ABSTRACT

Objective: To explore the long-term outcomes of clinically isolated syndromes (CIS) patients from the Barcelona cohort. Methods: From a consecutive CIS cohort, we selected patients with a follow up longer than 10 years to: 1) estimate the risks of MS and disability accumulation according to baseline T2 lesion number as well as to study the course of the disease for treated patients vs. untreated and early treated vs. delayed treated; 2) to study the prevalence and baseline features of patients with an aggressive MS phenotype, defined as patients with an EDSS $\geq$ 6.0 at 10 years. Results: 401 patients were included with a mean follow-up of 14.4 (standard deviation 2.9) years. A higher number of T2 lesions was associated with an earlier MS diagnosis and an earlier risk of irreversible disability. Early treatment was associated with a decreasing risk of EDSS 3.0 (adjusted HR, confidence interval 95%) aHR = 0.4 (0.2, 0.7). Less than 5% of the patients had an aggressive MS. Patients with an aggressive phenotype mainly differed in their baseline brain MRIs: the median (IQR) number of baseline T2 lesion was 71 (28-95) vs 7 (1-19) and contrast enhancing lesions (CEL) was 3 (1-24) vs 0 (0-1). The cut-offs that better classify patients with aggressive MS were 20 for T2 lesions and 2 for CEL. Conclusions: Although MS natural history is changing and aggressive MS is infrequent in the treatment era, a high lesion load (more than 20 T2 lesions and more than 2 CEL) at onset is helpful to identify patients at risk for an aggressive MS.

INTRODUCTION

MS is a chronic disease. Classical natural history studies have shown that more than 50% of the patients will develop severe disability after 15 to 30 years (1-4). However, when considering inception cohorts of patients seen from their very first attack, the rates for disability accumulation seem to be milder. In this sense, the clinically isolated syndromes (CIS) cohort from the National Hospital for Neurology and Neurosurgery, Queen Square showed that only 25% had reached an Expanded Disability Status Scale EDSS of 6.0 after 20 years of follow-up (5). Despite these figures, there is still a subgroup of MS patients with an aggressive disease who develop severe disability early in the disease course. Although there is not an established definition of aggressive MS, the early identification of this population would be ~~nowadays~~ of utmost importance for establishing an accurate and personalized treatment strategy (6).

The aim of the present study was to explore the long-term outcomes of CIS patients from the Barcelona inception cohort: for this objective, we selected patients with a follow up longer than 10 years to: 1) estimate the risks of MS and disability accumulation according to baseline T2 lesion number and to compare treated and untreated and patients with an early vs. delayed treatment; 2) to describe the prevalence and baseline features of patients with an aggressive MS phenotype, defined as patients with an EDSS $\geq$ 6.0 at 10 years of the disease.

PATIENTS AND METHODS

Study data and inclusion criteria

~~This is an observational study based on a prospective, open CIS cohort initiated in 1995~~

This is a retrospective analysis based on a prospective and open CIS cohort initiated in 1995. The cohort includes patients younger than 50 years who presented with a CIS suggestive of MS and with clinical symptom onset within three months of our first

assessment. At baseline, we recorded the demographics, topography of the CIS, and disability according to the EDSS score. EDSS score and the occurrence of relapses were evaluated every three to six months or annually depending on each patient's characteristics. For the purpose of this analysis, the database was locked on February 15th, 2016. For the main analysis of the study, we included patients with a CIS before 15.02.2006 and with at least 10 years of follow up. IgG oligoclonal bands (OB) were examined within the first three months of disease onset via agarose isoelectric focusing combined with immunoblotting (7). OB were considered positive when demonstrated in CSF only and at a number of at least two.

