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**Title****Prognostic indicators in pediatric clinically isolated syndrome****Authors**

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## Abstract

**Objective:** To assess prognostic factors for a second clinical attack and a first disability worsening event in pediatric clinically isolated syndrome (pCIS) suggestive of Multiple Sclerosis (MS) patients.

**Methods:** A cohort of 770 pCIS patients was followed-up for at least 10 years. Cox proportional hazard models and REcursive Partitioning and AMalgamation (RECPAM) tree-regression were used to analyze data.

**Results:** In pCIS, female sex and a multifocal onset were risk factors for a second clinical attack (HR, 95% CI: 1.28, 1.06-1.55; 1.42, 1.10-1.84, respectively), whereas disease modifying drugs (DMDs) exposure reduced this risk (HR, 95% CI: 0.75, 0.60-0.95). After pediatric onset MS (POMS) diagnosis, age at onset younger than 15 years and DMDs exposure decreased the risk of a first EDSS worsening event (HR, 95% CI: 0.59, 0.42-0.83; 0.75, 0.71-0.80, respectively), whereas the occurrence of relapse/s increased this risk (HR, 95% CI: 5.08, 3.46-7.46).

An exploratory RECPAM analysis highlighted a significant higher incidence of a first EDSS worsening event in patients with multifocal or isolated spinal-cord or optic neuritis involvement at onset in comparison to those with an isolated supratentorial or brainstem syndrome. A Cox regression model including RECPAM classes confirmed DMDs exposure as the most protective factor against EDSS worsening events and relapses as the most important risk factor for attaining EDSS worsening.

**Interpretation:** This work represents an important step forward in identifying predictors of unfavorable course in pCIS and POMS and supports a protective effect of early DMDs treatment in preventing MS development and disability accumulation in this population.

## Introduction

Patients with pediatric onset (before the age of 18 years) multiple sclerosis (POMS) represent 3-10 % of the total MS population.<sup>1-12</sup> An onset before age 10 is even less frequent, accounting probably for less than 1% of total MS cases.<sup>9, 13-15</sup> The estimated annual incidence of POMS ranges between 0.13 and 0.6/100.000 in different countries.<sup>10, 12, 15-18</sup> POMS usually starts with the occurrence of a first attack of demyelination, termed pediatric clinically isolated syndrome (pCIS),<sup>19</sup> characterized by a monofocal or multifocal clinical central nervous system event of presumed inflammatory demyelinating cause with acute or subacute onset in the absence of encephalopathy, not explained by fever or systemic illness and that does not meet the 2010 MS McDonald criteria on baseline MRI.<sup>20</sup> The majority of children with pCIS experience a second clinical attack and consequently convert to clinically definite MS (CDMS) within a variable time ranging between 11 and 71.3 months.<sup>6, 8-10, 21-23</sup> POMS subjects tend to have higher relapse rate,<sup>24, 25</sup> higher magnetic resonance imaging (MRI) lesion accrual<sup>26</sup> and more prominent cognitive deficits<sup>27</sup> early in their disease course than adult onset MS (AOMS).

Although time to conversion to a secondary progressive (SP) course is longer in POMS than in AOMS, SP patients' median age is lower in POMS in comparison to AOMS, suggesting that POMS is not a more benign disease<sup>7, 9</sup> in comparison to AOMS. Recent MRI data have demonstrated that POMS have a smaller overall brain volume than would be expected for age,<sup>28</sup> suggesting that demyelinating lesions may impact brain growth and development. For this reason, although the current available disease modifying drugs (DMDs) are not licensed for POMS, their off-label prescription is increasing in this sub-population.<sup>29, 30</sup>

Prognostic demographic, topographic, clinical (age, sex, symptoms at first presentation, relapses after the first attack), MRI (number of brain T2 lesions) and laboratory (Cerebrospinal fluid-restricted IgG oligoclonal bands - CSF OB -) factors predicting

conversion to CDMS or the risk of disability accumulation over time have been extensively studied in adult CIS.<sup>9,31-46</sup>

As POMS is a rare disease, very few studies on small populations tried to determine which patients with pCIS are at highest risk for CDMS and disability worsening. Predictors for an increased risk of time to second attack in the KIDMUS study,<sup>8,9,47</sup> the largest prospective series of pCIS to date, included demographic (age higher than 10 years) and topographic (optic neuritis–ON) characteristics, and MRI features (multiple well-defined periventricular or subcortical lesions suggestive of MS) at onset. Myelitis or altered mental status impairment at onset were associated with a decreased risk of conversion to CDMS.<sup>8,47</sup>

Abnormal cranial MRI, presence of CSF OB and age were confirmed as independent predictors of conversion to CDMS in a series of children with isolated ON.<sup>48</sup>

Occurrence of severe disability and SP course in pCIS were<sup>8,9,47,48</sup> more frequently found in children with disability sequelae after the first attack, a short interval between the first two demyelinating episodes, number of relapses and progressive onset. However, there was no consistent correlation between gender, age at onset, or a polysymptomatic vs monosymptomatic onset, in disease course prognosis,<sup>6,47,49,50</sup> that it is still challenging to identify children who could benefit from very early initiation of a DMD treatment.

