-remitting disease onset

	n	Patients developing secondary progression	Mean length of the relapsing-remitting phase (95% CI)	p*	Cox regression hazard ratio (95% CI)
All patients	219	140 (64%)	18.28 (16.26-20.30)	—	—
Familial MS					
non-familial cases	160	99 (62%)	18.73 (16.32-21.13)	0.46	1.0 (reference)
familial cases	59	41 (69%)	16.91 (13.31-20.52)		1.18 (0.80 - 1.73)
Degree of relatedness					
non-familial cases	160	99 (62%)	18.73 (16.32-21.129)	0.80	—
1st degree plus	10	5 (50%)	14.90 (9.71-20.09)		
1st degree	19	15 (79%)	16.27 (10.62-21.92)		
2nd or 3rd degree	30	21 (70%)	15.49 (11.28-19.71)		
Gender					
women	156	94 (60%)	19.49 (16.99-21.98)	0.10	1.0 (reference)
men	63	46 (73%)	14.91 (12.04-17.78)		1.37 (0.96-1.95)
Age at disease onset, years					
younger than 25	71	48 (68%)	17.56 (14.95-20.17)	0.73	1.0 (reference)
25 - 29	45	33 (73%)	15.81 (12.40-19.22)		1.11 (0.70 - 1.76)
30 - 34	42	22 (52%)	18.55 (14.37-22.74)		0.85 (0.51 - 1.42)
35 and older	61	37 (61%)	19.23 (14.91-23.55)		1.05 (0.68 - 1.63)

*: log-rank-test

Familial MS and length of the relapsing-remitting phase

The results of the survival and regression analyses are listed in Table 4.4. The length of the relapsing-remitting phase decreased significantly with increasing age at onset ($p<0.0005$). There were no significant differences in the length of the relapsing-remitting phase for any of the other investigated variables. A survival plot for familial MS and length of the relapsing-remitting phase is shown in Figure 4.2.

Figure 4.2: Influence of familial MS on the length of the relapsing-remitting phase in secondary progressive patients.

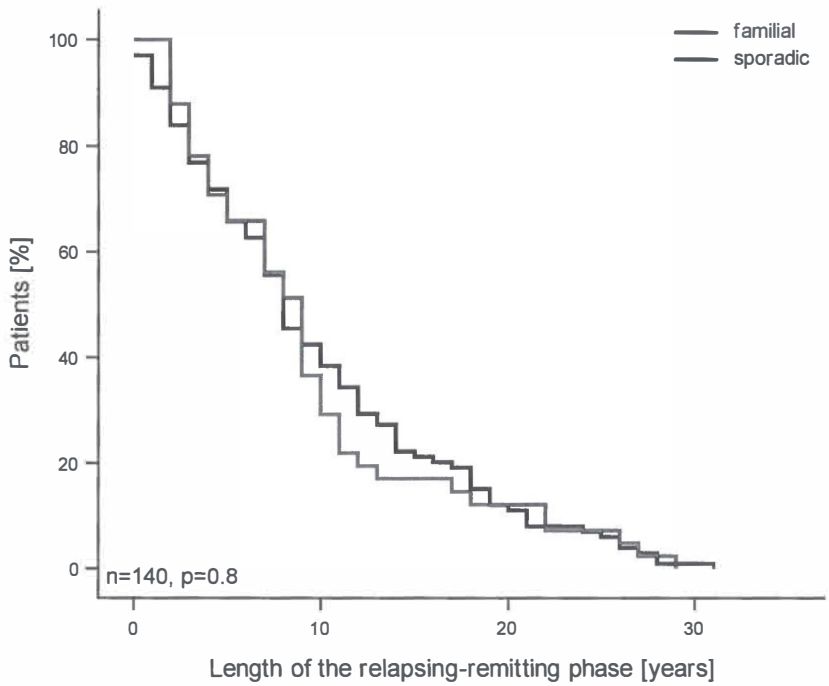


Table 4.4: Potential factors influencing the length of the relapsing-remitting phase in SPMS patients

	n	Mean length of the relapsing-remitting phase (95% CI)	p*	Cox regression hazard ratio (95% CI)
All patients	140	9.79 (8.57-11.02)	—	—
Familial MS				
non-familial cases	99	9.90 (8.42-11.38)	0.46	1.0 (reference)
familial cases	41	9.54 (7.35-11.72)		0.9 (0.61-1.33)
Degree of relatedness				
non-familial cases	99	9.90 (8.42-11.38)		
1st degree plus	5	6.8 (3.87-9.73)	0.67	—
1st degree	15	11.07 (7.28-14.85)		
2nd or 3rd degree	21	9.10 (5.88-12.32)		
Gender				
women	94	9.69 (8.29-11.10)	0.79	1.0 (reference)
men	46	10.00 (7.59-12.41)		0.81 (0.55 - 1.19)
Age at disease onset, years				
younger than 25	48	12.90 (10.49-15.30)		1.0 (reference)
25 - 29	33	10.15 (7.64-12.67)	<0.0005	1.34 (0.85-2.13)
30 - 34	22	6.55 (4.21-8.88)		2.54 (1.49-4.32)
35 and older	37	7.37 (5.76-9.00)		2.26 (1.42-3.62)

* log-rank test

Familial MS and age at progression

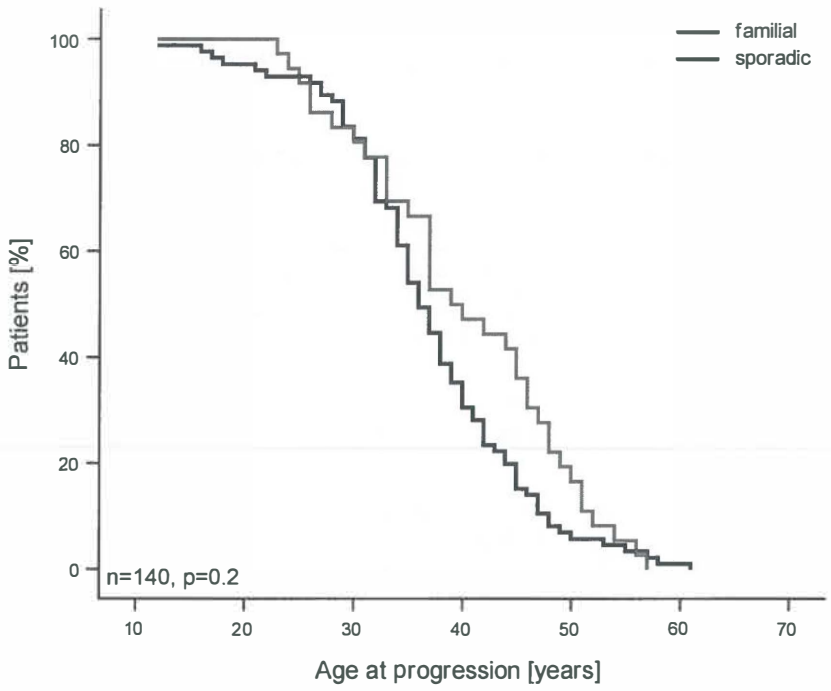
The results of the survival and regression analyses are shown in Tables 4.5 and 4.6. In SPMS patients, familial MS had no significant effect, but male patients were significantly older at progression (mean 40.44 years versus mean 37.37 years in women, p=0.03). The age at progression was significantly higher with increasing age at disease onset (p<0.0005). A survival plot for familial MS and age at secondary progression is shown in Figure 4.3.

Table 4.5: Potential factors influencing the age at progression in SPMS patients

	n	Mean age at progression (95% CI)	p†	Cox regression hazard ratio (95% CI)
All patients	140	38.38 (36.80 - 39.96)	—	—
Familial MS				
non-familial cases	99	37.55 (35.67-39.42)	0.20	1.0 (reference)
familial cases	41	40.39 (37.49-43.30)		0.70 (0.48 - 1.03)
Degree of relatedness				
non-familial cases	99	37.55 (35.67-39.42)		
1st degree plus	5	32.80 (27.79-37.81)	0.08	—
1st degree	15	42.40 (36.25-45.28)		
2nd or 3rd degree	21	40.76 (36.25-45.28)		
Gender				
women	94	37.37 (35.51-39.17)	0.03	1.0 (reference)
men	46	40.44 (37.38-43.49)		0.64 (0.42-0.90)
Age at disease onset, years				
younger than 25	48	32.04 (29.56-34.52)		
25 - 29	33	37.21 (34.64-39.78)	<0.0005	—
30 - 34	22	38.00 (35.73-40.27)		
35 and older	37	47.57 (45.89-49.84)		

† log-rank-test

Figure 4.3: Influence of familial MS on the age at secondary progression



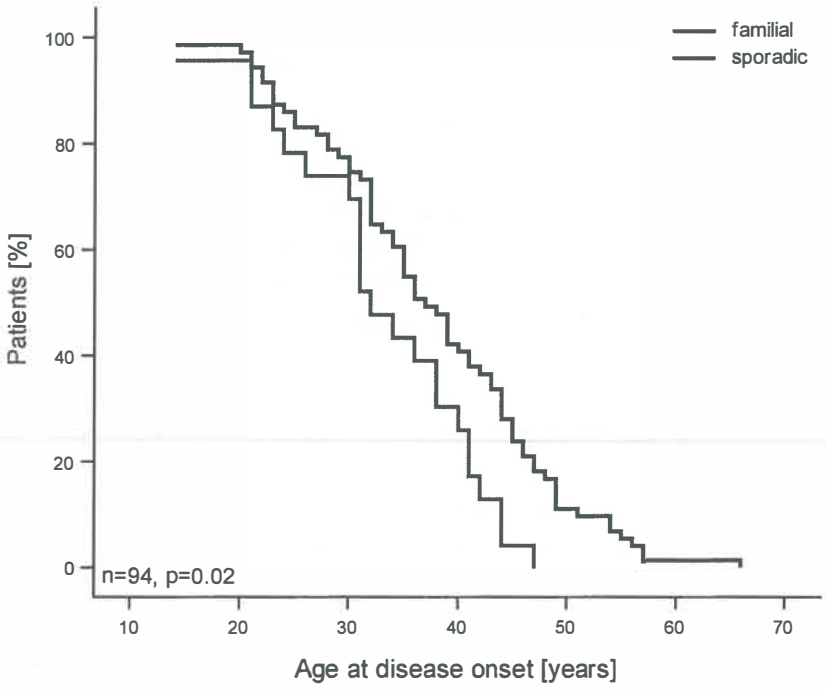
In PPMS patients, familial MS was associated with a significantly younger age at disease onset (mean 33.04 years in familial cases versus mean 37.73 years in non-familial cases). The Cox regression model showed a significant association of familial MS with a younger age at disease onset (hazard ratio familial versus non-familial cases: 1.78, 95% CI 1.09-2.92). A survival plot for familial MS and age at primary progression is shown in Figure 4.4.

Table 4.6: Potential factors influencing the age at disease onset in PPMS patients

	n	Mean age at disease onset (95% CI)	p†	Cox regression hazard ratio (95% CI)
All patients	94	36.59 (34.47-38.70)	—	—
Familial MS				
non-familial cases	71	37.73 (35.22-40.24)	0.02	1.0 (reference)
familial cases	23	33.04 (29.49-36.60)		1.78 (1.09-2.92)
Degree of relatedness				
non-familial cases	71	37.73 (35.22-40.24)	0.08	—
1st degree plus	2	38.00 (26.24-49.76)		
1st degree	8	31.38 (25.01-37.74)		
2nd or 3rd degree	13	33.31 (34.47-38.70)		
Gender				
women	60	36.25 (33.71-38.79)	0.58	1.0 (reference)
men	34	37.18 (33.38-40.97)		0.94 (0.61 - 1.44)

† log-rank-test

Figure 4.4: Influence of familial MS on the age at primary progression



Discussion

In this study, we found an earlier age at onset of PPMS in patients with a family history of MS, while there were no significant differences between familial and non-familial MS in any of the other investigated measures.

The endpoints age at primary or secondary progression have not been investigated in previous studies on familial MS. Ebers and coworkers evaluated the length of the relapsing-remitting phase in familial versus non-familial MS in their study of 1,044 patients, and found no significant differences. The proportion of patients with a family history of MS in our patient sample (26%) is comparable to that published by Ebers et al. (20%).[10]

A limitation of our study is the relatively small sample size, which precludes further analysis by degree of relatedness. The comparison of included versus not included patients revealed some imbalances: the shorter disease duration in included patients suggests that a detailed family history was more often recorded in new patients than in patients who had been attending the clinic for longer, and an exploratory analysis showed that significantly fewer of the included patients had been attending the clinic before 1985 (the beginning of database registration, data not shown). The difference in disease duration may also account for the difference in IMD usage, since IMD are preferably started early in the disease. The difference in age at disease onset is difficult to explain, but the absolute difference is small and likely not clinically relevant. The fact that disease subtypes and gender distribution were not different between included and not included patients, suggests that the study population was nevertheless representative of the overall patient population. In families with one or more affected family members, the familiarity with the disease may lead to a greater attention towards early symptoms, and this may bring further affected family members to medical attention earlier. This effect could be a confounder in our study and other studies on familial MS. Our findings need confirmation in other established patient cohorts.

The most apparent difference between PPMS and MS with a relapsing-remitting onset is a different gender distribution. The female to male ratio in our cohort was 1.7:1 in PPMS versus 2.5:1 in relapsing-remitting onset patients. This relative predominance of men in PPMS has been observed

earlier, and was even more pronounced in London, Ontario (female to male ratio 1.3:1)[13] and British Columbia (1.1:1).[14] The reason for this striking gender difference is unknown. The age at onset of PPMS in our cohort is slightly higher in men, but this difference was not statistically significant (p=0.58). In previous studies, male age at onset of PPMS was either slightly lower than in females,[13] or equal in both sexes.[14] Gender had no significant influence on the time to EDSS 6.0 in the British Columbia PPMS cohort.[14]

In keeping with previous studies,[6–8] the age at secondary and primary progression is strikingly similar in patients with non-familial MS. The age at secondary progression was significantly higher in men compared to women (p=0.03). In an earlier study on the timing of secondary progression including 228 SPMS patients, we also observed a higher age at secondary progression in men, although this difference was not statistically significant;[8] Tremlett and colleagues report similar ages at EDSS 6 for men and women in their cohort of 2,319 patients.[3]

In the last two decades, hereditary variants of several neurodegenerative diseases have been discovered. Parkinson's disease was traditionally regarded as a sporadic disease until the description of families with early-onset Parkinson's disease led to the discovery of mutations in the *alpha-synuclein* gene in 1997.[15] Targeted genetic research in patients with early-onset disease has subsequently led to the identification of a number of disease modifying genes in neurodegenerative diseases like the *parkin1* and *DJ-1* genes in familial Parkinson's disease, the *superoxide dysmutase 1* gene in familial amyotrophic lateral sclerosis, and of the *A-beta-precursor protein*, *preseneline1* and *preselenine2* genes in early-onset Alzheimer's disease (for review see Bertram and Tanzi[16]). An earlier disease onset is a characteristic of the familial variants of all of these neurodegenerative diseases.

There is currently much interest in differences in pathology and pathophysiology between the subtypes of MS. One pathologic study showed less inflammation in autopsy cases of PPMS patients compared to SPMS patients,[17] and magnetic resonance spectroscopy studies report significantly lower levels of N-Acetyl-Aspartate, a marker of neuronal integrity, in patients with PPMS compared to healthy control persons.[18] These findings have given rise to the suspicion that PPMS may be primarily a neurode-

generative disease. Our main finding that primary progression occurs at a younger age among patients with familial MS may reflect a genetic influence on the susceptibility to axonal degeneration in these patients.

If our findings can be confirmed in other patient cohorts, primary progressive patients with a family history of MS would be an important patient group for future genetic research.

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Chapter 5

Fatigue, depression and progression in multiple sclerosis

Marcus Koch, Maarten Uyttenboogaart, Annemarie van Harten, Marco Heerings, Jacques De Keyser

under peer review

Abstract

Objective: To investigate the effect of fatigue and depression on disease progression in multiple sclerosis (MS), and the long-term prognosis of these symptoms.

Methods: 228 patients with MS were investigated for fatigue and depression with the Fatigue Severity Scale (FSS) and Center for Epidemiologic Studies Depression Scale (CES-D). These patients regularly attended our MS clinic, where disability scores and the development of secondary progression were monitored. After ten years, the 149 patients remaining from the original cohort were asked to participate in a repeat assessment of fatigue and depression and 96 (64%) could be reevaluated. In relapsing-remitting patients, the influence of baseline fatigue and depression on the risk of secondary progression during the following ten years was assessed with Kaplan-Meier survival analyses. In the whole patient group, we investigated the influence of baseline fatigue and depression on progression of disability at ten years. We also investigated differences in fatigue, depression and disability scores between baseline and ten years.

Results: Fatigue and depression at baseline did not predict the development of secondary progression or progression of disability. Most patients who were fatigued or depressed at baseline remained so at ten years (74% and 67%), and the majority of patients not experiencing these symptoms remained free of them (59% and 61%). FSS and CES-D scores were not significantly different between baseline and ten years, while disability scores were significantly increased.

Conclusion: Our data suggest that fatigue and depression in MS are unrelated to disease progression in MS. Fatigue and depression tend to persist at roughly the same levels over time.

Introduction

The disease course of Multiple Sclerosis (MS) depends on two main pathophysiological processes. Focal inflammatory demyelination in the central nervous system (CNS) results in relapses, which dominate the clinical picture in the early stages of the disease. Diffuse axonal degeneration, the other process, appears to be responsible for chronic progression in MS. Axonal degeneration may start early in the disease course and remain clinically silent for many years, because the large functional reserve of the CNS counterbalances the loss of neurons. Once this functional reserve is exhausted, axonal degeneration becomes clinically apparent as chronic progression.[1]

Fatigue and depression are common complaints in patients with MS.[2;3] Although these symptoms have an important impact on the patients' lifes, very little is known about their underlying pathophysiology and long-term prognosis.

A number of studies explored the possible link between fatigue and depression and chronic progression in MS: patients with a progressive disease course had higher fatigue[4] and depression[5] scores in cross-sectional studies, and several imaging studies have suggested an association between fatigue and depression with measures of brain atrophy and axonal loss.[6;7]

The aim of this study was to investigate the effect of fatigue and depression on disease progression in MS, and the long term prognosis of these symptoms. We examined a group of patients who had been assessed for fatigue and depression ten years earlier. The patients attended our outpatient department, where levels of disability and the development of secondary progression are monitored. The remaining patients from the intial cohort were asked to participate in another assessment of fatigue and depression after ten years.