Since 1996, DMT was offered to patients presenting with at least two attacks in the previous three years according to the Catalonian Regulatory Agency guidelines. After 2001, patients presenting with a high-risk CIS (defined as the fulfilment of 3-4 Barkhof criteria) were also candidates for treatment. Age and sex of the patient, date of the CIS, CIS topography, steroid treatment, date of the second attack, EDSS measurements, date of DMT initiation and date of the most recent visit were prospectively collected. The number and location of T2 baseline lesions, the presence of contrast-enhancing lesions (CEL) and the number of new T2 lesions were recorded during the follow up. For the purposes of this study, the number of T2 baseline lesions was divided into 4 categories: 0, 1-3, 4-9 and 10 or more T2 lesions. Additionally, a normal brain MRI was defined as displaying zero T2 lesions.

Finally, the patients' clinical and MRI data were entered and updated on a regular basis. Quality controls were performed including a review of the data using the primary sources of 10 randomly selected patients every month. This study received approval from the local ethics committee, and all patients signed a written informed consent form.

MRI protocol

A diagnostic brain MRI at the time of the first event was performed three to five months after the CIS and repeated after 12 months and then every five years. Baseline spinal cord MRIs were systematically performed since the beginning of 2007 and therefore they were not considered in this study. Brain MRI was performed using a 1.5 or 3.0 T magnet and included the following sequences: transverse proton density and T2-weighted conventional or fast spin-echo, transverse and sagittal T2-FLAIR, and un-enhanced and contrast-enhanced (0.1-2.0 mmol/kg; scan delay, 5-10 minutes) T1-weighted spin-echo. All sequences were obtained using a contiguous 3-5 mm slice thickness covering the entire brain. ~~Brain MRI was not part of the inclusion criteria and therefore patients with a normal brain MRI could be included in the Barcelona CIS cohort if the clinical picture was suggestive of MS (as an example an optic neuritis with a normal brain MRI)~~ Having a normal brain MRI was not an exclusion criterion, since if the patient showed a clinical picture suggestive of MS (for instance, an optic neuritis with a normal brain MRI), this patient was included.

Definition of the outcomes

The 2017 McDonald criteria were applied to patients included after 2002 as lesion topography was not assessed individually before this date. For patients included from 1995 to 2002, the 2005 McDonald criteria were used (8, 9). CDMS was established when new symptoms suggestive of relapse occurred after an interval of at least one month (10). Disability was evaluated according to the EDSS scale at each visit during stability periods (11). The primary disability milestones were defined as reaching an EDSS score equal to or greater 3.0 or 6.0 in two distinct evaluations. Finally, the follow-up duration was computed as the time elapsed between the date of the CIS and the date of the most recent visit.

Statistical analysis

Kaplan-Meier risk estimates were obtained for the times to McDonald MS, CDMS, EDSS score of 3.0 and 6.0 according to the number of T2 baseline lesions. Instead of representing the resulting estimates using curves, we have used heat maps to represent the risk of achieving each of the four outcomes along the follow-up. In the heat maps, columns are the follow up in years and rows indicate the number of baseline lesions. Thus, each cell of the map shows the estimated risk (with its 95% confidence interval) and the number of patients under risk within the time interval for that number of baseline lesions. Heat maps offer the possibility of using colours as a scoring gradient that goes from blue (low risk) to red (high risk). Besides, the same range of colours have been used in the four heat maps to allow for an easier comparison of risks between outcomes. (Figure 2). Univariable and multivariable Cox PH proportional hazards regressions were built for the previous cited outcomes. Covariates including age, sex, CIS topography, OB, baseline number of T2 lesions MRI criteria and DMT status were considered. This last variable was firstly considered as a time dependent exposure and modelled later as an indicator of whether the DMT was initiated before or after the second attack. Possible interactions between age, sex, CIS topography, presence of OB, number of baseline T2 lesions and DMT were also evaluated (Figure 3). We defined the aggressive MS phenotype as reaching an EDSS $\geq$ 6.0 at year 10 years. We compared patients who present this phenotype to those who did not in terms of sex, age at CIS, CIS topography, OB status, and number of T2 and CEL at baseline (Table 3 and Figure 4). Next, we looked for the best cut-off in the number of baseline T2 lesions and CEL that predicted better the development of an aggressive MS. Both cut-offs were defined as those with the best balance between sensitivity and specificity rates. Finally, we have combined both MRI information in order to estimate the positive predictive values (PPV) of developing aggressive MS (Table 4).