Although several randomized clinical trials<sup>51-56</sup> (RCTs) and their extension phases<sup>57,58</sup> demonstrated that early treatment with DMDs can delay conversion to CDMS and accumulation of medium to long-term disability in adult onset CIS patients, comparable evidence is currently lacking in pCIS.

The aim of this multicenter, collaborative study was to assess prognostic factors, including DMDs exposure, for time to second clinical attack and to first disability worsening event in a large cohort of pCIS prospectively collected and followed up to 10 years in two large registries: The Italian iMedWeb registry and MSBase registry.

## Methods

### Ethics Statement

The Italian iMedWeb network was approved by the Policlinico of Bari Ethics Committee and by the local ethics committees in all participating centers. The MSBase Registry was approved by the Melbourne Health Human Research Ethics Committee and by the local ethics committees in all participating centers. Written informed consent was obtained from all enrolled patients, or in the case of pediatric patients from their parents, in accordance with the Declaration of Helsinki.

### Study population

This was a large, multi-center, retrospective observational study performed on prospectively acquired data. Longitudinal data from pCIS patients, with an age at onset before 18 years and with a first clinical visit within 1 year from the disease onset, were extracted from the Italian iMedWeb registry and the MSBase registry in June 2015.

All the participating centers use the iMed software to collect uniform information about all patients with MS who have been examined as outpatients or inpatients. Information is collected by well-trained neurologists in a retrospective manner at the first visit, and prospectively every six months thereafter. Quality assurance through online certification of Expanded Disability Status Scale (EDSS) competency is required at each participating site. Patients included in this analysis had a diagnosis of pCIS or POMS.<sup>19</sup> Patients with a diagnosis of monophasic or recurrent disseminated encephalomyelitis (ADEM) were not included in the analysis, whereas patients with an ADEM-like onset and a second non-encephalopathic clinical attack were considered.<sup>19</sup>

Patients with a progressive disease course from onset were excluded from this study.

Baseline data included demographics, date of onset and topography of pCIS (isolated ON, isolated spinal syndrome, isolated supratentorial syndrome - including ADEM-like onset -,

isolated brainstem syndrome; or multifocal if more than two of these locations were involved) and disability levels according to the EDSS score. Brain MRI features as well as CSF data regarding presence/absence of OB were also extracted, if available.

Follow-up data collected approximately biannually included: date of visit, date of MS diagnosis, EDSS score, relapses, DMD treatment prescription (date of start and end of each treatment) since the patient's last visit. Date of Brain MRI follow-up was also recorded.

A minimum of three visits per patient spanning a minimum 9 months, with full EDSS evaluation was required to define a minimum 3-month confirmed disability worsening event.

Disability worsening was defined as a minimum one-point increase in EDSS score above a baseline value, if the baseline EDSS was 1- 5.5, or one and a half-point increase if the baseline EDSS was zero, or half-point increase above baseline EDSS scores equal to or

higher than 6.0. A confirmation at repeat assessment at least 3 months later was required to confirm the EDSS worsening event. EDSS scores recorded during relapses were excluded.

Brain MRI data were included as a prognostic factor, if performed within 1 year from the onset and before the occurrence of a second attack or the first EDSS worsening event. Brain MRI T2 lesion load was classified according to the following criteria: 0-2 lesions, > 2 lesions.

CSF data were also retrieved. CSF data were recorded as presence/absence of CSF OB.

### **Statistical Analyses**

In descriptive analyses, continuous covariates were summarized as median and interquartile range (IQR), and categorical variables were expressed as frequency and percentages. Median times from onset to each outcome were based on Kaplan-Meier estimates.

Univariate and multivariate Cox proportional hazard regression models were performed to identify predictive factors for shorter time to second attack or first 3 months confirmed EDSS worsening event.

For the analysis to the 2<sup>nd</sup> attack, the date of onset was considered as time of origin in the Cox model. For the analysis to first EDSS worsening event, the date of MS diagnosis was used as time of origin in order to more properly evaluate predictor factors of disability worsening after excluding pCIS patients who did not convert to MS during the follow-up and thus with lower probability of having the disability worsening. Times to events were calculated from the date of origin to the date of outcome occurrence or last follow-up.