Patients and methods

Patients

A group of patients with a diagnosis of definite MS according to the Poser criteria[8] completed questionnaires on fatigue and depression in 1996. The patients were attending our MS outpatient clinic, where follow-up appointments are scheduled at least once a year and demographic factors, age at disease onset, use of immunomodulatory treatments, disability scores and the development of a progressive disease course are regularly assessed and recorded in an electronic database. Secondary progression was defined as the continuous worsening of neurologic symptoms unrelated to relapse for at least one year.[9] In 2006 the patients were reevaluated for fatigue and depression.

Instruments

Fatigue was measured with the Fatigue Severity Scale (FSS), a nine-item scale scored from 1.0 (least fatigued) to 7.0 (most fatigued).[10] Based on previous studies,[10;11] patients were divided into a group with fatigue (FSS of 5.0 or more) and without fatigue (FSS less than 5.0).

Depression was measured with the Center for Epidemiological Studies Depression Scale (CES-D), a twenty-item depression inventory scored from 0 (least depressed) to 60 (most depressed).[12] A score of 16 or more was considered to denote depression, according to previous recommendations.[5;12;13]

Disability was measured with the Expanded Disability Status Scale (EDSS).[14] All EDSS scores were recorded during a clinically stable phase. We computed MS Severity Scores (MSSS) from disease duration and EDSS scores. The MSSS relates an individual patient's disease duration and EDSS score to a very large patient population and gives an indication of the speed of disability accumulation. Values above 5.0 indicate a greater than average speed of disability accumulation.[15]

Statistical analyses

Risk of secondary progression

The risk of secondary progression was analysed in the group of patients with a relapsing-remitting disease course in 1996.

All patients with relapsing-remitting MS at baseline were followed up for ten years or censored at the time of loss to follow-up or the time of death. The time of onset of secondary progression was recorded during this period.

The analysis was done in two steps. In the first step we examined the data of all patients with a complete follow-up of ten years. Differences between patients developing secondary progression and stable relapsing-remitting patients were investigated with Fisher's exact test or the Mann-Whitney U test where appropriate.

In the second step, fatigue and depression scores and the variables with significant differences in the first analysis were dichotomised and differences in the time to secondary progression were assessed with Kaplan-Meier survival analyses. For dichotomisation, we chose cut-off scores of 3.0 or more (denoting significant disability) on the EDSS, and more than 5.0 (denoting greater than average speed of disability accumulation) for the MSSS.

Progression of disability

Progression of disability was investigated in the group of patients who were followed until ten years.

Progression of disability at ten years was defined as worsening of the EDSS score by at least one point in patients with a baseline score of less than 6.0 and by at least one half point in patients with a baseline score of 6.0 or higher. This definition was taken from two randomised controlled trials on interferon beta treatment in secondary progressive MS.[16;17]

Patients were dichotomised by fatigue and depression scores at baseline, and by baseline EDSS and MSSS score as described above. Group differ-

ences were investigated with Fisher's exact test.

Change in fatigue and depression status

Changes in fatigue and depression status between baseline and ten years were assessed with McNemar's test for paired dichotomous data.

Change in fatigue, depression and disability scores

Differences in FSS, CES-D and EDSS scores between baseline and ten years were investigated with Wilcoxon's paired samples test. The correlation between changes in FSS, CES-D and EDSS scores was assessed with Spearman's rank correlation analysis.

Statistical significance was taken to be at the two-tailed 0.05 level. All statistical analyses were performed with the SPSS statistical software package version 14.

Results

Patient sample

The baseline cohort comprised 228 patients: 76 with RRMS, 79 with secondary progressive MS (SPMS), and 73 with primary progressive MS (PPMS). After ten years, 21 patients had died and 58 were lost to follow-up. The characteristics of the initial cohort, of the lost patients and of the patients followed up until ten years are shown in Table 5.1.

Table 5.1: Characteristics of the initial cohort and of patients lost and followed until ten years.

	Patients at baseline	Patients followed until ten years	Patients not followed until ten years	p
n	228	149	79	—
Gender (n, %)				
Men	62 (27%)	38 (26%)	24 (30%)	0.44*
Women	166 (73%)	111 (74%)	55 (70%)	
Age at disease onset (median, IQR)	31, 24-39	31, 24-39	30, 23-41	0.62‡
Characteristics at baseline:				
Disease course				
RRMS (n, %)	76 (33%)	66 (44%)	10 (13%)	
SPMS (n, %)	79 (35%)	49 (33%)	30 (38%)	<0.0005†
PPMS (n, %)	73 (32%)	34 (23%)	39 (49%)	
Disease duration (median, IQR)	12, 7-19	11, 6-16	13, 9-23	0.002‡
Use of IMD (n, %)	10 (4%)	9 (6%)	1 (1%)	0.09*
EDSS (median, IQR)	6.0, 2.5-7.0	4.0, 2.0-6.5	6.5, 6.0-8.0	<0.0005‡
FSS (mean, SD)	5.39, 1.28	5.37, 1.17	5.42, 1.48	0.23‡
CES-D (median, IQR)	16, 13-21	16, 13-21	16, 12.25-21.75	0.83‡

*: Fisher's exact test, †: Pearson's chi square test, ‡: Mann-Whitney U test
IQR: interquartile range, SD: standard deviation
IMD: immunomodulatory drugs

The remaining 149 patients were asked to complete questionnaires on fatigue and depression. Ninety-nine patients replied to this request. Three of these 99 patients had become so disabled that they were unable to complete the questionnaires, leaving 96 patients for the analyses. The characteristics of responders and non-responders at ten-years are listed in Table 5.2.

At the baseline assessment, four patients did not complete the CES-D; at the ten-years assessment, one patient did not complete the FSS, and another patient did not complete the CES-D. No patient was experiencing a relapse at the time of the first or second assessment.

Table 5.2: Characteristics of the cohort at ten years

	Patients at ten years	Patients reassessed at ten years	Patients not reassessed at ten years	p
n	149	96	53	—
Gender (n, %)				
Men	38 (26%)	27 (28%)	11 (21%)	0.43*
Women	111 (74%)	69 (72%)	42 (79%)	
Age at disease onset (median, IQR)	31, 24-39	32, 26-41	27, 23-36	0.03‡
Characteristics at ten years:				
Disease course				
RRMS (n, %)	52 (35%)	32 (33%)	20 (38%)	
SPMS (n, %)	63 (42%)	38 (40%)	25 (47%)	0.25†
PPMS (n, %)	34 (23%)	26 (27%)	8 (15%)	
Disease duration (median, IQR)	21, 16-26	21, 16.25-26	19, 15-26	0.36‡
Use of IMD (n, %)	23 (15%)	16 (17%)	7 (13%)	0.64*
EDSS (median, IQR)	6.0, 3.0-7.0	6.25, 3.125-7.0	6.0, 3.0-7.5	0.97‡

*: Fisher's exact test, †: Pearson's chi square test, ‡: Mann-Whitney U test
IQR: interquartile range, SD: standard deviation
IMD: immunomodulatory drugs

Risk of secondary progression

Of the 76 relapsing-remitting patients, 18 developed secondary progression, and 5 were censored when lost to follow-up. Patients developing secondary progression had significantly higher baseline EDSS and MSSS scores, and there were significantly more men among this group, while there were no differences in FSS and CES-D scores (Table 5.3).

Table 5.3: Group of patients with a relapsing-remitting disease course at baseline: Characteristics of all patients and group differences between patients developing and not developing secondary progression

Baseline characteristics	All RRMS*	Patients developing SPMS	Patients remaining relapsing-remitting	p
n	71	18	53	—
Men (n, %n)	14 (8%)	7 (45%)	7 (13%)	0.035†
Age (median, IQR)	39, 34-45	36.5, 34.5-44.5	40, 33-45.5	0.64‡
Age at disease onset (median, IQR)	27, 23-35	28, 23.75-33	27, 23.35	0.75†
Disease duration (median, IQR)	7, 4-11	6.5, 4-9	8, 4-14.5	0.45‡
Use of IMD (n, %)	8 (11%)	3 (17%)	5 (9%)	0.41†
EDSS (median, IQR)	2, 1-2.5	3.25, 2-4	1.5, 1-2	<0.0005‡
MSSS (mean, SD)	2.96, 2.36	4.77, 2.58	2.36, 1.96	0.001‡
FSS (mean, SD)	5.12, 1.38	5.35, 1.06	5.22, 1.35	0.91‡
CES-D (median, IQR)	16, 14-21	16, 13.75-20.5	16, 13.5-21.5	0.91‡

*: excluding five patients lost to follow-up
†: Fisher's exact test, ‡: Mann-Whitney U test
IQR: interquartile range, SD: standard deviation
IMD: immunomodulatory drugs

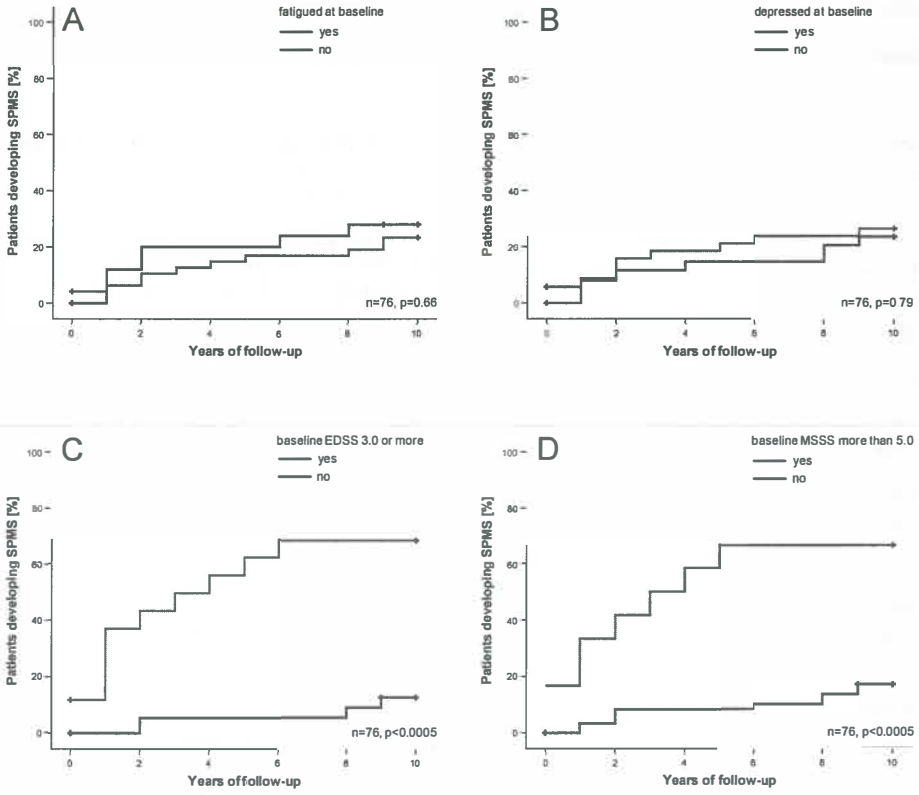
The results of the Kaplan-Meier survival analyses are given in Table 5.4. Men, patients with a baseline EDSS of 3.0 or more and patients with a baseline MSSS of more than 5.0 had a significantly faster onset of secondary progression, whereas fatigue and depression had no significant influence on the time to secondary progression. Survival plots for fatigue and depression status at baseline and for the dichotomised EDSS and MSSS scores are shown in Figure 5.1.

Table 5.4: Group of patients with a relapsing-remitting disease course at baseline: Kaplan-Meier analyses of factors affecting the risk of secondary progression

Factor	n	Patients developing SPMS	Mean time to progression (95% CI)	p*
All patients	76	18	8.40 (7.67-9.13)	—
Fatigued at baseline:				
yes	48	11	8.58 (7.72-9.44)	0.66
no	28	7	8.04 (6.69-9.39)	
Depressed at baseline:				
yes	40	10	8.21 (7.17-9.25)	0.79
no	35	8	8.60 (7.58-9.63)	
Gender:				
women	61	11	8.72 (7.99-9.45)	0.019
men	15	7	7.13 (5.08-9.19)	
Baseline EDSS 3.0 or more:				
yes	17	11	4.66 (2.73-6.60)	<0.0005
no	59	7	9.46 (8.99-9.42)	
Baseline MSSS more than 5.0:				
yes	12	8	4.67 (2.39-6.95)	<0.0005
no	64	10	9.13 (8.53-9.73)	

*: log-rank test, CI: confidence interval

Figure 5.1: Group of patients with a relapsing-remitting disease course at baseline: Influence of fatigue (A) and depresssion (B) status at baseline and dichotomised EDSS (C) and MSSS (D) scores at baseline on the risk of secondary progression.



Progression of disability

Significantly more patients with a baseline EDSS of 3.0 or more (p=0.002) and a baseline MSSS of more than 5.0 (p=0.01) experienced progression of disability over ten years. No significant differences were found for gender, fatigue and depression at baseline (Table 5.5).

Table 5.5: Group of patients followed until ten years: Factors affecting progression of disability at ten years

Factor	n	progression of disability (n)	no progression of disability (n)	p*
All patients	149	96	53	—
Fatigued at baseline				
yes	104	66	38	0.85
no	45	30	15	
Depressed at baseline				
yes	79	46	33	0.12
no	69	49	20	
Gender				
women	111	68	43	0.24
men	38	28	10	
Baseline EDSS 3.0 or more				
yes	95	70	25	0.002
no	54	26	28	
Baseline MSSS more than 5.0				
yes	78	58	20	0.01
no	71	38	33	

*: Fisher's exact test

Change in fatigue and depression status

Of the 68 patients fatigued at baseline, 50 (74%) remained fatigued at ten years. Of the 27 patients not fatigued at baseline, 16 (59%) remained free of fatigue at follow-up (McNemar test p=0.44).

Of the 45 patients depressed at baseline, 30 (67%) remained depressed at ten years. Of the 49 patients not depressed at baseline, 30 (61%) remained not depressed at follow-up (McNemar test p=0.61).

The combination of fatigue and depression was found in 36 of 95 (38%) patients at baseline and in 40 of 93 (43%) at ten years.

Change in fatigue, depression and disablity scores

There were no differences between baseline and ten-year FSS (n=95, baseline [mean, SD:] 5.33, 1.22; ten years: 5.23, 1.29; Wilcoxon p=0.47) and CES-D scores (n=94, baseline [median, IQR]: 15, 12-20; ten years 16, 12-20.25; Wilcoxon p=0.69). EDSS scores were higher at ten years (n=96, baseline [median, IQR]: 4.25, 2.0-6.5; ten years: 6.25, 3.13-7.0; Wilcoxon p<0.0005).

There were no correlations between the change in EDSS score and the change in either FSS (n=95, rho=-0.09, p=0.41) or CES-D scores (n=94, rho=0.1, p=0.35). There was a weak positive correlation between change in FSS and change in CES-D scores (n=93, rho=0.22, p=0.04).

Discussion

Our data show that fatigue and depression in MS persist at roughly the same levels over a long time. Fatigue and depression appear unrelated to progression in MS.

We investigated the influence of fatigue and depression on disease progression in two ways. On the one hand, we investigated progression in the sense of developing a secondary progressive disease course, and found no association between baseline fatigue and depression and the risk of secondary progression. Male gender, higher baseline disability and a higher score on the recently introduced MSSS scale were associated with a greater risk of secondary progression. Male gender has previously been associated with a higher risk of secondary progression.[18]

Our assessment of progression in the sense of worsening disability revealed no important influence of fatigue and depression either. Patients who were fatigued or depressed at baseline were no more likely to develop progression of disability than non-fatigued and non-depressed patients, whereas higher baseline EDSS and MSSS scores were associated with progression of disablity.

Our investigation of the changes in fatigue and depression over ten years showed that these symptoms tend to persist in the long term. Most patients suffering from fatigue or depression at baseline continued to experience these symptoms in the long term and vice versa. About 40% of patients at baseline and at ten years suffered from a combination of fatigue and depression, and there was a positive correlation between the change in fatigue and depression scores.

These findings are in keeping with a shorter longitudinal study in which FSS scores and scores on the Modified Fatigue Impact Scale and Beck's Depression Inventory did not significantly change over a period of 18 months.[19] In this previous study, the change in depression scores was also correlated with the change in fatigue scores. This correlation as well as the fact that fatigue and depression are commonly combined may suggest that they share common pathophysiological mechanisms.

Previous studies have shown that fatigue is a very common symptom in MS, with a prevalence of more than 80%.[20] The rate of depression in MS is typically reported to be around 50%, which is much higher than in other chronic diseases.[3;21] The pathophysiology of both symptoms is unknown. While most authors suspect a central mechanism in the pathophysiology of fatigue,[11] others suggest a peripheral cause, e.g. circulating cytokines.[22] The cause of depression in MS is equally uncertain: some view depression as reactive to the physical disability and psychosocial stress inherent to MS, while others believe that the depressive disorder is the direct result of biochemical changes in the brain caused by the disease process.

The suggestion that fatigue and depression may be caused by axonal degeneration stems from cross-sectional imaging studies. One cross sectional magnetic resonance spectroscopy study found an inverse correlation between FSS scores and the NAA/Cr ratio (a marker of neuronal integrity) in a group of 73 patients, but this correlation was weak (Spearman's rho=-0.36, p=0.02) and its relevance uncertain.[7]

Another cross-sectional study including 48 patients reports an association of depression scores and brain atrophy, most evident in the frontal lobes.[6] Preclinical and clinical observations suggest that the neurochemical changes associated with depression may lead to neuronal loss, especially in the prefrontal and hippocampal cortex.[23] It is therefore uncertain whether the atrophy observed in depressed MS-patients is due to the axonal degeneration of MS or due to the depression itself.

Strengths of our study are the lenght of follow-up, and the fact that patients were regularly reexamined at our outpatient department, which enabled us to monitor the beginning of secondary progression. A limitation of our study is the relatively large number of patients lost to follow-up in the reassessment for depression and fatigue at ten years.

The lacking influence of baseline fatigue and depression on progression in our study, and the fact that the mean FSS and CES-D scores remained unchanged between baseline and ten years (while EDSS scores significantly increased), suggest that mechanisms other than progressive axonal degeneration underlie fatigue and depression in MS.

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Chapter 6

Cigarette smoking and progression in multiple sclerosis

Marcus Koch, Annemarie van Harten, Maarten Uyttenboogaart, Jacques De Keyser

Neurology 2007;**69**:1515-1520.

Abstract

Objective: To investigate the influence of cigarette smoking on progression and disability accumulation in MS.