A p value of 0.05 was considered statistically significant. All analyses were performed using SPSS 22.0 (SPSS Inc. Chicago, IL, USA) and R 3.5.1 (R Foundation for Statistical Computing).

RESULTS

Study population

From January 1995 to February 15th, 2016, 1207 patients were enrolled in the prospective CIS cohort; 70 (5.8%) were ultimately excluded for various reasons: previous attack (n=13), age over 50 (n=4), exceeded entry window (n=26), and alternative diagnoses (n=27). These alternative diagnoses included acute disseminated encephalomyelitis (n=1), neuromyelitis optica spectrum disorder (n=5), chronic relapsing inflammatory optic neuritis associated with anti-MOG antibodies (n=1), brain tumour (n=5), ischemic stroke (n=2), CADASIL (n=1), anterior ischemic optic neuropathy (n=3), Leber's hereditary optic neuropathy (n=1), central nervous system vasculitis (n=1), atypical brainstem lesions (n=2), alcoholic polyneuropathy with vitamin B deficiency (n=1), musculoskeletal disorders (n=2), unspecified sensory symptoms (n=1), and unspecified ophthalmological condition (n=1).

According to the database lock date, we identified 562 cases with a CIS before February 15th, 2006 and at least one follow up visit (Figure 1). Of these, 161 (28.6%) patients had an incomplete follow up and 401 (71.3%) completed the 10 years follow up (Figure 1). Patients with an incomplete follow-up were similar in age but were more likely to have a normal baseline MRI and negative OB (Supplementary Table 1).

Ultimately, 401 patients were included in this analysis. Of these, one died during follow-up due to an acute myeloid leukaemia with a complex karyotype not related to MS treatment.

Table 1 shows the baseline characteristics of this long-term cohort: as expected two out of three were females, the mean age at onset was 30 years and a one third presented with an optic neuritis. The mean clinical follow-up duration was 14.4 (SD 2.9) years. Of the total cohort, 334 patients underwent a CSF tap, among whom 221 (66.2%) had positive OB. The baseline brain MRI was normal in 20.5% of the patients, almost half of the patients displayed 10 or more T2 lesions, and one third showed at least one CEL. Out of the entire cohort, 190 (47.4%) patients received no DMT; 55 (13.7%) had initiated DMT prior to their second attack and 156 (38.9%) after their second attack.

The median time from CIS onset to drug prescription was 23.5 months (IQR: 8.3-63.2).

Long-term outcomes: CDMS, McDonald MS, EDSS 3.0 and 6.0

Figure 2 shows the risk of reaching each of the four outcomes according to the number of baseline T2 lesions. A higher number of T2 lesions is associated with an earlier second attack and an earlier McDonald MS diagnosis. Additionally, the risk of irreversible disability is especially worrisome in patients with at least 10 baseline lesions in which 30% reached an EDSS of 3.0 and 7% needed a cane before 10 years. These numbers raised to 39% and 9% respectively within the first 15 years of the disease.

~~Sensitivity analysis incorporating cases with a potential follow-up of 10 years, showed very similar results (data not shown).~~

Long term outcomes: treated versus not treated patients

Patients who were treated had clearly a more aggressive phenotype (Supplementary Table 2). It is worth highlighting that covariates were not balanced across treated and untreated groups. Thus, time-dependent variables could not control for this unbalance and no further analysis comparing treated and untreated patients were performed.