In both univariate and multivariate analysis models the following covariates were tested: sex, age at onset ( $\leq 12$ , 12-15 and  $> 15$ ), symptoms at onset (isolated ON, isolated myelitis, isolated supratentorial syndrome, isolated brainstem syndrome or multifocal symptoms), brain MRI T2 lesions ( $\leq 2$  and  $> 2$ ), CSF OB (positive and negative), and decade of birth and treatment (handled as time-dependent covariate). For the time to a first confirmed EDSS worsening event, relapses occurring before disability progression were included as a time dependent covariate and relapses (1 vs 2) and DMDs exposure (Yes vs No) before the MS diagnosis were also considered.

For the multivariate models, multiple imputation with expectation-maximization (EM) and bootstrapping was used to overcome the presence of missing data.<sup>59</sup>

The missing values of the brain MRI T2 lesions and of the CSF OB were imputed based on a multivariate linear model using all the covariates included in the multivariate Cox proportional hazard regression models for each outcome. MRI T2 lesions and CSF OB status, respectively, were included as dependent variable in the multivariate linear regression models. For the covariates which were not normally distributed a transformation has been performed to make them roughly continuous and unbounded.

The multiple imputation with EM and bootstrapping was performed using the “Amelia package for R”. This R package implemented different algorithms. First, a dataset with the same dimension of the original data is obtained by a bootstrap ( $n=1,000$ ) procedure. Second,

the algorithm estimates the sufficient statistics (with priors if specified) by expectation-maximization (EM), and then imputes the missing values of sample. It repeats this process  $m$  times to produce the  $m$  complete datasets where the observed values are the same and the unobserved values are drawn from their posterior distributions. Finally, utilizing each of the multiply-imputed datasets separately, we carry out statistical analyses and combine the results of the  $m$  (in our case 15) statistical analyses to calculate a point estimate.

The assumptions we have applied (number of imputations 15; bootstraps 1,000) ensure a missing imputation with a relative efficiency greater than 95% and robust estimates.<sup>60</sup>

Results were expressed in terms of Hazard Ratios (HRs) with 95% confidence intervals (95% CI).

Furthermore, the RECURSIVE Partitioning and AMalgamation (RECPAM) method<sup>61, 62</sup> was used as an exploratory analysis to identify distinct and homogeneous subgroups of patients at different risk of EDSS progression, using as time of origin the date of MS diagnosis. This tree-based method integrates the advantages of main effects of standard Cox regression and tree-growing techniques. At each partitioning step, the method chooses the covariate and its best binary split to maximize the difference in the risk of reaching the outcome. The algorithm stops when user defined conditions (stopping rules) are met. In the RECPAM model, we tested the same set of variables used in the Cox regression analysis, except for the time dependent variables (treatment and relapses before progression) that were added in the final Cox model. In our RECPAM analysis, a minimum set of 0 confirmed progression of EDSS after MS diagnosis and 20 subjects per node were considered. A final exploratory Cox regression analysis including the RECPAM classes was carried out. P-values were 2-sided, and values  $<0.05$  were considered statistically significant. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc) and R version 3.2.0.

## Results

A cohort of 770 patients with pCIS was extracted from the Italian iMedWeb registry (44 contributing MS centers) and the MSBase registry (32 contributing MS centers) in June 2015 (**Figure 1**). See supplementary table 1 for the complete list of participating centers.

Demographic and clinical characteristics of this cohort are shown in **table 1**. Four hundred and ninety-three (64.0%) underwent a CSF tap and 494 (64.2%) had an MRI examination recorded within 1 year of onset symptoms.

The median (IQR) follow-up was 5.4 (1.9-10.8) years. Six hundred and two (78%) of patients experienced a 2<sup>nd</sup> attack and 299 (24.3%) experienced a confirmed EDSS worsening event during follow-up. Five hundred and twenty-one (66.7%) patients received one or more DMDs during follow-up, 200 (26.0%) of these received their first DMD prescription before the 2<sup>nd</sup> clinical attack (79.0% Interferons beta, 6.5% Glatiramer Acetate, 5.0% Natalizumab, 9.5% other immunomodulators/immunosuppressive drugs) and 468 (60.8%) before the first EDSS worsening event (76.7% Interferons beta, 4.7% Glatiramer Acetate, 4.5% Natalizumab, 3.0% Azathioprine, 11.1% other immunomodulators/immunosuppressive drugs).

### **Second Attack**

The median (IQR) time between the onset and the 2<sup>nd</sup> attack was 0.7 (0.3 - 2.2) years.

**Supplementary table 2** reports demographic and clinical characteristics in patients with and without a 2<sup>nd</sup> clinical attack during follow-up.

The univariate analysis showed that female patients (HR 1.23, 95% CI 1.02 - 1.48), patients with a multifocal disease onset (HR 1.32, 95% CI 1.03 - 1.70) and patients with at least 3 brain MRI T2 lesions (HR 1.72, 95% CI 1.11 - 2.64) were at higher risk to develop a 2<sup>nd</sup> attack. Neither presence of OB nor early DMD treatment were predictive of time to a 2<sup>nd</sup> attack (**Figure 2**).