Methods: Information on past and present smoking of 364 patients with MS was obtained through a structured questionnaire survey. We used Kaplan-Meier analyses and Cox regression models to evaluate the influence of smoking on the development and age at onset of secondary progression, on the age at onset of progression in patients with primary progressive MS, and on the time from disease onset to Expanded Disability Status Scale (EDSS) scores 4.0 and 6.0 in all patients. We also investigated the correlation between smoked pack-years and EDSS scores and the rate of progression as measured with the Multiple Sclerosis Severity Score (MSSS).

Results: We found no significant associations between cigarette smoking and any of the used measures.

Conclusion: Our data suggest that cigarette smoking has no influence on disease progression or accumulation of disability in MS.

Introduction

The disease course in multiple sclerosis (MS) is highly variable: while some patients remain free of exacerbations and disability for a very long time after a first episode, others experience unremitting worsening of symptoms from disease onset and quickly deteriorate.

Most patients with MS experience relapses with at least partial remissions in the years following disease onset (relapsing-remitting MS, RRMS), and later convert to a secondary progressive disease course (secondary progressive MS, SPMS). After conversion to SPMS, the worsening of symptoms is steady, relentless and more uniform in the patient population.[1] A minority of patients have a progressive disease course from disease onset (primary progressive MS, PPMS), while another subgroup of patients has little or no disability despite a long disease duration and are considered to have benign MS (BMS).

It appears that the difference between the relapsing-remitting and progressive disease course are expressions of distinct pathophysiologies. Diffuse axonal degeneration is believed to underlie the progressive disease course,[2] whereas relapses appear to result from focal inflammation.

Genetic and environmental factors influencing the incidence of MS have been studied in great detail, and a number of epidemiological studies found an increased incidence of MS among smokers.[3;4] Much less attention has been paid to factors influencing disease progression in MS, but cigarette smoking has also been suspected to increase the risk of developing secondary progression of MS.[5]

In this study, we investigated the influence of cigarette smoking on several aspects of progression and disability accumulation in MS.

Patients and methods

The Groningen MS Database

The Groningen MS database contains data on disease course and disability accumulation of 672 MS patients. Data have been collected prospectively since 1985 by means of 3- to 12-monthly follow-up at the MS clinic of the University Medical Center Groningen. The data collected include gender, age, disease subtype, time of disease onset, time of secondary progression, Expanded Disability Status Scale (EDSS) scores and use of immunomodulating drugs (IMD). All patients were diagnosed with definite MS according to the Poser criteria.[6]

Patient inclusion

Of the 672 patients in the database, 134 had died since the start of data collection and 42 patients had been lost to follow-up. 497 patients were available for questioning and received a structured questionnaire on present and past cigarette smoking. 337 patients returned the questionnaire and the smoking history of 32 additional patients could be obtained by telephone interview. Three cigar smokers and two pipe smokers were excluded, leaving a study population of 364 patients.

Smoking history

The smoking questionnaire contained questions on current smoking status, starting and quitting dates, intervening non-smoking periods and number of cigarettes smoked. With this information, the number of smoked pack-years until participation in this study, before onset of MS and after onset of MS was calculated for every patient. A pack-year was defined as 20 cigarettes smoked per day for 1 year. The average number of cigarettes smoked per day was calculated up to the time of progression, and up to the time of participation in this study. Patients were divided into three

groups according to the average number of cigarettes smoked per day: none, 1 to 10, and more than 10 cigarettes per day.

Patients were labeled as 'non-smokers' if they had never smoked in their lives or smoked only incidentally (less than one cigarette per week and less than one half pack-year in their entire lives).

Data used in this study

Gender, disease course, age at disease onset, age at secondary progression, IMD usage and EDSS scores were taken from the patient database. Patients with a missing 2006 EDSS score were contacted and the EDSS score was determined by telephone interview.

BMS was defined as an EDSS-score of no more than 3.0 despite at least 10 years disease duration.[7] A progressive disease course was defined as the continuous unremitting worsening of neurological impairment unrelated to relapses over a period of at least one year.[8]

We determined Multiple Sclerosis Severity Scores (MSSS) from EDSS scores and disease duration as described by Roxburgh and colleagues.[9]

Statistical analyses

Included and not included patients

The distribution of the quantitative variables was assessed with the Kolmogorov-Smirnov test. Differences between included and not included patients were investigated with Fisher's exact test, Pearson's chi square test and the Mann-Whitney U test where appropriate.

Smokers and non-smokers

Gender, disease subtype, IMD usage, age at disease onset, disease duration, EDSS scores and MSSS were compared between smokers and non-smokers (smoking status at the time of participation in this study) with Fisher's exact test, Pearson's chi square test, and the Mann-Whitney U test where appropriate.

Regression analyses

Since smoking is more prevalent among men,[10] gender was used as a mandatory covariate in all analyses, and the interaction of gender and smoking status was evaluated in all regression models.

The number of smoked pack-years is the best quantitative estimate of cigarette smoking, but since this variable is strongly influenced by the time a person has smoked, we chose not to use it in the survival and regression analyses; the average number of cigarettes smoked per day was used instead.

Smoking and the risk of secondary progression

For this analysis, the length of the relapsing-remitting phase was determined in all patients with a relapsing-remitting onset (BMS, RRMS and SPMS patients). Patients were followed from disease onset until onset of secondary progression or censored at the time of participation in this study. Group differences in the length of the relapsing-remitting phase were assessed with Kaplan-Meier survival analyses. We estimated the hazard ratio for conversion to secondary progression with a Cox regression model including gender, smoking status and age at disease onset as covariates.

Smoking and the age at progression

The analyses on the age at progression were performed for SPMS and PPMS separately. The influence of gender, smoking status (at onset of progression), number of cigarettes smoked per day and age at disease onset on the age at progression was assessed using Kaplan-Meier survival analyses and Cox regression analysis. Gender and smoking status were used in the regression analysis. In SPMS patients, age at disease onset was not included in the regression model, since age at progression must necessarily be greater than age at disease onset in every case.

Smoking and disability accumulation

The influence of gender, smoking status, number of cigarettes smoked per day and age at disease onset on the time to reach EDSS 4.0 and 6.0 was investigated with Kaplan-Meier survival analyses and Cox regression analysis.

In addition to these survival analyses, the correlation of smoked pack-years (total, before onset of MS and after onset of MS) and EDSS and MSSS scores at the time of participation in the study was investigated in the whole patient sample and for men and women separately with Spearman's nonparametric correlation analysis.

Significance was taken to be at the two-tailed 0.05 level. All statistical analyses were performed with the SPSS statistical software package version 14.

Results

Characteristics of included and not included patients

Characteristics of included and not included patients are listed in Table 6.1. Included patients had a significantly later disease onset and a higher age at progression, whereas EDSS and MSSS scores were no different between the two groups.

Table 6.1: Comparison of baseline characteristics between included and not included patients

	all patients	included	not included	p
n	497	364	133	—
Gender:				
Women	342	247	95	0.54*
Men	155	117	38	
Disease course:				
BMS	103	75	28	
RRMS	108	79	29	0.63†
SPMS	166	117	49	
PPMS	120	93	27	
Disease duration (median, IQR)	17, 12-26	17, 11.25-26	16, 12-24	0.59‡
Age at disease onset (median, IQR)	31, 25-40	32, 25-41	29, 24-37	0.04‡
Age at progression$ (median, IQR)	39, 32-47	41, 33.75-47	37.5, 31-44.75	0.026‡
Use of IMD (n)	108	82	26	0.54*
EDSS (median, IQR)	6.0, 2.5 - 7.0	6.0, 2.5 - 7.0	5.0, 2.5 - 7.0	0.98‡
MSSS (mean, SD)	5.21, 3.14	5.21, 3.14	5.22, 3.14	0.93‡

$: in 286 patients with SPMS or PPMS
*: Fisher's exact test, †: Pearson's chi-square-test, ‡: Mann-Whitney U test
IQR: interquartile range, SD: standard deviation

Characteristics of smokers and non-smokers

A comparison of characteristics between smokers and non-smokers is given in Table 6.2. There were more men among the group of smokers (p=0.002), but no significant differences in any other variable.

Table 6.2: Comparison of baseline characteristics between smokers and non-smokers

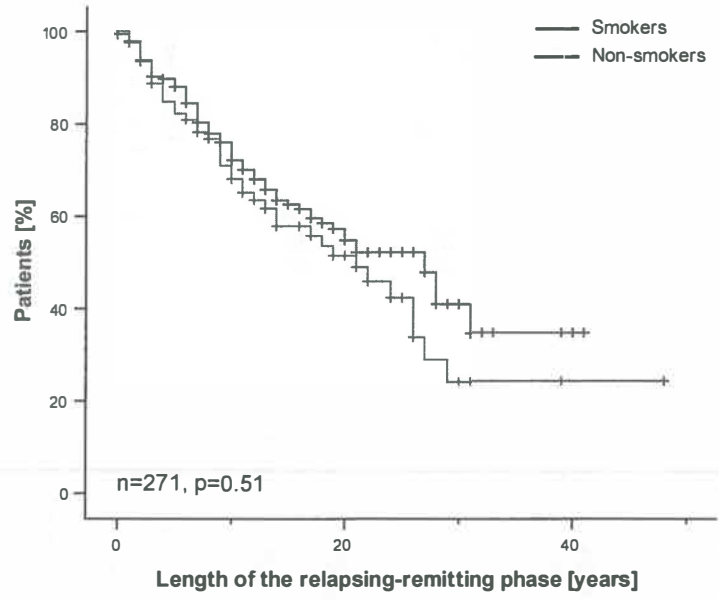
	Non-smokers	Smokers	p
n	101	263	—
Gender:			
Women	81	166	0.002*
Men	20	97	
Disease course:			
BMS	24	51	
RRMS	18	61	0.35†
SPMS	37	80	
PPMS	22	71	
Disease duration (median, IQR)	19, 11-27	17, 12-25	0.57‡
Age at disease onset (median, IQR)	31, 24.5-40	32, 25-41	0.56‡
Use of IMD (n)	24	58	0.78*
EDSS (median, IQR)	6.0, 2.5-6.5	6.0, 2.5-7.5	0.32‡
MSSS (mean, SD)	5.5, 3.34	5.09, 3.05	0.21‡

*: Fisher's exact test, †: Pearson's chi-square-test
‡: Mann-Whitney U test
IQR: interquartile range, SD: standard deviation

Smoking and the risk of secondary progression

The results of the survival analyses and Cox-regression model are listed in Table 6.3. There were no significant differences in length of the relapsing-remitting phase for any variable. Survival plots for smokers vs. non-smokers and for the number of cigarettes smoked per day are shown in Figure 6.1.

Figure 6.1: Influence of smoking on risk of secondary progression in patients with a relapsing-remitting disease onset



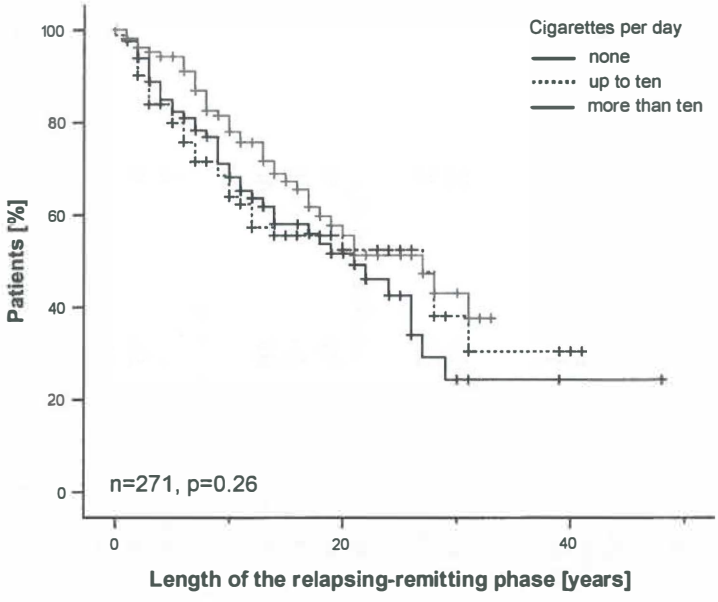


Table 6.3: Potential factors influencing the risk of secondary progression in all patients with relapsing-remitting disease onset

	n	Patients developing secondary progression	Mean length of the relapsing-remitting phase (95% CI)	p*	Cox regression hazard ratio (95% CI)†
All patients	271	117	25.42 (22.64-29.19)	—	—
Gender:					
Women	192	79	26.00 (22.56-29.44)	0.45	1.0 (reference)
Men	79	38	21.27 (18.06-25.45)		1.15 (.78-1.71)
Smoking status:					
Non-smokers	79	37	23.54 (18.73-28.35)	0.51	1.0 (reference)
Smokers	192	80	23.73 (21.11-26.35)		.89 (.60-1.32)
Number of cigarettes smoked per day:					
None	79	37	23.54 (18.73-28.35)		
Up to ten	86	40	21.98 (18.03-25.93)	0.26	—
More than ten	106	40	22.16 (19.69-24.62)		
Age at disease onset, years:					
Younger than 25	77	36	27.61 (23.3-31.92)		1.0 (reference)
25-29	55	26	19.44 (16.07-22.8)	0.13	1.35 (.81 - 2.25)
30-34	48	16	21.85 (18.28-25.43)		.95 (.53 - 1.73)
35 and older	91	39	18.68 (22.64-28.19)		1.58 (.99 - 2.52)

*: log-rank test, †: Interaction term (gender/smoking statuts) p=0.2

Smoking and age at progression

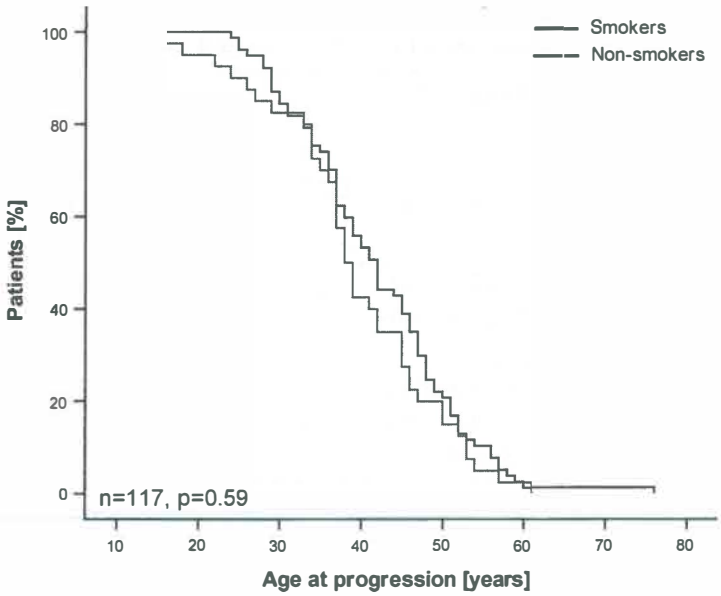
The results of the survival and regression analyses are listed in Tables 6.4 and 6.5. In PPMS patients, none of the investigated variables had a significant effect on the age at progression. In SPMS patients, age at progression was significantly higher with increasing age of disease onset. Survival plots for smokers vs. non-smokers and for the number of cigarettes smoked per day are shown in Figures 6.2 and 6.3.

Table 6.4: Potential risk factors influencing the age at secondary progression.

	n	Mean age at progression (95% CI)	p*	Cox regression hazard ratio (95% CI)†
All patients	117	41.09 (39.25-42.94)	—	—
Gender:				
Women	79	40.22 (38.06-42.37)	0.08	1.0 (reference)
Men	38	42.92 (39.47-46.37)		.72 (.48 - 1.08)
Smoking status:				
Non-smokers	37	40.35 (37.29-43.42)	0.59	1.0 (reference)
Smokers	80	41.44 (39.14-43.74)		.97 (.65 - 1.46)
Number of cigarettes smoked per day:				
None	37	40.35 (37.29-43.42)		
Up to ten	40	40.50 (37.21-44.00)	0.74	—
More than ten	40	42.37 (39.15-45.60)		
Age at disease onset, years:				
Younger than 25	36	34.47 (31.39-37.56)		
25-29	26	38.77 (35.76-41.78)	<0.0005	—
30-34	16	39.88 (36.08-43.67)		
35 and older	39	49.26 (46.92-51.60)		

*: log-rank test, :†Interaction term (gender/smoking status) p=0.71

Figure 6.2: Influence of smoking on age at secondary progression.