Long term outcomes in treated patients: DMT before or after second attack

Table 2 compares the baseline characteristics of patients who received an early treatment (median time: four months) and patients starting treatment after the second attack (median time: 36 months). Although patients who received an early treatment had a baseline MRI with higher number of T2 and CEL lesions, uni- and multivariable analyses showed that treatment before the second attack was associated with a decreased risk (aHR = 0.4 (0.2, 0.7)) of reaching EDSS 3.0 (Figure 3). When analysing EDSS 6.0 as the outcome, the hazards proportionality assumption was violated (p-value of 0.2 in the log-rank test) and therefore no further research on this outcome was performed.

Aggressive MS: EDSS 6.0 at 10 years

Table 3 compares the baseline characteristics for patients who presented this aggressive phenotype to those who did not. There were no differences in sex and age at CIS. In terms of CIS topography, there was a lower proportion of optic neuritis and a higher proportion of spinal cord CIS in patients with aggressive MS although these differences were not statistically significant. On the other hand, there were evident differences in the baseline MRI variables: The median (IQR) number of baseline T2 lesion was 71 (28-95) compared to 7 (1-19) and for CEL it was 3 (1-24) compared to 0 (0-1) ($p<0.0001$, for both tests) (Figure 4).

Patients displaying 20 T2 lesions at baseline and having 2 CEL were the cut-offs that best discriminate patients with aggressive MS to those who did not present this phenotype. Table 4 shows the PPVs for developing aggressive MS for several combinations of baseline T2 and CEL. It is important to note that both information (number and activity of the lesions) seem to contribute to better predict patients with an aggressive phenotype. If MRI information is not considered, the risk of having aggressive MS for patients in our cohort is 4.7% (Table 4). For patients having more than 20 T2 lesions and more than 2

CEL, this risk increases to 19.0% (10.4%, 28.1%) and to 40% (14.3%, 71.4%). for those having more than 20 T2 lesions and more than 10 CEL .

DISCUSSION

Our study confirmed that when the baseline MRI is abnormal a high proportion of patients with CIS presented with a second attack in the long-term. Moreover, almost all patients developed McDonald MS. The heat maps show that a higher number of lesions is associated with an earlier outcome. Less than 5% of our patients developed an aggressive MS (reaching an EDSS status of 6.0 at 10 years). It is worth highlighting that baseline characteristics such as sex and age were not helpful in identifying patients at risk although a lower proportion of optic neuritis and higher proportion of spinal cord CIS was observed. In patients with aggressive MS, a higher proportion of OB was found in comparison to patients with a milder MS phenotype (90.0% vs. 65.4%). Comparing patients with an aggressive phenotype to those with a more standard evolution, the former mainly differed in their baseline brain MRIs: the median number of baseline T2 lesion was 71 (28-95) compared to 7 (1-19) and the median for CEL was 3 (1-24) compared to and 0 (0-1). The cutoffs that better classify patients with aggressive MS were 20 for T2 lesions and 2 for CEL. It is important to note that there is a lack of an accepted definition of what constitutes and defines aggressive MS and a lack specific guidelines for its treatment.

A high proportion of our patients received DMT during their follow up, but for the majority of them, there was a long time between the first clinical event and treatment onset. These data reflect our clinical practice two decades ago when the concept of early treatment was in its infancy. Treated patients had a more aggressive disease phenotype than not treated patients and for this reason we were unable to match both populations. In