In the multivariate model, female patients (1.28, 95% CI 1.06-1.55) and a multifocal disease onset (HR 1.42, 95% CI 1.10-1.84) were confirmed as independent risk factors for the 2<sup>nd</sup>

attack. Moreover, this model showed a significant lower risk for a 2<sup>nd</sup> attack in patients who started DMDs (from the time of initiation of DMDs), relative to patients who did not start DMDs (0.75, 95% CI 0.60 - 0.95). (**Figure 2**)

### **First 3-months confirmed EDSS worsening event**

The median (IQR) time between MS diagnosis and the first EDSS worsening event was 3.2 (1.1-6.7) years.

Demographic and clinical characteristics of all pCIS patients and pCIS patients converted to MS, stratified by the occurrence of a 3 months confirmed EDSS worsening event, are shown in **supplementary tables 3**.

In Figure 3 the univariate and multivariate Cox models are reported.

In the univariate model the occurrence of relapse/s was a strong determinant of an increased risk of a first EDSS worsening event (HR 4.48, 95% CI 3.11 - 6.46), whereas an age at onset lower than 15 years and a supratentorial syndrome at onset were found to be protective (HR 0.69, 95% CI 0.50 - 0.96; HR 0.67, 95% CI 0.45-0.98, respectively). No effect of sex, CIS topography, brain MRI T2 lesions, OB and DMDs exposure was detected in the univariate model.

In the multivariate Cox model, age at onset lower than 15 years (HR 0.59, 95% CI 0.42-0.83) and DMDs exposure before the first worsening event (HR 0.75, 95% CI 0.71-0.80) prolonged the time to confirmed EDSS worsening, whereas the occurrence of relapse/s was a strong significant risk factor associated with a shorter time to EDSS worsening (HR 5.08, 95% CI 3.46-7.46).

### **RECPAM analysis for the first 3-months confirmed EDSS worsening event**

An exploratory RECPAM analysis, was used to identify distinct and homogeneous subgroups of POMS patients at different risk of reaching a first EDSS worsening event. RECPAM

analysis for the incidence of EDSS worsening led to the identification of 3 heterogeneous risk classes from a "pruned" tree (**Figure 4**).

The most important variable in discriminating this risk was the decade of birth, followed by the pCIS topography, with the lowest incidence in patients born before the 1990 followed by those with a supratentorial or a brainstem syndrome at onset (reference category: Class 3; HR = 1).

In comparison with patients belonging to class 3, those born before the 1990 but with a isolated ON or spinal syndrome, or multifocal symptoms at onset had a six-fold increased risk (Class 2; HR = 6.49, 95% CI 1.48 - 28.44) and those born after the 1990 (Class 1; HR = 9.81, 95% CI 2.28 - 42.18) had a ten-fold higher incidence of EDSS progression. The characteristics for each class were reported in the table below the **Figure 4**.

POMS patients belonging to the lowest risk Class (class 3) compared to those belonging to the Class 1 and 2 had less frequently an age at onset younger than 12 years (0% vs 19.2% and 6%) and an isolated ON (0% vs 20.2% and 42%) or spinal syndrome (0% vs 16.2 and 16%) or a multifocal involvement (0% vs 22.2% and 42%) at onset, whereas they had more frequently an isolated supratentorial (51.4% vs 17.2% and 0%) or brainstem syndrome (48.6% vs 24.2% and 0%) at onset. Notably, POMS patients belonging to the highest risk Class (Class 1) more frequently than those in the Classes 2 and 3 had more relapses before the EDSS worsening (18.2% vs 2.0% and 2.9%).

The final Cox regression model, including RECPAM classes, confirmed DMDs exposure as the most important protective factor (HR = 0.33, 95% CI 0.14 - 0.77) and relapses after diagnosis as the most important risk factor (HR 5.91, 95% CI 2.47-14.14) for EDSS worsening events in this POMS population. (**Table 2**). Moreover, this model confirmed a higher risk of an EDSS worsening event for patients belonging to RECPAM Classes 1 and 2 in comparison to patients belonging to RECPAM Class 3 (HR 18.66, 95% CI 4.04 - 86.15,

HR 8.42, 95% CI 1.89 - 37.43, respectively), and a lower risk for patients with an age at onset younger than 12 years in comparison to those with an age at onset older than 15 years (HR 0.30, 95% CI 0.10-0.94). (Table 2).