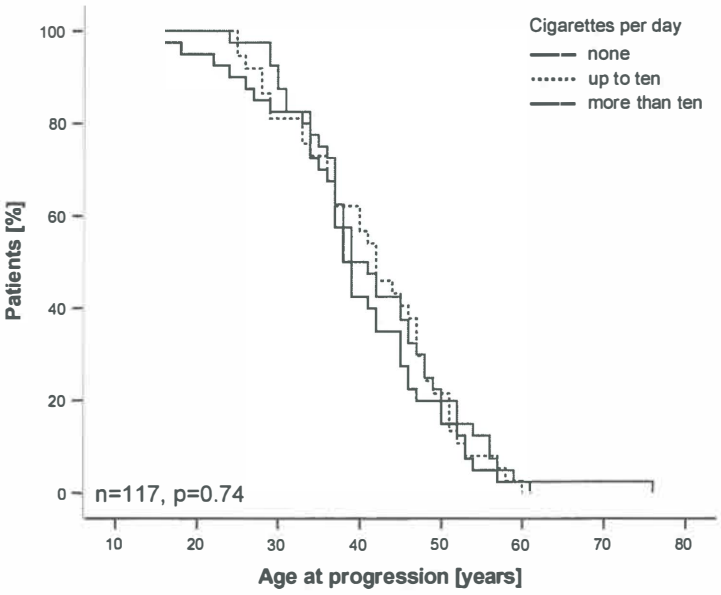
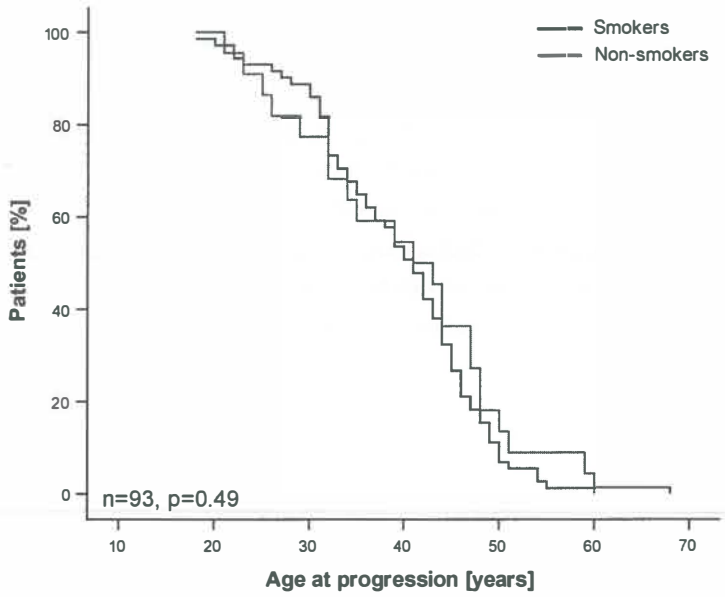


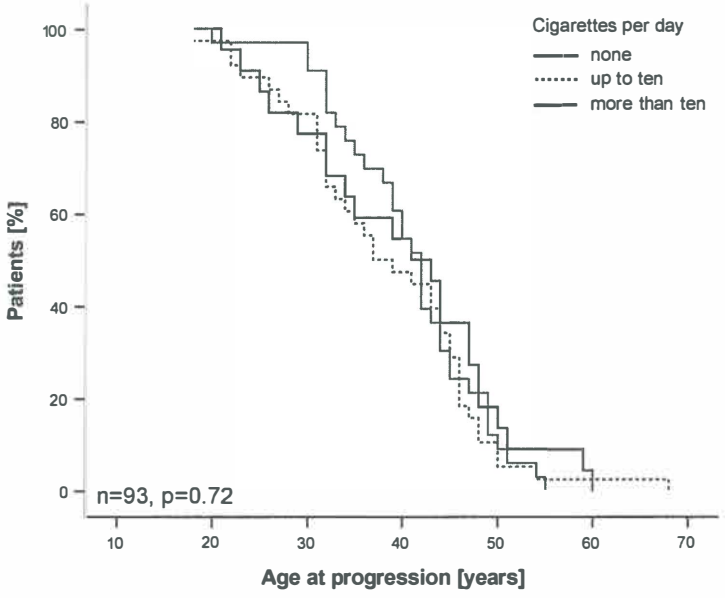
Table 6.5: Potential risk factors influencing the age at primary progression.

	n	Mean age at progression (95% CI)	p*	Cox regression hazard ratio (95% CI)†
All patients	93	39.59 (37.61-41.57)	—	—
Gender:				
Women	55	39.98 (37.18-42.78)	0.28	1.0 (reference)
Men	38	39.03 (36.37-41.68)		1.21 (.4-3.62)
Smoking status:				
Non-smokers	22	39.91 (35.23-44.59)	0.49	1.0 (reference)
Smokers	71	39.49 (37.33-41.66)		1.11 (.63 - 1.97)
Number of cigarettes smoked per day:				
None	22	39.91 (35.23-44.59)		
Up to ten	38	38.42 (35.11-41.73)	0.72	—
More than ten	33	40.73 (38.06-43.39)		

*: log-rank test, †: Interaction term (gender/smoking status) p=0.98

Figure 6.3: Influence of smoking on age at primary progression.





Smoking and disability accumulation

The time to EDSS 4.0 was unavailable in 28 patients (7.7%), and time to EDSS 6.0 was unavailable in 20 patients (5.5%). The results of the survival and regression analyses are listed in Tables 6.6 and 6.7. There were no significant differences in the time to EDSS scores 4.0 and 6.0 between smokers and non-smokers. Men reached EDSS 4.0 after a significantly shorter time than women. There was no significant influence of gender on the time to EDSS 6.0. The time to both EDSS 4.0 and 6.0 was significantly shorter with increasing age at disease onset. In the regression analyses, age at disease onset was the only variable associated with a shorter time to both EDSS 4.0 and 6.0.

Table 6.6: Potential factors influencing the time to EDSS 4.0

	n	Patients reaching EDSS 4.0	Mean time to EDSS 4.0 (95% CI)	p*	Cox regression hazard ratio (95% CI)†
All patients	336	214	15.37 (13.74-17.00)	—	—
Gender:					
Women	229	136	16.24 (14.25-18.22)	0.04	1.0 (reference)
Men	107	78	13.53 (10.89-16.17)		.88 (.46 - 1.7)
Smoking status:					
Non-smokers	93	60	16.02 (13.03-19.02)	0.69	1.0 (reference)
Smokers	243	154	15.20 (13.25-17.15)		.93 (.66 - 1.33)
Number of cigarettes smoked per day:					
None	93	60	16.02 (13.03-19.02)		
Up to ten	114	75	14.13 (11.34-16.93)	0.40	—
More than ten	129	79	15.46 (13.23-17.70)		
Age at disease onset, years:					
Younger than 25	68	37	21.21 (17.77-24.65)		1.0 (reference)
25-29	58	30	17.81 (14.55-21.07)	<0.0005	1.26 (.77 - 2.06)
30-34	60	35	15.78 (12.67-18.90)		1.55 (.96 - 2.48)
35 and older	150	112	9.92 (8.23-11.61)		1.61 (.77 - 3.34)

* log-rank test, †: Interaction term (gender/smoking statuts) p=0.21

Table 6.7: Potential factors influencing the time to EDSS 6.0

	n	Patients reaching EDSS 6.0	Mean time to EDSS 6.0 (95% CI)	p*	Cox regression hazard ratio (95% CI)†
All patients	344	173	21.94 (19.88-24.00)	—	—
Gender:					
Women	237	118	21.87 (19.42-24.31)	0.66	1.0 (reference)
Men	107	55	20.47 (17.50-23.45)		.74 (.32 - 1.75)
Smoking status:					
Non-smokers	96	51	21.43 (17.88-24.97)	0.72	1.0 (reference)
Smokers	248	122	21.04 (19.09-22.99)		.88 (.61 - 1.28)
Number of cigarettes smoked per day:					
None	96	51	21.42 (17.88-24.97)		
Up to ten	118	65	18.83 (16.16-21.49)	0.12	—
More than ten	130	57	21.64 (19.40-23.88)		
Age at disease onset, years:					
Younger than 25	75	27	31.03 (26.86-35.20)		1.0 (reference)
25-29	59	24	22.19 (19.10-25.28)	<0.0005	1.75 (.99 - 3.07)
30-34	61	26	20.93 (18.08-23.78)		1.95 (1.12 - 3.40)
35 and older	149	96	14.02 (12.43-15.61)		4.55 (2.88 - 7.18)

*: log-rank test, †: Interaction term (gender/smoking statuts) p=0.37

Total pack-years were not significantly correlated with EDSS or MSSS in the whole study population and male patients. In women (n=247), there was a weak correlation between total pack-years and MSSS (rho=-0.14, p=0.03) and EDSS (rho=-0.15, p=0.02).

Pack years smoked before the onset of MS were significantly correlated with EDSS in women (rho=-0.16, p=0.01). Pack years smoked after the onset of MS were significantly correlated with MSSS in the whole patient group (rho=-0.11, p=0.03) and in women (rho=-0.15, p=0.02).

Discussion

In this study, we found no influence of cigarette smoking on the development of the progressive phase, disability accumulation, or progression rate in patients with MS.

We minimised the influence of recollection bias by using smoking status rather than average number of cigarettes smoked per day in the regression analyses, and by using very broad categories for average number of cigarettes smoked per day. The fact that the study cohort is hospital-based rather than population-based may limit the generalisability of our findings. The characteristics of included and not included patients were not exactly equal. Although these differences were statistically significant, the absolute differences between included and not included patients were small and probably not clinically relevant. The fact that EDSS and MSSS scores were similar in both groups argues against important selection bias in our study population.

The fact that there were significantly more men in the group of smokers is not surprising, since smoking is more prevalent among men.[10] Because most previous studies on smoking in MS were performed in female-only populations, we took special care in correcting for the possible influence of gender in our analyses. We included gender as a mandatory covariate in all Cox regression models and analysed the correlation of pack years and EDSS and MSSS in the whole study population as well as separately in men and women.

We found no significant influence of smoking on the development of secondary progression. This finding is in conflict with the only previous study investigating this association, in which a hazard ratio of 3.6 (95% CI 1.3-9.9) was found in a similar analysis in 179 patients.[5] Differences in methodology and patient sample may account for this discrepancy. In the previous study, the time of secondary progression was determined from general practitioners' paper records, whereas we systematically assessed the patients' condition at their follow-up appointments. Only 20 of the 179 patients (5 of the 98 non-smokers and 15 of the 81 smokers) included in the previous study progressed to SPMS. We believe that the greater sample size (271 patients versus 179) and the smaller proportion of cen-

sored patients (56.8% versus 88.8%) make our estimate of the hazard ratio more accurate.

It is now well established that the age at onset of the progressive phase is very similar in SPMS and PPMS patients.[1;11;12] This striking similarity seems to confirm the earlier idea that diffuse axonal degeneration may be an ongoing process from an early phase of the disease which is largely independent of relapses and becomes clinically apparent when axonal degeneration reaches a threshold.[13;14] If smoking had an important influence on the pathophysiological mechanisms underlying progression in MS, one would expect smokers to reach that threshold earlier than non-smokers.

Our analyses failed to show a significant influence of cigarette smoking on the age at progression in SPMS or PPMS patients. To investigate a possible influence of smoking on disability accumulation in MS, we investigated the time from disease onset to EDSS 4.0 and 6.0 as well as the EDSS and MSSS scores at the time of participation. The MSSS score is currently the best method to estimate the speed of disability progression, it is determined by comparing an individual patient's disease duration and EDSS score to those of a very large patient database. Neither disability itself (as measured by the EDSS) nor the speed of disability accumulation (as measured with the MSSS and time to landmark disability scores) were significantly different between smokers and non-smokers.

The correlation analysis of total smoked pack-years and accumulating disability (EDSS) and progression rate (MSSS) yielded no significant results in the whole patient group, but in women, there was a significant correlation with both measures. Pack-years smoked after the onset of MS were correlated with MSSS in the whole patient group and in women. All of these correlations were statistically significant but the correlation coefficients were very weak. This could be an area of further investigation.

Our data suggest that cigarette smoking has no important influence on disease progression or the development of a progressive disease course in MS.

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Chapter 7

Timing of birth and disease progression in multiple sclerosis

Marcus Koch, Jaqcues De Keyser, Helen Tremlett

under peer review

Abstract

Background: The timing of birth has recently been associated with the risk of developing MS in later life. Whether the timing of birth also influences the disease course of MS is unknown.

Objective: To investigate if the season or month of birth influences the timing of secondary progression or the time to landmark disability outcomes in MS.

Methods: To allow confirmation of findings, all analyses were performed in duplicate in two large natural history cohorts from geographically distinct, but seasonally similar locations in Europe and North America. Kaplan-Meier survival analyses were used to investigate the influence of month and season of birth on (a) the time to and age at the development of secondary progression in patients with a relapsing disease onset, and (b) the time to reach an EDSS score of 6.0 in patients with primary progressive and relapsing MS.

Results: No association between the month or season of birth and disease progression could be found which was reproducible in both natural history cohorts. A seasonal trend was observed for the time to and age at secondary progression in Groningen, with March babies exhibiting a shorter time to and younger age at secondary progression. The birth month affected time to EDSS 6 for those with relapsing MS in British Columbia, with January babies exhibiting a longer time to EDSS 6. Neither finding could be reciprocated in the other natural history cohort.

Conclusion: The season or month of birth does not appear to influence disease progression of MS.

Introduction

The timing of birth has recently been shown to influence the risk of developing MS later in life. A large-scale study showed that significantly more persons born in May and significantly fewer born in November developed MS.[1] These observations were taken to support the hypothesis that factors influencing gestation and the early postnatal period play an important role in the risk of developing MS.[1]

Previous studies suggest that the timing of birth may also have an effect on the disease course. In the MS population of British Columbia, Canada, the month of birth appeared to affect the time to reach a score of 6.0 ('requires a cane for walking') on the Expanded Disability Status Scale (EDSS).[2] In another Canadian study, birth month affected the risk of developing relapsing MS, but not primary progressive MS.[3]

The aims of this study were twofold. Firstly, to confirm that birth timing affects the time to EDSS 6 by repeating previous analyses[2] in a second independent patient sample. Secondly, to assess the influence of birth timing on (a) the onset of secondary progression and (b) disease progression in primary progressive and relapsing forms of MS.

All analyses were performed in two large natural history cohorts from geographically distinct, but seasonally similar locations in Europe and North America.

Patients and methods

Patients

In this study, we used data collected in two large patient databases from: British Columbia, a province on the west coast of Canada, and Groningen, a northern province in the Netherlands. The natural history of both populations have been previously described.[2;4–11] To allow confirmation of findings, analysis of these distinct geographical populations was carried out separately.

All patients had a diagnosis of definite MS according to the Poser diagnostic criteria.[12] Disease course was classified clinically into either a primary progressive or relapsing course from onset. Both databases collate retrospective clinical information (events occurring prior to a patients first clinic visit, obtained from physician referral letters and the clinical history obtained by the MS specialist neurologist) and prospective data (including EDSS scores, occurrence of relapses and drug treatment, and the onset of secondary progression).

British Columbia MS cohort

The British Columbian MS database is a longitudinal database capturing over 80% of the MS population in the province.[4;5] A cohort of 2,837 MS patients followed prospectively between 1980 and 1st July 2003 have formed a cohort for extensive natural history studies.[6–9] This cohort comprises of patients with first onset symptoms prior to July 1988 (to maximize the possibility of a substantial and meaningful follow-up time); at least one EDSS score and registered with a British Columbian MS clinic before July 1998 (to enable establishment of the disease course). No minimum active follow-up time was required, such that those with a rapid disease course or premature death could still be eligible.

Groningen MS cohort

The Groningen MS database is a prospective computerised database containing the clinical data of all patients attending the MS Clinic at the University Medical Center Groningen (UMCG). Patients are seen at the clinic at 3- to 12 month intervals with intercurrent visits if necessary. The UMCG is the main secondary and tertiary referral centre for MS in the province of Groningen (population approximately 575,000). Data acquisition started in 1985. Currently, the database contains data on 810 patients.

Disease progression outcomes

Disease progression was examined in two ways. Firstly, in patients with a relapsing disease onset, we examined the time from onset to the development of secondary progression. In the British Columbian cohort, the onset of secondary progression was assigned by the treating neurologist and concurred with the definition outlined by Lublin and Reingold in 1996 ('initial RR disease course followed by progression with or without occasional relapses, minor remissions, and plateaus'[13]), although we acknowledge that these guidelines were not published until after the database was established. In the Groningen cohort progression was defined as the continuous worsening of neurological symptoms unrelated to relapse for at least one year (the same definition later used by Kremenchutzky and coworkers[14]).

Secondly, we investigated the time from onset to sustained EDSS 6. This outcome had to be confirmed and sustained, with every subsequent disability score being greater or equal to EDSS 6 (which had to be at least 150 days later in the British Columbia cohort and at least one year later in the Groningen cohort).

Statistical analyses

Using Kaplan-Meier survival analysis (which allows consideration of right-censored data i.e. those patients who have not reached the outcome of interest), we examined (a) the time from MS onset to the onset of secondary progression and the age at secondary progression in all patients with a relapsing disease onset, and (b) sustained EDSS 6.0 for (i) patients with a relapsing onset and (ii) patients with a primary progressive onset. All months were compared simultaneously using log-rank tests. Furthermore, each month of birth was compared to all other months combined. This was repeated using the birth season, with seasons grouped as follows: Spring (March-May), Summer (June-August), Autumn (September-November), Winter (December-February).

Statistical significance was taken to be at the two-tailed 0.05 level. We made no correction for multiple testing. All statistical analyses were performed with the SPSS statistical software package version 14 or 15.

Results

Patient cohorts

Disease progression and demographics of both natural history cohorts have been detailed elsewhere.[6–11] An overview of the baseline characteristics of the two patient cohorts is given in Table 7.1. The Groningen natural history cohort had a higher proportion of primary progressive patients (238 of 810, 29.4%) compared to British Columbia (353 of 2,837, 12.4%). Consequently, the Groningen cohort also had a slightly higher proportion of males and an older average age at onset of MS (Table 7.1).

Table 7.1: Demographics of the British Columbian and Groningen natural history populations

	British Columbia	Groningen
n	2,837	810
Gender		
males	840 (29.6%)	270 (33.3%)
females	1,997 (70.4%)	540 (66.6%)
Initial disease course		
primary progressive	353 (12.4%)	238 (29.4%)
relapsing-remitting	2,484 (87.6%)	572 (70.6%)
Age at onset (mean years, SD)	30.6 , 10.0	32.9, 10.8
Disease duration (mean years, SD)	20.1, 9.9	17.98, 10.4
Onset symptoms n (%)		
Motor	495 (17.4%)	169 (20.9%)
Sensory	1,156 (40.7%)	313 (38.6%)
Optic neuropathy	526 (18.5%)	196 (24.2%)
Cerebellar, ataxia or brainstem	476 (16.8%)	94 (11.6%)
Other	—	35 (4.3%)

SD: standard deviation

Timing of birth and onset of secondary progression

British Columbia

Time to secondary progression exhibited a gradual increase from a nadir for those born in spring, to a peak for those born in winter (Figure 7.1A). However, this incremental increase was small, and did not reach significance, with none of the seasons (or months of birth, data not shown) showing a significant effect.

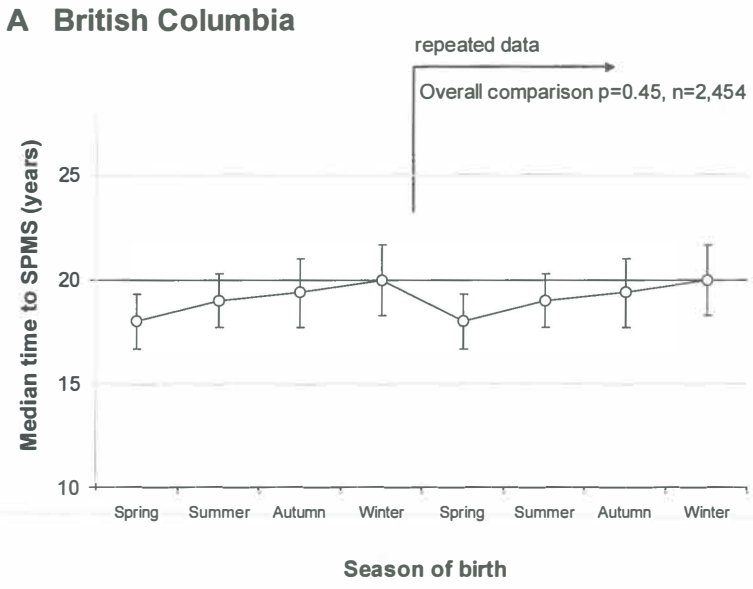
Groningen

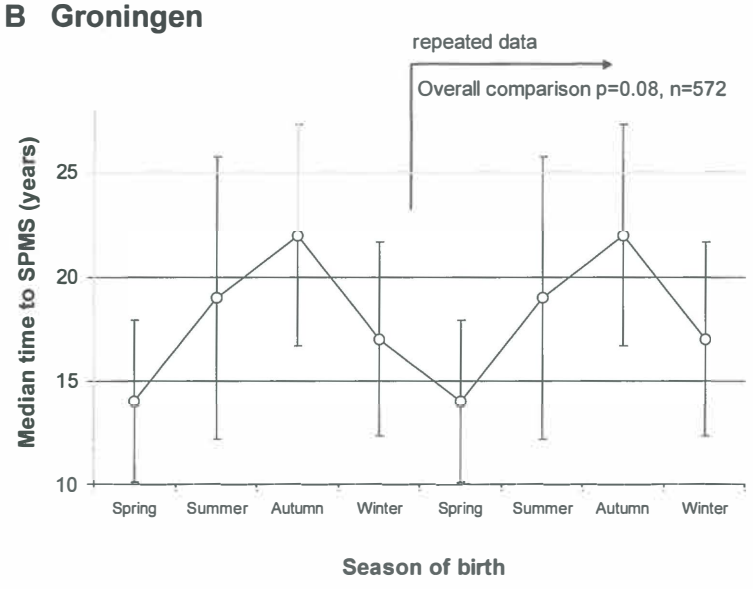
The time to secondary progression was shortest for patients born in spring, and longest in those born in autumn. These differences were not significant (Figure 7.1B, p=0.08, all seasons compared simultaneously). The shorter time to secondary progression in patients born in spring was primarily due to the effect of patients born in March, who had a significantly shorter time to secondary progression than patients born in the other months of the year (p=0.02, compared to all other months combined).