our study, after excluding non-treated patients, it appeared that treatment before second attack could decrease the risk of disability accumulation, highlighting the importance of early treatment. As a rule patients were treated with first line treatments. This study is in line with our previous work analysing our whole cohort of 1,058 CIS patients followed up for a mean of 6.5 years, in which we showed that MRI features (number and topography of T2 lesions) were the main prognostic factor at disease onset and that initiation of a DMT before a second attack could reduce the risk of disability accumulation(12). Focusing on patients with more than 10 T2 lesions at baseline, compared to the London cohort we found similar rates of patients presenting with a second attack (80% versus 85% at 10 years) (13, 14). Although a small proportion of our patients started DMT before their second attack (n=55), compared to the London CIS cohort largely untreated, these similar rates of conversion probably reflect the fact that first line DMTs (mainly interferons and glatiramer acetate in our cohort) only delay the occurrence of second attack in the short medium term. Again, in patients with at least 10 baseline T2 lesions we found lower rates of disability accumulation than the London cohort (30% versus 75% for EDSS 3.0 and 7% versus 35% for EDSS 6.0 at 10 years). Our rates of disability are closer to other treated cohorts: The BENEFIT cohort reported 30% of patients with an EDSS of 3.0 and 6% with an EDSS of 6.0 after 11-year follow-up (15). This is also in line with the EPIC cohort, a prospective single centre cohort including actively managed CIS and early MS patients which showed 4.7% of patients reaching an EDSS of 6.0 after 10 years (16). Taking all together, and considering other important factors such as the Will-Rogers phenomenon derived from diagnostic criteria modifications and improvement in general health among others, our data seem to confirm that natural history is changing in the treated era. Others have addressed the impact of first line treatments on long-term disability in relapsing MS patients (17)(18). This

protective effect is modest in patients treated with first line drugs with and estimated a prevention of 1.0 EDSS point increase for every 11.6 years of interferon-beta/glatiramer acetate exposure (19).

Limitations of our study include the following aspects: MRI criteria to define MS have changed overtime and therefore not all patients had the required information to apply the 2017 McDonald criteria. To overcome this limitation a combination of 2005 and 2017 criteria has been used. Another concern relates to the lack of spinal cord imaging in this analysis. Unfortunately, this limitation reflects our practice at a time where spinal cord MRI was only performed in patients presenting with a myelitis. The treatments that were available at that time were mainly interferons and glatiramer acetate. The use of other treatments was restricted to exceptional cases. Again, only an intention to treat analysis has been performed taking into consideration the date of initiation of DMT. However, time on treatment or changes among preparations have not been evaluated. We also acknowledge that this is a cohort culturally and genetically homogeneous, as non-caucasian people were an exception in our place. We are also aware that the conclusions referring to patients with aggressive MS are based on a limited number of patients. However, this limitation also refers to the exceptionality of this endpoint.

The long-term Barcelona cohort confirms that patients with a normal baseline MRI have a low risk of developing MS after 10 or 15 years of follow-up. Conversely, patients with an abnormal MRI have a very high risk of developing further attacks or McDonald MS. In line with other cohort studies, we showed that early treatment seems to prevent disability accrual. Our long-term study confirms that MS natural history has changed and that aggressive MS defined as reaching an EDSS 6.0 at 10 years is infrequent in the treatment era. A high lesion load (more than 20 T2 lesions and more than 2 CEL) at onset was helpful to identify patients at risk for an aggressive MS. Establishing a lesion number

cut-off could help to identify patients that are candidates to receive highly effective drugs (20).

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DISCLOSURES

M Tintore has received compensation for consulting services and speaking honoraria from Almirall, Bayer Schering Pharma, Biogen-Idec, Genzyme, Merck-Serono, Novartis, Roche, Sanofi-Aventis, and Teva Pharmaceuticals. MT is co-editor of Multiple Sclerosis Journal-ETC

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X Montalban has received speaking honoraria and travel expenses for participation in scientific meetings, has been a steering committee member of clinical trials or participated in advisory boards of clinical trials in the past with Actelion, Amirall, Bayer, Biogen, Celgene, Genzyme, Hoffmann-La Roche, Novartis, Oryzon Genomics, Sanofi-Genzyme and Teva Pharmaceutical. MJ Arévalo, L Midaglia, I Galán, C Espejo, J Castilló, P Mulero, B Rodríguez-Acevedo, S Pérez-Hoyos, R Mitjana, M Rodriguez, E Anglada, R Menendez, Ana Zabalza and A de Barros report no disclosures.

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