## Discussion

Our study is the first attempt to evaluate predictors, including DMDs exposure, for the risk of a 2<sup>nd</sup> attack and a first EDSS worsening event in a cohort of more than 700 patients with pCIS, prospectively followed for a median of over 5 and up to 10 years in two large MS registries: the Italian iMedWeb registry and MSBase registry. Accordingly, to previous studies<sup>4-6,9,21,30,63</sup> about 80% of our patients experienced a 2<sup>nd</sup> attack in a median time of 0.7 years with a range between 0.3 - 2.2 years. About a quarter of them experienced an EDSS worsening event in a median time of 3.4 years and 67% of them received at least one DMD treatment. In line with other reports<sup>64,65</sup> we found that 81% of pCIS who underwent CSF examination showed a positive CSF OB status and 88% of those who underwent MRI examination had at least 3 brain MRI lesions.

Comparing demographic and clinical characteristics between patients with and without a 2<sup>nd</sup> clinical attack during the follow-up, we found significant greater percentages of females and patients with CSF OB and a lower frequency of patients with a first DMD prescription in the group of pCIS who experienced a 2<sup>nd</sup> attack. These results are in accordance with previous studies in adult-onset and pediatric onset CIS patients.<sup>37,39,40,50</sup>

The univariate analyses for the risk of a 2<sup>nd</sup> attack confirmed that female patients have a higher risk, but also highlighted an increased risk in patients with a multifocal disease onset and with at least 3 brain MRI T2 lesions as already demonstrated in adult onset CIS<sup>39</sup> and in other series of pCIS.<sup>8,47</sup>

The multivariate model further confirmed the higher risk for a 2<sup>nd</sup> clinical attack in females and in patients with a multifocal onset, but also showed a significant impact of DMDs

exposure in patients who started DMDs before the 2<sup>nd</sup> attack. The prognostic implications of gender in determining the risk of a second clinical attack has been already demonstrated in adult CIS,<sup>39</sup> whereas results regarding CIS topography are at the best mixed in pediatric populations.<sup>65</sup>

Several previous studies on adult onset CIS as well as on pCIS showed that the presence of brain T2 lesions was associated with a higher risk of future clinical events.<sup>36, 37, 41, 42, 48</sup> In our multivariate model a trend for a higher risk of a 2<sup>nd</sup> attack was found in patients with at least 3 brain MRI T2 lesions, but it did not reach a statistical significance.

As already demonstrated in RCTs<sup>51-58</sup> and observational cohorts<sup>37, 66</sup> of adult CIS patients, we found a significant protective effect of a DMD treatment, started after the first attack, against the occurrence of a 2<sup>nd</sup> attack.

The presence of CSF OBs was not a significant predictor of the time to a 2<sup>nd</sup> attack in our cohort. So far contradictory results on the effect of OB positivity for time to relapse in selected cohorts of children with isolated ON have been reported by the same group.<sup>48, 67</sup>

Both the univariate and multivariate Cox models for attaining EDSS worsening showed that the occurrence of relapse/s after the MS diagnosis was the only significant factor for this outcome. In particular, the presence of at least 1 relapse after the MS diagnosis, increased this risk almost 5-fold in comparison to patients with no subsequent relapses. The role of relapses on the accumulation of disability is still somewhat controversial in adult onset MS.<sup>9, 31, 33, 44, 45, 68</sup> However a higher number of relapses during the first year or the first 2 years of the disease has been shown to be associated with a higher rate of SP and severe disability milestones in previous studies in AOMS<sup>33, 44, 45</sup> and POMS.<sup>8, 9</sup> It is noteworthy that in the present study we have investigated the role of relapse occurrence as a time-dependent covariate, whereas previous studies usually have included the number of relapses during the first years (e.g. the first two-five years) of the disease.

A younger age at onset was found to be a significant protective factor against the risk for EDSS worsening, especially for patients with onset between 12 and 15 years. This was confirmed in the multivariate Cox analysis, after the adjustment for all the other covariates, POMS patients with an onset between 12 and 15 years had a 41% lower risk of EDSS worsening in comparison to patients with a disease onset between 15 and 18 years.

These findings are in line with the results of previous studies on AOMS and POMS in which patients younger than 18 years of age took 10 years longer than AOMS to reach disability milestones and SP course.<sup>7,9</sup>

Most important, the most significant protective factor shown by the multivariate models for the risk of EDSS worsening was an early DMDs exposure.

This finding is novel and clearly demonstrates the importance of early treatment in pCIS and POMS, as already reported for adult CIS.<sup>51-58</sup>

Finally, the RECPAM analysis, which integrates the advantages of main effects of standard Cox regression and tree-growing techniques, allowed us to better identify distinct and homogeneous subgroups of POMS patients at different risk of reaching an EDSS worsening event.

The most important variable in discriminating this risk was the decade of birth, followed by the pCIS topography, with the lowest incidence in patients born before the 1990 followed by those with a supratentorial or a brainstem syndrome at onset, and the highest incidence (9.8 times higher) in those born after 1990.