Key to the figures

P-values are derived from the overall simultaneous comparison of all seasons of birth (log-rank test). Medians and the 95% confidence intervals are shown in the figures. To facilitate viewing any seasonal patterns, a repeated year was placed back to back.

Figure 7.1: Median time to secondary progression





Timing of birth and age at secondary progression

British Columbia

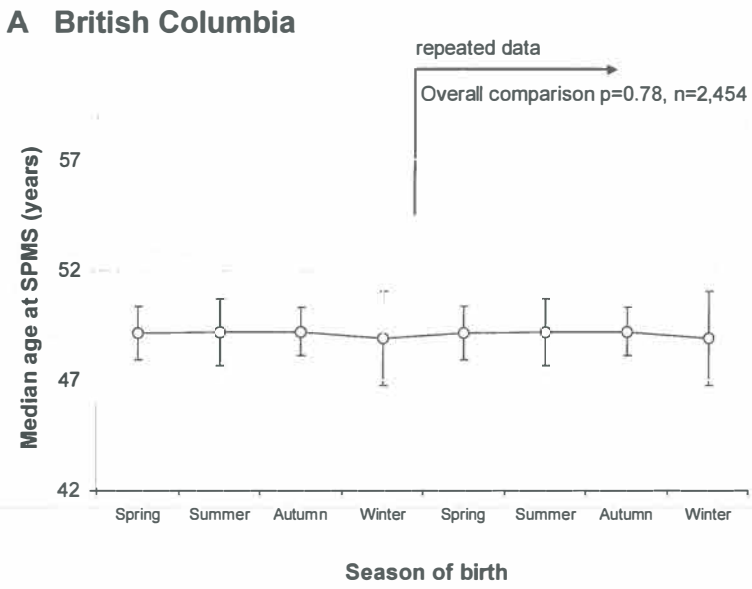
The age at secondary progression exhibited little seasonal variation (Figure 7.2A, p=0.78). Neither was a discernable trend found when the month of birth was considered (p=0.69, all months compared simultaneously).

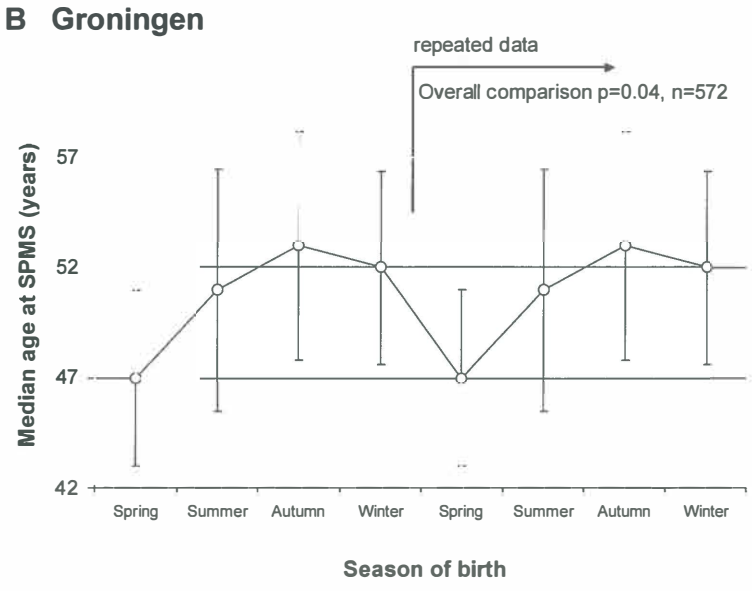
Groningen

There was a significant seasonal and monthly variation in the age at secondary progression (Figure 7.2B, p=0.04, all seasons compared simultaneously) with patients born in spring significantly younger at secondary progression (Figure 7.2B, p=0.008, compared to all other seasons combined). Patients born in autumn were the oldest at secondary progression, this was borderline significant (Figure 7.2B, p=0.05, compared to all other seasons combined).

These seasonal differences were primarily due to the effects of the months March and October (both p=0.04, compared to all other months combined).

Figure 7.2: Median age at secondary progression





Timing of birth and progression to EDSS 6.0

(i) Patients with a relapsing onset

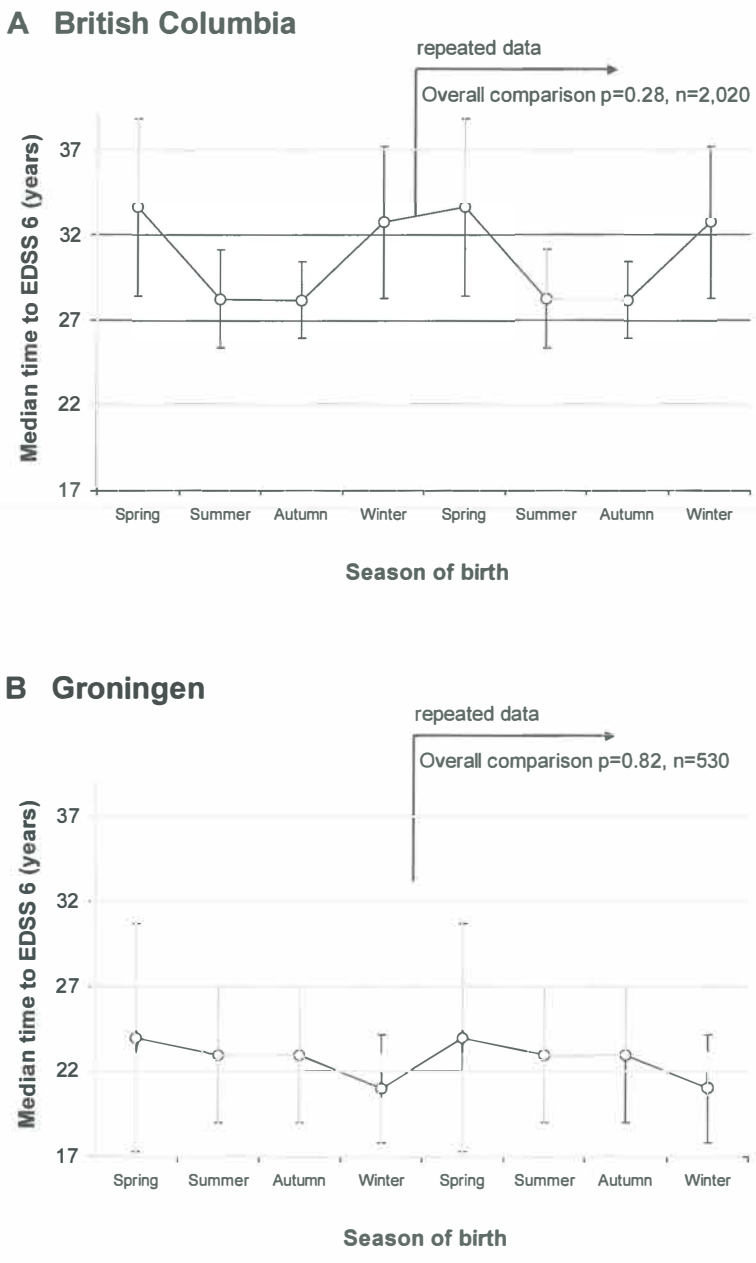
British Columbia

There was a non-significant trend for winter and spring to be the most favorable birth seasons and summer and autumn the least favorable (Figure 7.3A, p=0.28, all seasons compared simultaneously). Those born in January took the longest to reach EDSS 6 (p=0.006 compared to all other months combined), no other months showed a significant effect, and neither was there a significant overall difference between the months (p=0.63).

Groningen

Spring was the most and winter the least favorable birth season, but the seasonal differences were not significant (Figure 7.3B, p=0.82, all seasons compared simultaneously). There were no significant differences for month of birth (data not shown).

Figure 7.3: Median time to EDSS 6.0 - Relapsing at onset only



(ii) Patients with a primary progressive onset

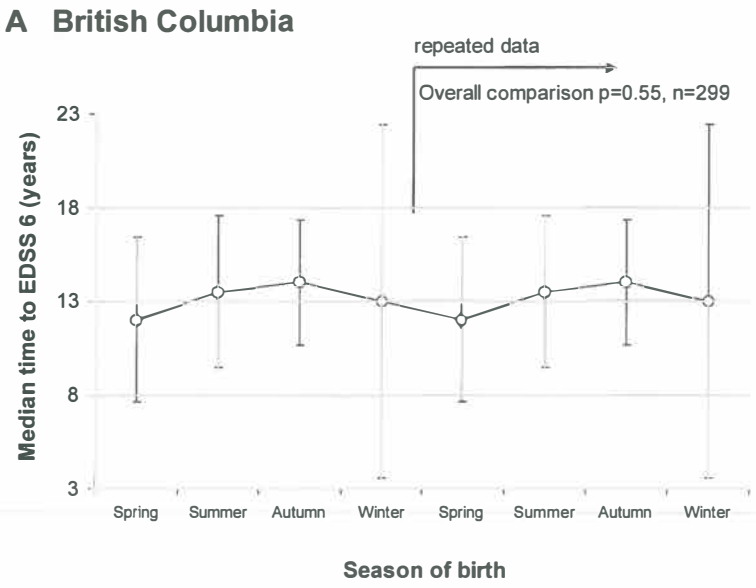
British Columbia

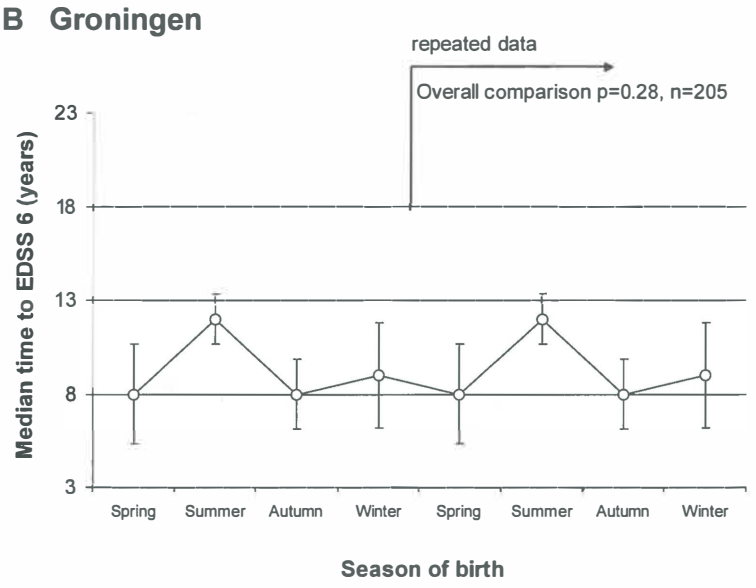
Time to EDSS 6.0 for those with a primary progressive onset exhibited little monthly (data not shown) or seasonal variation (Figure 7.4A).

Groningen

Patients born in January reached EDSS 6.0 significantly earlier (p=0.002, compared to all other months combined). There were no other significant differences between months or seasons of birth (Figure 7.4B).

Figure 7.4: Median time to EDSS 6.0 - Primary progressive only





Discussion

We were unable to find a convincing effect of the month or season of birth on several measures of disease progression in MS.

If, as previous studies suggest, the risk of developing MS is modified by the month of birth,[1] our findings suggest that exposure to these early-life factor(s) does not affect later disease progression and that these factors maybe distinct from those influencing disease progression.

While findings from each of the individual cohorts (Groningen or British Columbia) indicated that the month or season of birth might influence disease progression, the inability to reproduce findings in both cohorts leads us to the conclusion that there is no effect of the timing of birth on disease progression; statistically significant results from the individual cohorts are assumed to be chance findings (a type I statistical error). The possibility remains that a seasonal or monthly effect may exist, but exerts such a small influence that it evaded detection in our cohorts. However, this possibility appears unlikely given that a significant finding in one cohort typically did not even exhibit a reciprocal trend in the other.

Varying seasonal epidermal exposure to sunlight, with the eventual synthesis of the hormonally active metabolite 1,25-dihydroxyvitamin D[15] is one theorized causal pathway in the month of birth and risk of MS effect.[1–3] If this were true and an intervention, such as vitamin D supplementation, would reduce the risk of developing MS, our findings suggest that such treatment would be unlikely to affect disease progression in those patients who develop MS in spite of it.

Our study also provides a timely reminder of the importance and need to reproduce and confirm findings in epidemiological studies.

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Part III

Laboratory measures and progression in multiple sclerosis

Chapter 8

Cerebrospinal fluid oligoclonal bands and progression of disability in multiple sclerosis

Marcus Koch, Dorothea Heersema, Jop Mostert, Albert Teelken, Jacques De Keyser

Eur J Neurol 2007;**14**:797-800.

Abstract

Background: Antibody-mediated inflammation is believed to contribute to tissue injury in MS. The majority of patients with MS have oligoclonal bands (OCB), corresponding to antibodies against a variety of antigens, in their cerebrospinal fluid (CSF). The relation of CSF OCB and disease progression in MS is uncertain.

Objective: To investigate whether there is a relation between CSF OCB and a more aggressive disease course of MS.

Patients: 143 patients with definite MS according to the Poser diagnostic criteria and CSF analysis at time of diagnosis were followed for five years.

Results: There were no differences in presence or number of CSF OCB between patients with significant worsening of disability and stable patients. There were no differences in presence or number of CSF OCB between patients with stable relapsing-remitting MS and patients developing secondary progression during follow-up.

Conclusion: The presence or number of CSF OCB does not seem to influence early disease progression in MS.

Introduction

The disease course in multiple sclerosis (MS) is highly variable; while some patients remain free of disability for a long time, others quickly deteriorate. One of the most devastating events in MS is the development of a progressive disease course, after which disability no longer worsens in bouts followed by some degree of recovery, but progresses relentlessly.

Cerebrospinal fluid (CSF) analysis shows oligoclonal bands (OCB) in the great majority of patients with MS. OCB unique to the CSF argue for an immunologic reaction to a small number of antigens in the intrathecal space. Antibodies that have the capacity to recognise particular targets in the CNS are believed to contribute to tissue injury in patients with MS:[1] Kuhle and coworkers found increased CSF and magnetic resonance imaging signs of inflammation in patients with CSF antibodies against myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP).[2] Peripheral blood serum antibodies against MOG are especially prevalent in patients with primary progressive MS (PPMS), and have the ability to induce cell death in MOG-expressing cells in vitro.[3]

A minority of patients with proven MS have no OCB, and it has been suggested that patients with this 'OCB negative' form of MS may have a better prognosis.[4;5] Furthermore, it has been suggested that a low OCB number in 'OCB positive' MS patients predicts a better prognosis.[6]

In order to learn more about the possible influence of CSF OCB on disease progression of MS, we examined the relation between CSF OCB and progression of disability during five years in a hospital-based MS cohort.

Patients and methods

Groningen MS Database

The Groningen MS Database is a prospective database of all MS patients attending the University Medical Center Groningen (UMCG) MS Clinic, where patients are followed at 3- to 12-monthly intervals with intercurrent visits if necessary. The UMCG is the main secondary and tertiary referral centre for MS in the province of Groningen (population approximately 575,000). Data acquisition started in 1985, and a diagnosis of definite MS was established according to the Poser criteria.[7] Currently, the database contains clinical data of 672 patients.

Patients

We identified all patients in the Groningen MS Database who had undergone a lumbar puncture as part of their diagnostic workup before the year 2000, and who had not been treated with immunomodulatory or immunosuppressive drugs (with the exception of high dose steroid courses for the treatment of relapses).

We identified 154 patients; 87 with relapsing-remitting MS (RRMS), 15 with secondary progressive MS (SPMS), and 52 with PPMS. During the follow-up period of 5 years, two RRMS patients died due to causes unrelated to MS and were excluded from the final analysis. Seven RRMS patients and two PPMS patients were lost to follow-up, leaving 143 patients for the final analyses.

Measurements

Matched CSF and plasma samples were analysed, during the diagnostic workup, by isoelectric focusing and IgG-specific immunofixation.[8] The presence or absence of OCB unique to the CSF (thus, absent in serum) was reported in all patients, OCB number was determined in 104.

We recorded age, gender, disease duration (the time since the first likely symptom of MS), Expanded Disability Status Scale (EDSS) score at baseline and at follow-up visits. We calculated Multiple Sclerosis Severity Scores (MSSS) from EDSS scores and disease duration as described by Roxburgh and coworkers.[9]

Clinical endpoints

Clinically relevant worsening of disability during follow-up in all patients was defined as an increase in EDSS score of at least one full point for patients with a baseline score of less than 6.0, and at least one half point for patients with a baseline score of 6.0 or greater. This definition of relevant worsening of disability was taken from the two main randomised controlled trials on interferon beta therapy in secondary progressive multiple sclerosis.[10;11] In view of the possible special relevance of OCB in PPMS patients, we included an analysis on this patient subgroup.

In addition, RRMS patients were evaluated for the development of a progressive disease course, defined as the progressive worsening of symptoms for at least one year unrelated to relapses.[12]

Statistical analyses

Group differences were assessed with Fisher's exact test or the Mann-Whitney U test where appropriate. Statistical significance was taken to be at the two-tailed 0.05 level. All statistical analyses were performed with the SPSS statistical software package version 12.