These results seem to support the hypothesis that a first attack with cognitive deficit (included in the supratentorial class) may predict a lower incidence of physical disability accumulation.

Historically, the topography of the first demyelinating event has been deemed an important clinical factor related to multiple sclerosis prognosis in AOMS.<sup>31-33</sup>

The RECPAM analysis revealed that the highest risk class (Class1) included POMS patients who reported additional relapse/s more frequently than those in the Classes 2 and 3 (18.2% vs 2.0% and 2.9%).

Notably, the final Cox regression model including RECPAM classes confirmed the DMDs exposure as the most important protective factor against the EDSS worsening, and relapses after diagnosis as the most important risk factor for attaining an EDSS worsening in this POMS population.

In conclusion, the strength of this study is its cohort size, one of the biggest ever studied, with acquisition of data performed prospectively. Our pCIS cohort is both multicenter and multinational, enabling, by a rigorous statistical approach, better identification of prognostic indicators. The major limits of this multicenter study are the lack of standardized protocols for CSF analysis and the lack of a systematic MR acquisition and analysis protocol, and also the quite large number of missing information on CSF and MR features which could be responsible of their poor significance as prognostic factors unlike the results found in adult onset CIS cohorts collected at a single centre.<sup>37</sup>

This work represents an important step forward in identifying risk factors for conversion to CDMS in patients with pCIS and disability worsening in POMS. Moreover, for the first time, the results consistently support a beneficial effect of an early DMD exposure in preventing the 2<sup>nd</sup> attack in pCIS and medium to long-term disability accumulation in POMS. In particular, the multivariate model showed that in patients receiving a DMD, there was a 25% reduction of the risk of EDSS worsening during the follow-up compared to untreated patients. This result was further confirmed and reinforced by the Cox regression model including RECPAM classes which demonstrated that, independently by the other risk factors, the DMDs treatment significantly reduces disability worsening.

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## Author Contributions:

Study conception and design: PI, MS, GL and MT. Contributed substantially to data acquisition and analysis: PI, MS, GL, GA, GC, VBM, GS, FP, AL, GI, RB, JACG, CP, EM, RA, CB, EP, GBZ, PS, GL, DM, CA, EC, SAS, GC, PD, VS, TP, RFB, DP, CT, TS, LM, MPA, GC, HB and MT. Drafted the manuscript and prepared the figures: PI, MS, GL and MT.

## Potential conflicts of interest

The authors report no conflicts of interest with respect to the contents of the current study.

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**Figure legends:**

## Figure 1

Title: Patients disposition.

## Figure 2

Title: Risk of a 2nd clinical attack during the follow-up in pCIS patients. Univariate (A) and multivariate (B) Cox proportional hazard regression models

Abbreviations: pCIS = Pediatric Clinically Isolated Syndrome.

## Figure 3

Title: Risk of attaining a 3-months confirmed EDSS worsening event during the follow-up. Univariate (A) and multivariate (B) Cox proportional hazard regression models in POMS patients.

Abbreviations: POMS = pediatric onset multiple sclerosis.

## Figure 4

Title: RECPAM - Risk classes from a "pruned" tree: 3-months confirmed EDSS worsening in POMS patients.

Legend: Circles represent nodes; Square represent leafs. The first number of each figures represent the total number of patients with the event, the second number represent the total number of patients included in the group.

Abbreviations: POMS = pediatric onset multiple sclerosis.

**Table 1. Demographic and clinical characteristics of patients with pCIS**

<b>Baseline Features</b>	
Sex, F/M	544/226
Age at Onset (years) median (IQR)	16.0 (14.1 - 17.2)
Classes of Age at Onset (years) <i>n</i> (%)	
0 - ≤ 12	92 (12.0)
> 12 - ≤ 15	190 (24.7)
> 15 - ≤ 18	487 (63.3)
pCIS topography, <i>n</i> (%)	
Isolated Optic Neuritis	196 (26.2)
Isolated Brain-Stem Syndrome	149 (19.9)
Isolated Spinal Syndrome	101 (13.5)
Isolated Supratentorial Syndrome	173 (23.1)
Multifocal	129 (17.3)
Patients with CSF examination, <i>n</i> (%)	493 (64.0%)
Patients with CSF OB, <i>n</i> positive OB/total (%)	399/493 (80.9)
Patients with MRI examination, <i>n</i> (%)	494 (64.2%)
Patients with number of brain MRI T2 lesions: 0 – 2, <i>n</i> (%)	58 (11.7)
Patients with number of brain MRI T2 lesions: > 2, <i>n</i> (%)	436 (88.3)
First EDSS Evaluation, mean (SD)	1.9 (1.4)
<b>Follow-up Features</b>	
Follow-up, year, median (IQR)	5.4 (1.9 - 10.8)
Patients with a 2 <sup>nd</sup> attack during the follow-up, <i>n</i> (%)	602 (78.2)
Patients with an EDSS worsening during the follow-up, <i>n</i> (%)	299 (24.3)
Patients treated with at least one DMD during the follow-up	614 (79.7)
Patients with a first drug prescription before 2 <sup>o</sup> Attack, <i>n</i> (%)	200 (26.0)