Results

Progression of disability

Baseline characteristics of the 143 MS patients are shown in Table 8.1. Ninety one (64%) had a clinically relevant worsening of disability during follow-up. The proportion of OCB positive and OCB negative patients was not significantly different between worsening and stable patients. OCB number was not significantly different between worsening and stable patients (Table 8.1). Patients experiencing significant worsening of disability during follow-up were older than patients who remained stable (p=0.042) and had higher EDSS scores at baseline (p=0.001) (Table 8.1).

Table 8.1: Comparison of patients with significant worsening of disability during follow-up and stable patients.

	All patients	Significant worsening of disability	Stable disability	p
n	143	91	52	—
Gender: Men/Women (n)	45/98	29/62	16/36	1*
Age at baseline (median, range)	39, 14-71	39, 14-71	35, 16-68	0.042†
Years since first symptom (median, range)	2, 0-26	2, 0-26	2, 0-21	0.13†
Baseline EDSS (median, range)	3.0, 0.0-7.5	3.5, 0.0-6.5	2.0, 1.0-7.5	0.001†
Baseline MSSS (mean, SD)	5.83, 2.69	6.09, 2.71	5.37, 2.61	0.076†
Oligoclonal bands: Negative/positive	33/110	19/72	14/38	0.42*
Number (median, range)	4, 0-25$	5, 0-25	2, 0-17	0.11†

*: Fisher's Exact test, †: Mann-Whitney test
$: measured in 104 patients (65 with significant worsening of disability, 39 with stable disability)

There were no statistically significant differences in any variable for the subgroup of PPMS patients (Table 8.2)

Table 8.2: Subgroup of primary progressive patients: comparison of patients with significant worsening of disability during follow-up and stable patients.

	All PPMS	Significant worsening of disability	Stable disability	p
n	50	40	10	—
Gender: Men/Women (n)	16/34	12/28	4/6	0.7*
Age at baseline (median, range)	45.5, 25-71	43.5, 25-71	48, 44-68	0.07†
Years since first symptom (median, range)	3, 0-18	3, 0-18	3, 2-10	0.54†
Baseline EDSS (median, range)	4.0, 1.0-7.5	4.0, 1.0-6.5	3.75, 1.0-7.5	0.61†
Baseline MSSS (mean, SD)	7.27, 1.96	7.39, 1.81	6.81, 2.55	0.68†
Oligoclonal bands:				
Negative/positive	16/34	11/29	5/5	0.26*
Number (median, range)	2, 0-25$	4, 0-25	0, 0-3	0.08†

*: Fisher's Exact test, †: Mann-Whitney test
$: measured in 36 patients (28 with significant worsening of disability, 8 with stable disability)

Development of secondary progression

Nineteen of the 78 RRMS patients (24%) developed a progressive disease course during follow-up. The proportion of OCB positive and OCB negative patients as well as OCB number did not differ between patients developing progression and stable RRMS patients (Table 8.3). Patients developing a progressive disease course had significantly higher EDSS (p=0.011) and MSSS (p=0.009) scores at baseline than patients who remained relapsing-remitting.

Table 8.3: Comparison of patients developing secondary progression during follow-up and stable patients.

	all RRMS	patients developing secondary progression	patients remaining relapsing-remitting	p
n	78	19	59	—
Gender: Men/Women (n)	24/54	8/11	16/43	.26*
Age at baseline (median, range)	34, 14-57	34, 20-56	34, 14-57	0.55†
Years since first symptom (median, range)	1, 0-21	1, 0-12	1, 0-21	0.61†
Baseline EDSS (median, range)	2.0, 0.0-4.5	2.0, 1.0-4.5	2.0, 0.0-4.5	0.01†
Baseline MSSS (mean, SD)	4.81, 2.69	6.27, 2	4.34, 2.72	0.009†
Oligoclonal bands: Negative/positive	15/63	5/14	10/49	0.5*
Number (median, range)	5, 0-17$	4.5, 0-11	5.5, 0-17	0.31†

*: Fisher's Exact test, †: Mann-Whitney U test
$: measured in 56 patients (16 developing progression, and 40 with stable RRMS)

Discussion

In this study, we found no relation between the presence or number of CSF OCB and short-term progression of disability, indicative of a more aggressive disease course of MS.

A possible limitation of our study is the use of the Poser diagnostic criteria, which excludes patients with a single relapse but with MRI findings which would allow a diagnosis according to the McDonald diagnostic criteria. Our findings can therefore not be generalised to this patient group.

Tintoré and coworkers examined OCB status in 112 patients with a clinically isolated syndrome suggestive of MS. In their study, the presence of OCB had a relatively high sensitivity (0.81) but low specificity (0.43) for predicting the development of definite MS.[13] The importance of OCB in established MS was evaluated by Amato and colleagues: in their cohort study, patients without CSF OCB reached landmark disability scores and developed secondary progression significantly later than OCB positive patients.[5] These findings are in conflict with our results. Possible explanations for this may be the different endpoints and the different definition of a progressive disease course used. A previous report by Zeman and colleagues suggested that OCB negative MS may have a relatively benign prognosis, but this conclusion was based on the analysis of only 12 patients.[4] In a more recent study by Imrell and colleagues on 1,505 patients, there were no significant differences in Multiple Sclerosis Severity Scores (MSSS) between patients with and without OCB.[14]

Avarasala and coworkers in a study on 44 MS-patients found a lower number of OCB in patients with EDSS scores of less than 3.5 as compared to patients with scores of 7.5 or more, but this difference was not statistically significant.[6] The small patient number, and the use of arbitrary criteria for 'benign' versus 'severe' MS are weaknesses of their study.

Our data suggest that the presence and number of antibodies in the CSF do not influence the disease course of MS.

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Chapter 9

Plasma lipid peroxidation and progression of disability in multiple sclerosis

Marcus Koch, Jop Mostert, Alexander Arutjunyan, Michael Stepanov, Albert Teelken, Dorothea Heersema, Jacques De Keyser

Eur J Neurol 2007;**14**:529-33.

Abstract

Background: Oxidative stress has been implicated in the pathophysiology of multiple sclerosis (MS), but its relation to disease progression is uncertain.

Objective: To evaluate the relationship of plasma lipid peroxidation with progression of disability in MS.

Methods: We measured blood plasma fluorescent lipid peroxidation product (PFLPP) levels in 23 patients with relapsing remitting MS with a benign course, 32 with secondary progressive MS, 24 with primary progressive MS, and 30 healthy controls. None of the patients had a relapse within the previous 3 months. Progression of disability was evaluated during a follow-up period of five years by the Expanded Disability Status Scale (EDSS) and the Multiple Sclerosis Severity Score (MSSS).

Results: We found plasma PFLPP levels elevated in patients with MS compared to controls ($p<0.0005$), but there was no difference between patients with a benign and progressive disease course. There was no correlation between PFLPP levels and worsening of disability on the EDSS and speed of progression on the MSSS.

Conclusion: Our data suggest that there is no relation between the degree of oxidative stress in plasma and progression of disability in MS.

Introduction

Multiple sclerosis (MS) is an inflammatory neurodegenerative disease of the central nervous system (CNS). The disease course of MS varies considerably between individual patients. In most patients the disease begins with a relapsing-remitting disease course in which attacks of neurological worsening are followed by full or partial recovery (relapsing-remitting MS, RRMS). A subgroup of RRMS patients has only little disability despite more than ten years disease duration. These patients are considered to have benign multiple sclerosis (BMS).[1]

Most patients with RRMS, however, convert to a secondary progressive disease course characterised by slow and relentless worsening of neurological symptoms independent of relapses (secondary progressive MS, SPMS). Within the first ten years, 30 to 40 percent and within twenty years 70 percent of RRMS patients will have converted to SPMS.[2;3] A minority of patients is progressive from disease onset (primary progressive MS, PPMS).

Large observational studies on the natural history of MS consistently show patients with a primary progressive disease course and male patients to have a quicker progression of disability;[4;5] other clear factors influencing the speed of progression have not been identified.

Many aspects of MS aetiology and pathophysiology are unresolved, but there is increasing evidence that the relapsing-remitting phase and the progressive phase of the disease are caused by two distinct mechanisms. Focal inflammation is believed to be the cause of relapses, whereas diffuse axonal degeneration appears to be the main contributor to progression.[2] The accumulation of disability in MS is thus caused by neuronal damage at inflammatory foci and by diffuse axonal degeneration. Current treatments of MS can reduce the number of relapses but are ineffective in the progressive phase.

Oxidative stress is a state in which the production of reactive oxygen species (ROS) exceeds their elimination by antioxidant mechanisms. In a tissue under oxidative stress, ROS may cause damage to cellular components like the cell membrane, proteins and DNA. Oxidative stress in MS is believed to contribute to tissue injury in focal inflammatory lesions and

to be involved in diffuse axonal degeneration.[6;7]

Measures of oxidative stress in blood and cerebrospinal fluid (CSF) of patients with MS were consistently found increased in a number of studies.[8–11] The relevance of oxidative stress for disease course and progression of disability in MS is, however, unclear since most of these studies comprised only small patient numbers and lacked a follow-up period.

In order to evaluate the relation of oxidative stress to disease course and progression of disability in MS, we measured plasma fluorescent lipid peroxidation products (PFLPP), a robust marker of total lipid peroxidation,[12] in patients with different disease courses of MS and in healthy controls. Changes in disease course and the accumulation of disability were evaluated during a follow-up period of five years.

Patients and Methods

Patients

The study was approved by the local medical ethics committee and all patients gave their written informed consent before inclusion into the study. Venous blood samples were obtained between 8 AM and noon through an intravenous cannula in the forearm from 30 healthy controls and 79 patients with definite MS, according to the Poser criteria.[13] The patients were attending our outpatient clinic, where follow-up appointments were made at least once a year. Five years later, 14 patients were lost to follow-up and 4 patients had died.

Of the 79 patients, 23 had BMS, defined as a score on Kurtzke's Expanded Disability Status Scale (EDSS)[14] of 3.0 or less despite at least 10 years disease duration.[15] Thirty-two patients had SPMS and 24 had PPMS. Eleven patients were using interferon beta; no other immunomodulatory drugs were used. None of the patients or controls was following a special diet or used antioxidant medication or food supplements, and none of the patients had a relapse within the previous 3 months.

We recorded disease duration, number of previous relapses, use of interferon beta, EDSS score at baseline, number of relapses during follow-up, and EDSS-scores at five-year follow-up. We calculated global Multiple Sclerosis Severity Scores (MSSS) from EDSS scores and disease duration as described by Roxburgh and coworkers.[16] The MSSS is currently the best measure of the speed of disability accumulation in MS, it denotes the speed of disability accumulation of an individual patient compared to a very large patient cohort.

A relevant worsening of disability was defined as worsening of the EDSS score by at least one full point for patients with a baseline score of less than 6.0, and as worsening by at least one half point for patients with a baseline score of 6.0 or higher. EDSS scores were recorded in the absence of relapses.

BMS patients were furthermore evaluated for the development of a pro-

gressive disease course, defined as the progressive worsening of symptoms for at least one year unrelated to relapse.[17]

Measurement of plasma FLPP

Plasma FLPP were measured with the method described by Fletcher and colleagues,[18] with a slight modification. Briefly, 1.8 ml of a 2:1 mixture ethanol:chloroform was added to 0.5 ml of plasma, the samples were then mixed and centrifuged; 0.5 ml of a 0.1% NaCl solution was added to the supernatants. After phase separation, 0.5 ml of the chloroform-rich layer was mixed with 4.5 ml of distilled chloroform. Fluorescence intensity of this final mixture was measured at the emission maximum of 450 nm and expressed as arbitrary fluorescence units (FU) per milligram protein content. Protein content was measured by the method of Lowry and coworkers.[19]

Statistical analyses

Group differences between three or more groups were evaluated with one-way analysis of variance followed by Tukey's post-hoc test for inter-group comparisons. Group differences between two groups were assessed with the independent samples t-test. Spearman's rank correlation was used for correlation analyses. Significance was taken to be at the two-tailed 0.05 level. All statistical analyses were performed with the GraphPad Prism (Version 4) and SPSS (Version 12) statistical software packages.

Results

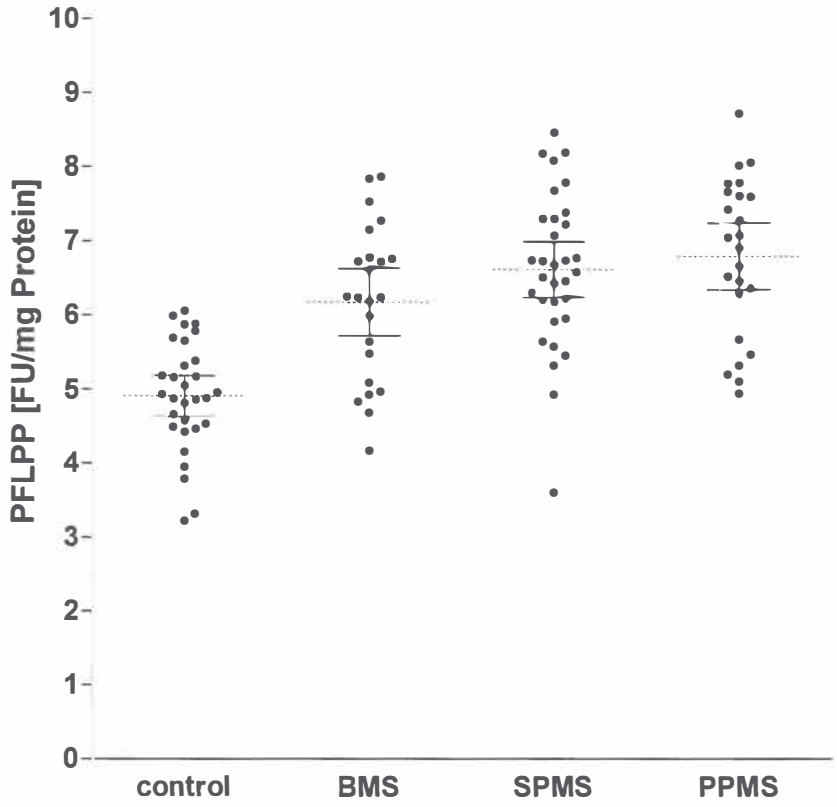
Patient characteristics at baseline and at five-year follow-up are given in Table 9.1.

Table 9.1: Characteristics of patients and control persons at baseline and at five-year follow-up

	control	BMS	SPMS	PPMS
Baseline				
n	30	23	32	24
Gender (Men/Women)	13/17	8/15	11/21	8/16
Age (median, range)	47, 31-65	48, 29-69	49.5, 28-72	51.5, 36-70
Disease duration (median, range)	—	21, 10-40	18.5, 7-35	11.5, 3-34
Number of relapses (median, range)	—	4, 1-9	6, 2-20	—
Use of interferon beta (n)	—	1	9	1
EDSS (median, range)	—	2.0, 0.0-3.0	7.0, 4.0-8.5	6.0, 2.5-8.0
MSSS (mean, SD)	—	0.96, 0.78	7.41, 1.75	7.36, 1.54
PFLPP levels [FU/mg] (mean, SD)	4.9, 0.74	6.17, 1.05	6.61, 1.04	6.79, 1.07
95% Confidence Interval	4.63-5.18	5.72-6.63	6.24-6.99	6.34-7.24
5-year follow-up				
Lost to follow-up (n)		4	3	7
Death due to MS (n)		—	2	2
n		19	27	15
Relapses during follow-up (median, range)		0, 0-4	0, 0-5	—
EDSS (median, range)		2.0, 1.0-6.5	7.0, 4.5-9.0	6.0, 3.5-9.0
MSSS (mean, SD)		1.23, 1.19	7.51, 1.76	7.03, 1.82
Relevant worsening of disability (n, %)		4, 21%	12, 44%	5, 33%

SD: Standard deviation
PFLPP: plasma fluorescent lipid peroxidation products

Figure 9.1: PFLPP levels in healthy controls and patients with different disease courses of MS. The dotted line represents the mean, the error bars represent the 95% confidence interval.



Baseline

PFLPP levels were significantly different between MS patients and controls (one-way analysis of variance: $p<0.0005$). Pairwise comparisons showed that PFLPP levels in each disease course was significantly higher than in controls (control vs. BMS, control vs. SPMS, control vs. PPMS: all $p<0.01$). There were no significant differences in PFLPP levels between the three disease courses (BMS vs. SPMS: $p=0.36$, BMS vs. PPMS: $p=0.14$, SPMS vs. PPMS: $p=0.91$) (Table 9.1, Figure 9.1). There was no significant correlation of PFLPP with EDSS or MSSS scores with number of previous relapses, age or disease duration (Table 9.3).

5-year follow-up

There were no differences in PFLPP levels between patients with relevant worsening of disability during follow-up and patients with stable disease. Relapses during follow-up, gender or the use of interferon beta were not associated with differences in PFLPP levels (Table 9.2). None of the BMS patients developed a progressive disease course during follow-up. There was no significant correlation of PFLPP with EDSS or MSSS scores with number of relapses during follow-up, age or disease duration (Table 9.3).

Table 9.2: Plasma FLPP levels in different patient groups.

Group	n	Plasma FLPP levels [FU/mg]	p
Relevant worsening of disability:			
Yes	29	6.53, 0.99	0.84
No	36	6.47, 1.17	
Relapses during follow-up:			
Yes	16	6.45, 0.96	0.5
No	30	6.23, 1.09	
Gender:			
Men	40	5.91, 1.14	0.27
Women	69	6.18, 1.27	
Use of interferon beta:			
Yes	11	6.54, 0.58	0.98
No	68	6.53, 1.13	

Table 9.3: Spearman's correlation analyses of plasma FLPP levels.

	n	Spearman's rho	p
Baseline:			
Age	109	0.10	0.33
Disease duration	79	0.15	0.19
Number of relapses	54	- 0.01	0.94
EDSS	79	0.17	0.13
MSSS	79	0.09	0.44
5 year follow-up:			
Number of relapses during follow-up	46	0.08	0.59
EDSS	61	0.19	0.15
MSSS	61	0.16	0.22

Discussion

In this study we found significantly higher PFLPP in patients with MS compared to healthy controls. We chose PFLPP as a marker of oxidative stress, because it is a robust and reliable marker of overall lipid peroxidation[12] used in several previous cross-sectional studies on oxidative stress in MS (see below).

Interestingly, there was no significant difference between patients with a benign and progressive disease course. The analysis of follow-up data showed that patients with relevant worsening of disability during follow-up did not have higher levels of PFLPP than patients with stable disease. Neither disability itself (EDSS scores) nor the speed of disability accumulation (MSSS scores) were correlated with PFLPP levels.

Strengths of our study compared to previous studies are the comparison between patients with a benign and progressive disease course, the larger number of patients and the length of follow-up. The fact that we did not perform serial measurements of PFLPP or use imaging techniques to detect clinically silent inflammatory disease activity, are limitations of our study.

Previous research has consistently shown increased measures of lipid peroxidation in patients with MS. Hunter and coworkers found significantly higher measures of lipid peroxidation in CSF but not plasma of MS patients compared to control persons.[8] The main weakness of their study was that blood and CSF experiments used different control groups; whereas plasma of MS-patients was compared to that of healthy control persons, the CSF experiments used patients with other neurological diseases (such as the Guillain-Barré syndrome and brain tumour) as the control group. Another weakness was the very small number of patients included (CSF: 4 patients with definite MS vs. 5 to 6 controls; plasma: 5 to 11 patients with definite MS vs. 10 controls). Other studies on oxidative stress in plasma and CSF of MS patients came to different conclusions. Naidoo and Knapp compared measures of lipid peroxidation in CSF and plasma of 9 MS-patients with that of 19 control persons. They found significantly higher levels of the lipid peroxidation markers thiobartituric acid reactive substances and FLPP in serum, but not in CSF,[10] confirming the results

of an earlier study on the lipid peroxidation marker malondialdehyde by Rogovina and Khokhlov.[20] Besler and coworkers found and increase in plasma levels of oxidised lipoproteins and a decrease of antioxidant vitamins in their study on 24 MS-patients,[21] and Ferretti and colleagues report an increase in plasma lipid peroxidation in patients in an early stage of the disease (mean disease duration in 24 patients: 3.1 years).[22] Taken together, these studies suggest that peripheral oxidative stress is present from an early stage in the disease process; most previous studies comparing plasma and CSF markers suggest that lipid peroxidation is more prevalent in the periphery than in the CNS.

What is the significance of our findings? Increased measures of oxidative stress have been described in many neurodegenerative diseases, including Alzheimer's dementia, amyotrophic lateral sclerosis, Parkinson's disease and Huntington's disease[23;24] The question arises whether oxidative stress in all these diseases, including MS, contributes to pathology or whether it is a non-specific epiphenomenon. Evidence for an important role of oxidative stress in the pathogenesis of these diseases should come from clinical trials with antioxidant drugs. However, so far, clear proof of the effectiveness of antioxidant treatments in MS and other neurodegenerative diseases is lacking.[25;26]

Plasma markers of oxidative stress are consistently found increased in MS patients, but there appears to be no relation to disease course and progression of disability. It is currently uncertain whether oxidative stress is generated by cells in the CNS or in the periphery. Further research is needed to determine whether oxidative stress is an important feature of MS pathophysiology or merely an epiphenomenon of neuro-degeneration or inflammation.

Our data do not support the idea that the use of antioxidant drugs or specialised antioxidant diets influence the progression of disability in MS.

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Chapter 10

Plasma S100beta and NSE levels and progression in multiple sclerosis

Marcus Koch, Jop Mostert, Dorothea Heersema, Albert Teelken, Jacques De Keyser

J Neurol Sci 2007;**252**:154-158.

Abstract

Background: Plasma levels of the glial cell marker S100beta and the neuronal marker neuron-specific enolase (NSE) are elevated in various conditions of central nervous system damage.

Objective: In this study we investigated whether plasma levels of S100beta and NSE are related to disease progression in multiple sclerosis (MS).

Methods: Plasma levels of S100beta and NSE were measured in 25 patients with relapsing remitting MS (RRMS), 23 with secondary progressive MS (SPMS) and 16 with primary progressive MS (PPMS). All MS patients were in a clinically stable phase. Progression and disability were evaluated during a follow-up period of five years.

Results: Plasma NSE levels were lower in patients with clinically relevant worsening on the Expanded Disability Status Scale (EDSS) ($p=0.04$), and in patients with a progressive disease course ($p=0.01$). There was a significant negative correlation between plasma NSE levels and both EDSS and Multiple Sclerosis Severity Scores (MSSS) at baseline and after five years of follow-up. There were no significant differences between patient groups in plasma S100beta levels.

Conclusion: Plasma NSE levels appear inversely related to disease progression in MS.

Introduction

Nervous tissue damage in multiple sclerosis (MS) is the result of two distinct mechanisms. Focal inflammatory demyelination is a subacute event, which leads to circumscript tissue destruction in the central nervous system and presents clinically as an acute relapse. Diffuse axonal degeneration on the other hand is a chronic process that leads to slow but relentlessly progressing neurodegeneration. Axonal degeneration is thought to be the most important mechanism underlying chronic progression in MS.[1]

The disease course in MS varies considerably between individual patients. Patients with so-called benign MS have only minor symptoms even after a very long disease duration, whereas patients with primary progressive MS (PPMS) experience steadily accumulating disability from disease onset. In most patients, the disease begins with a period of relapses and remissions (relapsing-remitting MS, RRMS) but later converts to a chronic progressive disease course (secondary progressive MS, SPMS).

MS is a disease that evolves over many years, and it has proven impossible to make any reliable prediction of the future disease course in individual patients in the early stages of the disease. Once patients reach a certain level of disability, however, the accumulation of disability is much more uniform.[2]

Given the prognostic diversity of MS, many researchers have attempted to identify markers of disease activity in cerebrospinal fluid or blood plasma. In most of these studies, the authors compared body fluid levels of marker substances between patients with MS and control persons, and investigated the correlation with other markers of disease activity, such as magnetic resonance imaging (MRI).

In this study we were interested in the influence of progression on two marker proteins in patients with MS. We therefore evaluated plasma levels of the glial marker protein S100beta and the neuronal marker protein neuron-specific enolase (NSE) in patients with different disease courses of MS. The patients were followed at our outpatient department, and we related the initial measurements to disease development over a period of five years.

Patients and Methods

Patients

The study was approved by the medical ethics committee of the University Medical Center Groningen. All patients gave their written informed consent before inclusion into the study. Venous blood samples were obtained through an intravenous cannula in the forearm from 64 patients with definite MS, according to the Poser criteria.[3] The patients were attending the Groningen MS outpatient clinic, where follow-up appointments were made at least once a year. Over five years of follow-up, 15 patients were lost to follow-up and two patients died.

Of the 64 patients, there were 25 with RRMS, 23 with SPMS and 16 with PPMS. Eight patients were using interferon beta; no other immunomodulatory treatments were used. No patient had experienced an exacerbation or received treatment with systemic corticosteroids in the previous three months.

We recorded age, gender, disease duration, number of relapses, use of interferon beta and the Expanded Disability Status Scale (EDSS) score at baseline. The number of relapses during follow-up and EDSS scores after five years were noted in our MS database and the patient records. We calculated Multiple Sclerosis Severity Scores (MSSS) from EDSS scores and disease duration as described by Roxburgh and coworkers.[4] For further analysis we divided patients into groups according to their baseline EDSS and MSSS scores: an EDSS score of more than 3.0 was considered to mark relevant disability, and an MSSS score of more than 5.0 was considered to indicate more than average disease progression.[4]

Clinically relevant worsening of disability during follow-up was defined as EDSS score progression by at least one full point for patients with a baseline score of less than 6.0, and by at least one half point for patients with a baseline score of 6.0 or greater. Death was included as an EDSS score of 10.

RRMS patients were evaluated for the development of a progressive disease

course, defined as the progressive worsening of symptoms for at least one year unrelated to relapse.[5]

Measurement of marker proteins

Plasma S100beta and NSE concentrations were measured with commercially available luminescence immunoassays (LIAISON Sangtec 100 and LIAISON Sangtec NSE, both by Byk-Sangtec, Dietzenbach, Germany). We were unable to obtain plasma S100beta levels (technical reason) in eight patients, and plasma NSE levels (due to haemolysis) in three patients.

Statistical analyses

Group differences between three or more groups were evaluated with one-way analysis of variance, using Dunett's post-hoc test for inter-group comparisons. Group differences between two groups were assessed with the independent samples t-test. Normal distribution of the measured variables was assessed with the Kolmogorov-Smirnov test. MSSS-scores and EDSS-scores were not following a normal distribution, and we therefore used the non-parametric Spearman's rank correlation for correlation analyses. Significance was taken to be at the two-tailed 0.05 level. All statistical analyses were performed with the GraphPad Prism (Version 4) and SPSS (Version 12) statistical software packages.

Results

Patient characteristics at baseline and at five-year follow-up are given in Table 10.1. Plasma NSE levels were lower in SPMS patients than in RRMS patients, and lower in PPMS patients than in SPMS patients (p=0.035). Dunett's post hoc analysis showed a significant difference in plasma NSE levels between RRMS and PPMS patients (p=0.041), but not between RRMS and SPMS or SPMS and PPMS patients (Table 10.1, Figure 10.1).

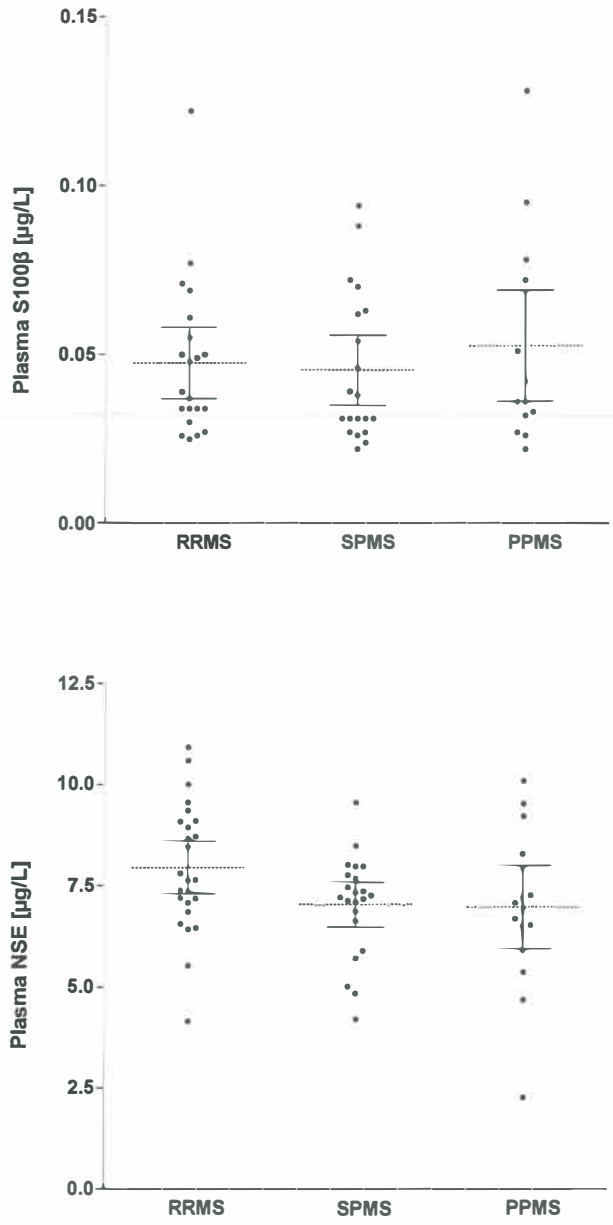
There was no difference in plasma S100beta levels between the different disease courses (p=0.69) (Table 10.1, Figure 10.1).

Table 10.1: Characteristics of patients and control persons at baseline and at five year follow-up.

	RRMS	SPMS	PPMS
Baseline			
n	25	23	16
Gender (Men/Women)	10/15	6/17	6/10
Age (median, range)	48, 29-70	51, 28-72	51, 36-70
Disease duration (median, range)	21, 1-40	20, 7-33	11.5, 3-34
Number of relapses (median, range)	4, 1-9	6, 2-20	—
Use of interferon beta (n)	1	6	1
EDSS (median, range)	1.5, 0.0-3.5	6.5, 5.5-8.5	6.0, 3.5-8.0
MSSS (mean, SD)	1.9, 1.44	7.18, 1.71	7.3, 1.62
Plasma S100beta [µg/L] (mean, SD)	0.048, 0.023	0.045, 0.022	0.053, 0.03
95% Confidence Interval	0.037 - 0.058	0.035, 0.056	0.036, 0.069
Plasma NSE levels [µg/L] (mean, SD)	7.94, 1.59	6.98 - 1.27	6.75 - 1.8
95% Confidence Interval	7.29 - 8.6	6.4 - 7.56	5.76 - 7.75
5 year follow-up			
Lost to follow-up (n)	6	3	6
Death due to MS (n)	0	1	1
n	19	19	9
Relapses during follow-up (median, range)	1, 0-4	1, 0-5	—
EDSS (median, range)	2.0, 1.0-6.5	7.0, 5.5-9.0	6.5, 3.5-8.0
MSSS (mean, SD)	1.58, 1.89	7.53, 1.7	6.83, 2.01
Relevant worsening of disability (n)	5	10	5

SD: Standard deviation

Figure 10.1: Plasma S100beta and plasma NSE levels in RRMS, SPMS and PPMS patients. The dotted line represents the mean, the error bars represent the 95% confidence interval.



Analysis of the dichotomised measures showed that patients with a clinically relevant worsening on the EDSS, with a progressive disease course, with a baseline EDSS score of more than 3.0 and a baseline MSSS score of more than 5.0 had significantly lower levels of plasma NSE. There were no significant differences in these patient groups for plasma S100beta (Table 10.2).

Table 10.2: Plasma S100beta and plasma NSE levels in different patient groups.

	n	Plasma S100beta [μg/L] (mean, SD)	p	n	Plasma NSE [μg/L] (mean, SD)	p
Clinically relevant worsening of EDSS:						
Yes	16	0.059, 0.032	0.18	18	6.81, 1.24	0.042
No	29	0.045, 0.023		28	7.6, 1.24	
Progressive disease course:						
Yes	35	0.048, 0.026	0.9	36	6.89, 1.49	0.01
No	21	0.048, 0.023		25	7.94, 1.58	
Relapses during follow-up:						
Yes	14	0.046, 0.029	0.88	17	7.33, 1.25	0.75
No	20	0.045, 0.021		19	7.47, 1.35	
Gender:						
Men	19	0.04, 0.014	0.09	19	7.27, 1.85	0.88
Women	37	0.052, 0.028		42	7.34, 1.5	
Use of interferon beta:						
Yes	8	0.043, 0.022	0.56	6	7.33, 1.24	0.99
No	48	0.049, 0.025		55	7.32, 1.65	
Baseline EDSS greater than 3.0:						
Yes	36	0.049, 0.025	0.83	37	6.95, 1.52	0.023
No	20	0.047, 0.024		24	7.89, 1.6	
Baseline MSSS greater than 5.0						
Yes	32	0.049, 0.026	0.84	33	6.79, 1.47	0.004
No	24	0.047, 0.023		28	7.95, 1.55	

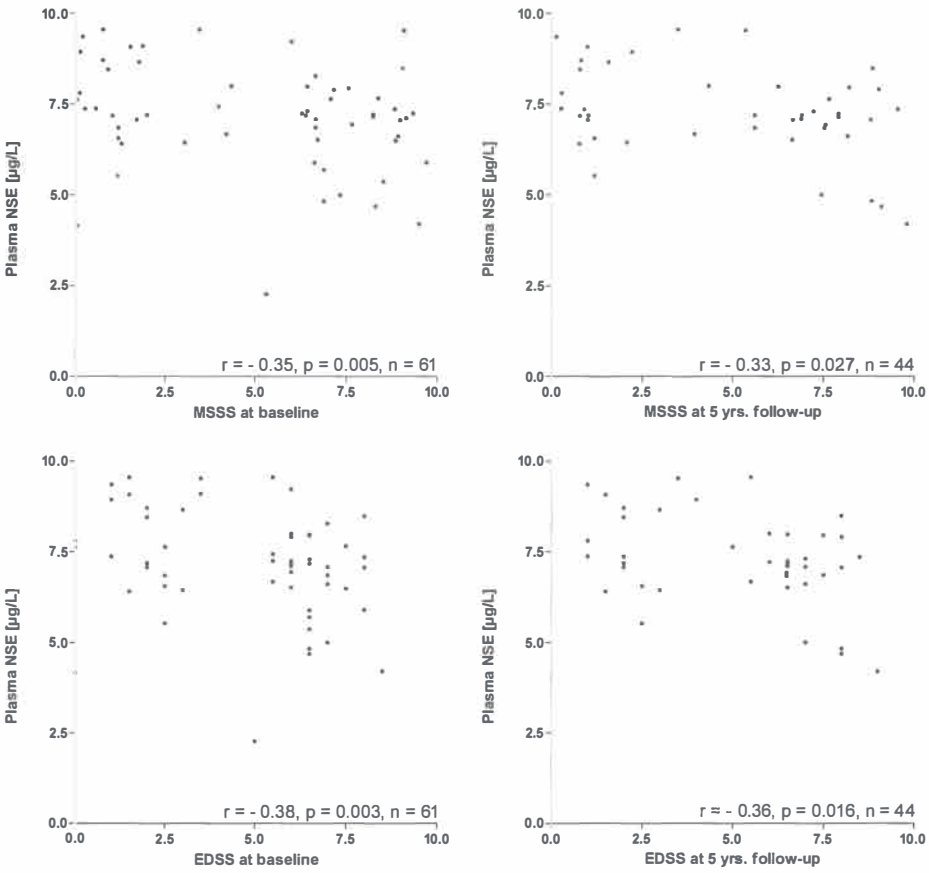
There was a significant negative correlation between plasma NSE and MSSS and EDSS scores at baseline and at five-year follow-up. There was no correlation of age, disease duration number of previous relapses

or number of relapses during follow-up with plasma NSE levels. Plasma S100beta levels were not correlated with any of these measures (Table 10.3, Figure 10.2).

Table 10.3: Spearman's correlation analyses of plasma S100beta and plasma NSE levels with several measures of disease progression in MS.

	n	Plasma S100beta Spearman's rho	p	n	Plasma NSE Spearman's rho	p
Baseline						
Age	56	0.04	0.77	61	0.02	0.88
Disease duration	56	-0.09	0.5	61	0.14	0.28
EDSS	56	-0.07	0.63	61	-0.38	0.003
MSSS	56	-0.03	0.81	61	-0.35	0.005
5 year follow-up						
EDSS	43	-0.15	0.36	44	-0.36	0.016
MSSS	43	-0.07	0.64	44	-0.33	0.027

Figure 10.2: Correlation of plasma NSE levels with EDSS and MSSS scores at baseline and at five-year follow-up.