Patients with a first drug prescription before first EDSS worsening event, <i>n</i> (%)	156 (52.2)
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Abbreviations: pCIS = Pediatric Clinically Isolated Syndrome; IQR = Interquartile Range; EDSS = Expanded Disability Status Scale; OB = Oligoclonal Band; DMD = Disease Modifying Drug.

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**Table 2. Post-RECPAM Cox regression model for EDSS worsening events in POMS patients with RECPAM classes included in the model.**

VARIABLE	HR (95% CI)	<i>P</i>
RECPAM Class 1 vs 3	18.66 (4.04-86.15)	0.0002
RECPAM Class 2 vs 3	8.42 (1.89-37.43)	0.0052
Female vs Male	0.62 (0.32-1.22)	0.1702
Class of Age at Onset		
0 - ≤12	0.30 (0.10-0.94)	0.0392
> 12 - ≤15	0.81 (0.39-1.66)	0.5581
Brain MRI T2 lesions >2 vs 0-2	0.80 (0.26-2.46)	0.6992
OB positive vs negative	2.69 (0.60-11.98)	0.1947
DMD exposure before diagnosis	0.26 (0.06-1.19)	0.082
Relapse before diagnosis 2 vs 1	1.23 (0.62-2.41)	0.5523
DMD exposure before EDSS worsening	0.33 (0.14-0.77)	0.0103
Relapses prior EDSS worsening	5.91 (2.47-14.14)	<0.0001

Abbreviations: RECPAM = Recursive Partitioning and Amalgamation; POMS = pediatric onset multiple sclerosis; DMD = disease modifying drug; EDSS = Expanded Disability Status Scale; OB = Oligoclonal Banding;

**Supplementary Table 1: Number of included patients per centre**

Centre	City	Country	Patients
University of Bari "Aldo Moro"	Bari	Italy	139
S.Antonio Abate Hospital	Gallarate (VA)	Italy	45
"Madonna delle Grazie" Hospital	Matera	Italy	36
University "Federico II"	Napoli	Italy	33
University of Palermo	Palermo	Italy	30
University of Catania	Catania	Italy	30
University of Chieti	Chieti	Italy	29
Hospital Universitario Virgen Macarena	Sevilla	Spain	28
Vita-Salute San Raffaele University	Milan	Italy	27
National Institute of Neurology Foundation	Pavia	Italy	25
Centro Internacional de Restauracion Neurologica	Havana	Cuba	18
S.Andrea Hospital	Rome	Italy	17
Policlinico Umberto I	Rome	Italy	16
Amiri Hospital	Kuwait City	Kuwait	15
Karadeniz Technical University	Trabzon	Turkey	15
Azienda Sanitaria Unica Regionale Marche	Macerata	Italy	15
Operative Unit of Neurology, "Dimiccoli" General Hospital	Barletta	Italy	15
University of Modena and Reggio Emilia	Modena	Italy	13
Second University of Naples	Napoli	Italy	12
Ospedale Garibaldi-Nesima	Catania	Italy	12
University of Foggia	Foggia	Italy	11
University of Cagliari	Cagliari	Italy	11
Golestan Hospital	Golestan	Iran	11

University of Florence	Florence	Italy	11
Ospedali Riuniti	Foggia	Italy	8
Hôpital Notre Dame	Montreal	Canada	8
Isfahan University of Medical Sciences	Isfahan	Iran	7
Aarhus University Hospital	Aarhus	Denmark	6
Hospital Universitario Virgen de Valme	Seville	Spain	6
Box Hill Hospital, Monash University	Melbourne	Australia	6
University of Melbourne			5
AORN San Giuseppe Moscati Avellino	Avellino	Italy	5
OO.RR Bergamo	Bergamo	Italy	5
"Miulli" Hospital	Acquaviva delle Fonti	Italy	5
Ospedali Riuniti di Salerno	Salerno	Italy	5
Ospedale Civile di Fidenza Fidenza (PARMA)	Fidenza	Italy	5
University of Ferrara	Ferrara	Italy	4
Second University of Naples - Neurology II	Napoli	Italy	4
"S. Luigi Gonzaga" Hospital	Orbassano	Italy	4
University of Parma	Parma	Italy	4
Maaslandziekenhuis	Maastricht en omgeving	Netherlands	4
Hospital São João	Porto	Portugal	4
19 Mayıs University, Medical Faculty	Samsun	Turkey	4
"S. Filippo Neri" Hospital	Rome	Italy	3
Ospedale Civile	Padova	Italy	3
University of Torino	Torino	Italy	3
NEUROMED Institute	Pozzilli (IS)	Italy	3
BMRI	Sydney	Australia	3