Discussion

In this study we found that plasma levels of the astrocytic marker protein S100beta in patients with MS were unrelated to progression of disability. This result is in agreement with a previous study showing that serum S100beta levels were unrelated to disease progression in PPMS patients.[6]

More interesting, however, is our finding that plasma NSE levels were inversely related to disability and progression in patients with MS. A previous study on plasma NSE levels in MS focused on comparisons with healthy control persons, and found no significant differences.[7]

We found that patients with a progressive disease course as well as patients with clinically relevant EDSS progression after five years of follow-up had lower plasma NSE levels than those who were not in the progressive phase and stable on the EDSS. Furthermore there was a significant weak correlation of plasma NSE levels with EDSS and MSSS scores at baseline and after five years. The MSSS score is a measure of the speed of progression in relation to a very large patient sample,[4] and is currently the best available measure of disease progression in MS.

A general limitation of all studies on plasma levels of S100beta and NSE in neurological disorders is the fact that there are other sources of these proteins besides neurons: S100beta occurs in fat tissue, cartilage and skin, and NSE can be found in erythrocytes, thrombocytes and neuroendorine cells. Both marker proteins can be released from non-neural tissues, e.g. through tissue damage (including haemolysis) and inflammation. A study on S100beta and NSE in acute care patients has raised concern of their brain specificity in patients at risk of multi-organ dysfunction.[8]

We minimised the influence of these non-neuronal sources of S100beta and NSE by including only patients in a clinically stable phase and by excluding haemolytic blood samples from our analyses.

Other limitations of our study are the relatively small patient number and the fact that we did not relate our measurements to other markers of disease progression such as MRI measures of brain atrophy and lesion load.

An increase in plasma NSE levels is considered a marker of damage to neuronal cells, as reported in traumatic brain injury and stroke.[9] The reason why serum NSE levels are lower in MS patients with progressive disease and more severe disability, which are correlates of axonal degeneration, is unclear. NSE is a critical enzyme in neuro-axonal glycolysis, where it converts 2-phospho-D glycerate to phosphoenolpyruvate.[9]

It is tempting to speculate that the lower plasma NSE levels in MS patients with a progressive disease course and more severe disability reflect a reduced neuronal metabolic activity. Reduced neuronal metabolic activity could be secondary to axonal loss.

The data presented in this study suggest that plasma NSE might be a useful marker to monitor disease progression in individual MS patients. Our findings need confirmation in other patient samples. For future studies, it would be interesting to compare plasma NSE levels with measures of brain atrophy and cerebral metabolic activity in patients with MS.

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Part IV

Appendices

Summary

Patients with a progressive disease course have the worst prognosis in MS. They can expect that their symptoms will steadily worsen, and there is currently no treatment that has a proven effect on progressive MS. In this thesis, we aimed to learn more about progression by evaluating the influence of a range of potential predictive variables on disease course.

Epidemiological aspects of progression

In **Chapter 2**, we investigated patients with primary and secondary progression of MS, and evaluated the influence of several potential risk factors on the timing of progression in MS. We found that progression is an age-dependent phenomenon and that the age at progression is no different between patients with secondary and primary progressive MS. Furthermore, the time to secondary progression is highly dependent on the time at disease onset, with patients with an early disease onset taking longer until the development of secondary progression. In this chapter, we concentrated on those patients who had already developed progression, and as a consequence, we could not include the influence of immunomodulatory drugs (IMD) in the analyses, since too few patients were using them for any meaningful analysis.

In **Chapter 3** we evaluated all patients with a relapsing-remitting disease onset, in order to find out which factors may influence the risk of developing secondary progression. The inclusion of all patients with a relapsing-remitting disease onset enabled us to include the use of IMD as a potential predictive factor. Besides providing confirmation for our previous finding that progression depends on the age at disease onset, we also found evidence that the use of IMD was independently associated with a lower risk of secondary progression. This finding should be regarded as hypothesis-generating rather than as proof of a treatment effect, and in the chapter we tried to highlight the need for further randomised controlled trials on the effect of IMD on progression.

Previous studies on the epidemiology of MS often used the time from disease onset to certain landmark disability scores as the most important outcome. The special prognostic importance of the development of pro-

gression and its pathophysiological difference from relapsing-remitting MS call for the use of other outcomes in such studies. We therefore strived to establish the outcomes time to progression and age at progression as relevant outcomes in natural history studies in MS in the first two chapters.

In **Chapters 4 through 6**, we used the outcomes time to progression and age at progression to investigate several other potentially predictive variables. In **Chapter 4** we evaluated the influence of a positive family history of MS on these outcomes, and found that patients with familial primary progressive MS are significantly younger at disease onset than patients without a family history of MS, which suggests that this patient group may be especially important for future genetic research in MS.

The influence of the very common but ill understood symptoms fatigue and depression on the prognosis of MS was assessed in **Chapter 5**. We could not find an important influence of these symptoms on progression in MS, and we furthermore found that fatigue and depression scores did not significantly change in the course of ten years, while disability increased. This argues against the assumption that fatigue and depression in MS are the consequence of progressive axonal degeneration.

Chapter 6 deals with cigarette smoking and the risk of progression. Cigarette smoking is a risk factor in many diseases, and previous studies suggested that the risk of developing MS is increased among smokers. One previous study also suggested that cigarette smoking could influence the risk of secondary progression. We found no important effect of smoking on disease progression in MS.

In **Chapter 7** we investigated whether the timing of birth had an effect on disease progression in MS. It had recently been found that significantly more persons born in May and significantly fewer born in November developed MS. Whether progression in established MS is also influenced by such a timing of birth effect was uncertain. For this analyses we cooperated with researchers from the University of British Columbia, Vancouver, Canada, to investigate possible timing of birth effects in two large patient cohorts. There were significant timing of birth effects on several outcomes in both cohorts, but none of these potential effects could be replicated in both databases. We conclude that the timing of birth has no important effect on disease progression in MS, and that the statistically significant

effects found in each database were most probably due to the play of chance. This cooperation also is an important reminder that findings in epidemiological studies need to be replicated in more than one patient cohort.

Laboratory measures and progression

In **Chapters 8 through 10** we evaluated the influence of several laboratory measures on progression in the following five years.

Chapter 8 deals with the importance of cerebrospinal fluid oligoclonal bands for progression in MS. We found no significant differences in disease progression between patients with and without oligoclonal bands, and this argues against the previously held opinion that patients without oligoclonal bands may have a better prognosis.

Oxidative stress is a factor that is commonly suspected to influence the disease course of MS. In **Chapter 9** we investigated whether differences in the plasma levels of lipid peroxidation products, a robust marker of oxidative stress, are associated with the progression of disability in the following five years. We found no such association, and cannot support the use of antioxidants or special antioxidant diets in patients with MS.

Several studies have tried to identify laboratory markers (so-called biomarkers) associated with progression in MS. In **Chapter 10** we present our findings on two plasma biomarkers, S100beta and neuron-specific enolase (NSE). While there were no significant differences in S100beta between progressing and stable patients, NSE levels appeared to be inversely related to disease progression in MS. This suggest that peripheral NSE may be useful as a biomarker of progression in MS, although further study is needed.

Future perspectives

With this thesis, we aimed to contribute to a better understanding of the progressive phase of MS. As always in science, some of the answers we found have led to new questions and ideas for future studies in this underrepresented area of MS research.

Given the importance of the onset of progression, we tried to highlight the importance of using the outcome measures age at progression and time to progression in epidemiological studies. The onset of progression is probably the best epidemiological measure of axonal degeneration, and using it as an outcome enables us to learn more about this process.

A treatment that could postpone the onset of secondary progression would be an important therapeutic breakthrough. There is an unfortunate lack of information on the effect of IMD on progression, since randomised controlled trials on IMD treatment were too narrowly focussed on inflammation and the reduction of relapses in the short term. Studies aimed at investigating the effect of IMD on progression were of too short duration and relied on surrogate outcome measures with uncertain validity. Our finding that IMD treatment may reduce the risk of of secondary progression suggests a beneficial effect of IMD on the processes underlying progression, but findings from observational studies should not be taken as proof of a treatment effect. Well conducted and preferably publicly funded randomised controlled trials, which use the onset of secondary progression as primary outcome, are needed to evaluate the effect of IMD on progression.

An important step towards a better understanding of the pathophysiology of progression in MS could come from the identification of genes associated with progression. Most genetic research in MS did not distinguish between disease subtypes. Our investigation of the influence of a positive family history of MS on progression suggests that such a distinction by disease subtype may be useful for future genetic research, and identified primary progressive MS as the disease subtype that may be especially promising for future genetic studies.

While the other epidemiological studies did not provide 'positive' results, they showed that fatigue, depression, cigarette smoking and the timing of birth are probably all unrelated to the disease process of progression.

Our laboratory studies on oligoclonal bands and oxidative stress found no important effect of these measures on progression. Oligoclonal bands are the result of an inflammatory reaction in the intrathecal space and oxidative stress is believed to occur most prominently in the circumscript inflammatory plaques. Our finding that both measures are unrelated to progression argue against a major role of inflammation in the pathophysiology of progression. Future research on the pathophysiology of progressive MS and axonal degeneration should therefore shift its focus from inflammation and explore alternative underlying mechanisms.

There is currently no simple laboratory marker of progression in MS. A biomarker of axonal degeneration in MS would be very useful as a surrogate marker in clinical trials, but also as a simple method to monitor axonal degeneration and evaluate the effect of future treatments in individual patients. In the last study included in this thesis, we found that plasma NSE levels are inversely correlated with progression in MS. This suggests that peripherally measured NSE may be a measure of the axonal reverse. Its usefulness as a biomarker of progression in MS needs to be evaluated in subsequent longitudinal studies.

Samenvatting in het Nederlands

MS-patiënten met een progressief ziektebeloop hebben de slechtste prognose. Zij kunnen verwachten dat hun symptomen geleidelijk toe zullen nemen en er is op dit moment geen behandeling met een bewezen effect op progressieve MS. Ons doel was om met dit proefschrift meer te weten te komen over progressie, door de invloed van een aantal mogelijk voor het ziektebeloop voorspellende variabelen te onderzoeken.

Epidemiologische aspecten van progressie

In **Hoofdstuk 2** hebben wij patiënten met primair en secundair progressieve MS geïncludeerd en de invloed van verschillende mogelijke risicofactoren op het tijdstip van progressie onderzocht. Wij hebben vastgesteld dat progressie een leeftijdsafhankelijk fenomeen is en dat de leeftijd waarop de progressie begint niet verschilt tussen patiënten met primaire en secundaire progressie. Bovendien bleek de tijd tot het begin van secundaire progressie sterk afhankelijk te zijn van het tijdstip van ziektebegin. In dit hoofdstuk hebben wij ons beperkt tot de patiënten die reeds een progressief ziektebeloop hadden ontwikkeld en als gevolg daarvan konden wij de invloed van immuunmodulerende middelen (IMM) niet beoordelen.

In **Hoofdstuk 3** hebben wij bij patiënten met een relapsing-remitting ziektebegin gepoogd te achterhalen welke factoren een rol spelen bij het optreden van secundaire progressie. De inclusie van alle patiënten met een relapsing-remitting begin maakte het mogelijk om dit keer ook IMM als mogelijk voorspellende factor in de analyses mee te nemen. Naast de bevestiging van onze eerdere bevinding dat het ontstaan van progressie afhangt van de leeftijd waarop de ziekte begint, hebben wij ook gevonden dat IMM onafhankelijk geassocieerd waren met een kleiner risico op secundaire progressie. Deze bevinding kan beter als hypothesegenererend worden beschouwd dan als bewijs voor een behandelingseffect en wij hebben dan ook geprobeerd te benadrukken dat nieuwe gerandomiseerde en gecontroleerde studies noodzakelijk zijn om het effect van IMM op progressie te kunnen beoordelen.

Eerdere epidemiologische studies op het gebied van MS hebben vaak de tijd van het ziektebegin tot het ontwikkelen van bepaalde invaliditeitss-

cores als belangrijkste uitkomstmaat gebruikt. Het speciale belang voor de prognose van het ontwikkelen van een progressief ziektebeloop en het pathofysiologische verschil met de relapsing-remitting vorm van de ziekte benadrukken dat men in zulke studies beter de uitkomstmaten 'tijd tot ontwikkelen van progressie' en 'leeftijd bij het begin van progressie' kan gebruiken. Wij hebben in de eerste twee hoofdstukken deze twee uitkomstmaten gebruikt om het natuurlijke beloop van de ziekte epidemiologisch te bepalen.

In de **Hoofdstukken 4 tot en met 6** hebben wij van deze uitkomstmaten gebruik gemaakt om een aantal mogelijk voorspellende factoren te onderzoeken. In **Hoofdstuk 4** hebben wij de invloed van een positieve familieanamnese voor MS op deze uitkomstmaten onderzocht en gevonden dat patiënten met een familiaire primair progressieve MS significant jonger zijn aan het begin van de ziekte dan patiënten zonder een familieanamnese van MS. Dit suggereert dat deze patiëntengroep bijzonder interessant zou kunnen zijn voor toekomstig genetisch onderzoek naar MS.

De invloed van de zeer frequent vóórkomende symptomen vermoeidheid en depressie op de prognose van MS werd onderzocht in **Hoofdstuk 5**. Wij konden geen belangrijke invloed van deze symptomen op progressie vinden en bovendien hebben wij vastgesteld dat de mate van vermoeidheid en depressie niet significant veranderde in de loop van tien jaar, terwijl de invaliditeit toenam. Dit pleit tegen het idee dat vermoeidheid en depressie het gevolg zijn van progressieve axonale degeneratie.

Hoofdstuk 6 behandelt roken en het risico van progressie. Roken is een bekende risicofactor bij vele ziekten en eerdere studies hebben gesuggereerd dat het risico om MS te ontwikkelen verhoogd is bij rokers. Eén eerdere studie heeft gesuggereerd dat roken ook het risico op secundaire progressie zou beïnvloeden. In onze studie konden wij geen belangrijk effect van roken op progressie bij MS aantonen.

In **Hoofdstuk 7** zijn wij nagegaan of het tijdstip van de geboorte een effect heeft op progressie bij MS. Recent werd ontdekt dat het tijdstip van de geboorte het risico op het krijgen van MS beïnvloedt, maar het was onbekend of het tijdstip van de geboorte ook effect heeft op het beloop van de ziekte.

Voor deze analyses hebben wij samengewerkt met onze collegae van de Universiteit van Brits Columbië, Vancouver, Canada, om mogelijke effecten van het tijdstip van de geboorte in twee grote cohorten te onderzoeken. Er waren significante effecten van het tijdstip van de geboorte op meerdere uitkomstmaten, maar geen van de potentiële factoren die in de ene cohort opvielen konden in de andere bevestigd worden. Wij concluderen dat het tijdstip van de geboorte geen belangrijk effect heeft op progressie bij MS en dat de statistisch significante effecten die in elke cohort gevonden werden waarschijnlijk op toeval berustten.

Deze samenwerking maakt tegelijkertijd duidelijk dat bevindingen in epidemiologische studies in meerdere cohorten bevestigd dienen te worden.

Biochemische merkers en progressie

In de **Hoofdstukken 8 tot en met 10** hebben wij de invloed van meerdere biochemische merkers op progressie in de volgende vijf jaar onderzocht.

Hoofdstuk 8 behandelt het belang van oligoclonale banden in de liquor voor de progressie bij MS. Wij konden geen significante verschillen in ziekteprogressie vaststellen tussen patiënten met en zonder oligoclonale banden. Dit pleit tegen de eerdere mening dat patiënten zonder oligoclonale banden een betere prognose zouden hebben.

Traditioneel denkt men dat oxidatieve stress een factor is die het ziektebeloop van MS ongunstig zou beïnvloeden. In **Hoofdstuk 9** hebben wij onderzocht of verschillen in plasmaconcentraties van lipiden peroxidatie producten, een robuuste merker van oxidatieve stress, geassocieerd zijn met de toename van invaliditeit in de volgende vijf jaar. Wij vonden geen associatie en kunnen dan ook het gebruik van antioxidatieve middelen of diëten bij patiënten met MS niet ondersteunen.

Verschillende studies hebben gepoogd om biochemische merkers (zogenaamde 'biomarkers') te identificeren, die met de progressie van MS geassociëerd zijn. In **Hoofdstuk 10** presenteren wij onze bevindingen voor twee plasma biomarkers, S100beta and neuron specifieke enolase (NSE). Terwijl er geen verschillen waren in S100beta concentraties tussen patiënten die progressief of stabiel waren, bleken de NSE concentraties invers gecorreleerd te zijn met ziekteprogressie bij MS. Dit suggereert dat perifeer gemeten NSE een bruikbare biomarker van progressie bij MS zou kunnen zijn, maar verder onderzoek hiernaar is noodzakelijk.

Dankwoord

Allereerst wil ik mijn promotor prof. De Keyser danken voor zijn geduldige en inspirerende begeleiding bij dit onderzoek.

De leden van de leescommissie, professores De Deyn, Dierckx en Kuks wil ik bedanken voor het beoordelen van het manuscript.

Jop Mostert, Thea Heersema en alle overige co-auteurs van mijn artikelen wil ik bedanken voor hun nuttige commentaren en hun inhoudelijke bijdrage aan dit onderzoek.

Maarten Uyttenboogaart, die zichzelf met enige trots "Strokoloog" noemt, wil ik bedanken dat hij zich heeft laten verleiden over multiple sclerose mee te denken en een belangrijke bijdrage te leveren aan een deel van de artikelen in dit proefschrift.

Annemarie van Harten was de eerste studente-onderzoekster die ik als supervisor heb mogen begeleiden. Ik wil haar danken voor haar inzet tijdens haar wetenschappelijke stage en voor de prettige samenwerking.

De typisch Nederlandse traditie van de paranimf was voor mij nieuw. Inmiddels ben ik te weten gekomen dat één van de traditionele taken van de paranimf erin bestaat de promovendus in het geval van een hoog oplaaiende en tot handgemeen leidende academische strijd lichamelijk te beschermen. Jeroen de Vries en Harold Weitenberg wil ik bedanken dat zij deze taak desondanks zonder aarzelen op zich hebben willen nemen.

Curriculum Vitae

Marcus Koch was born on the 21st of february 1977 in Lübeck in northern Germany. He graduated from grammar school (Ernestinenschule, Lübeck) in 1996. After one year of 'alternative service' (in lieu of military service), he began his preclinical medical studies at the University of Freiburg in 1997, and later changed to the Medical University Lübeck for the clinical part of the study. He obtained his medical degree in 2003 and has been working as a resident in clinical neurology at the University Medical Center Groningen since april 2004.

He has been involved in research since the time of his study, and he obtained the degree MD for research in microbiology in 2005. His current research interests are multiple sclerosis and stroke.

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