Flinders Medical Centre	Bedford Park	Australia	3
Cliniques Universitaires Saint-Luc	Bruxelles	Belgium	3
"Vito Fazzi" Hospital	Lecce	Italy	2
Divisione Universitaria di Neurologia at "S. Luigi Gonzaga" Hospital	Torino	Italy	2
University of Milan IRCCS Ospedale Maggiore Policlinico	Milan	Italy	2
"S. Anna" Hospital	Como	Italy	2
"San Carlo Voltri" Hospital	Genova	Italy	2
Consultorio Privado	Buenos Aires	Argentina	2
John Hunter Hospital	New Castle	Australia	2
Centre de réadaptation déficience physique Chaudière-Appalache	Charny	Canada	2
Bombay Hospital Institute of Medical Sciences	Mumbai	India	2
University Hospital Nijmegen	Nijmegen	Netherlands	2
"Niguarda Ca' Granda" Hospital	Milan	Italy	1
"San Giovanni Battista" Hospital	Foligno	Italy	1
University of Trieste	Trieste	Italy	1
"Regionale" Hospital	Treviso	Italy	1
"Maggiore" Hospital	Crema	Italy	1
"A. Manzoni" Hospital	Lecco	Italy	1
FLENI	Buenos Aires	Argentina	1
Hospital Italiano	Buenos Aires	Argentina	1
St Vincents Hospital	Melbourne	Australia	1
Royal Brisbane and Women's Hospital	Herston	Australia	1
Neuro Rive-Sud	Greenfield Park	Canada	1
Hospital Donostia	Gipuzkoa	Spain	1
MS Clinic, Hopital Tenon	Paris	France	1

University of Debrecen	Debrecen	Hungary	1
Hospital Angeles de las Lomas. Instituto Mexicano de Neurociencias.	Mexico City	Mexico	1
Groene Hart ziekenhuis	Gouda	Netherlands	1

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**Supplementary table 2. Demographic and clinical characteristics of pCIS stratified by the occurrence or not of the 2<sup>nd</sup> clinical attack**

VARIABLE	Second Attack ( <i>n</i> = 602)	No Second Attack ( <i>n</i> = 168)
Females, <i>n</i> (%)	439 (72.9)	105 (62.5)
Classes of Age at Onset, years, <i>n</i> (%)		
0 - ≤ 12	70 (11.6)	22 (13.8)
> 12 - ≤ 15	148 (24.6)	42 (25.2)
> 15 - ≤ 18	384 (63.8)	103 (61.7)
pCIS topography, <i>n</i> (%)		
Isolated Optic Neuritis	146 (24.7)	50 (31.9)
Isolated Brain-Stem Syndrome	122 (20.6)	27 (17.2)
Isolated Spinal Syndrome	73 (12.4)	28 (17.8)
Isolated Supratentorial Syndrome	140 (23.7)	33 (21.0)
Multifocal	110 (18.6)	19 (12.1)
First DMD prescription before 2 <sup>nd</sup> Attack, <i>n</i> (%)	119 (19.8)	81 (48.2)
Brain MRI T2 lesions, lesions within the first year and before 2nd Attack, <i>n</i> (%)		
0 - 2	25 (13.2)	13 (16.9)
> 2	165 (86.4)	64 (83.1)
OB Positive, <i>n</i> (%)	346 (83.8)	53 (66.3)
First EDSS Evaluation, mean (SD)	1.9 (1.4)	1.8 (1.31)

Abbreviations: pCIS = Pediatric Clinically Isolated Syndrome; DMD = Disease Modifying Drug; OB = Oligocolonal Band.

**Supplementary table 3. Demographic and clinical characteristics of pCIS who converted to MS stratified by the occurrence or not of a 3-months confirmed EDSS worsening event**

VARIABLE	EDSS Worsening event ( <i>n</i> = 238)	No EDSS Worsening event ( <i>n</i> = 287)
Females, <i>n</i> (%)	170 (71.4)	219 (76.3)
Age at Onset, years, <i>n</i> (%)		
0 - ≤12	22 (9.2)	32 (11.2)
> 12 - ≤15	50 (21.0)	82 (29.6)
> 15 - ≤18	166 (69.8)	173 (60.3)
pCIS topography, <i>n</i> (%)		
Isolated Optic Neuritis	61 (26.3)	68 (24.1)
Isolated Brain-Stem Syndrome	50 (21.6)	57 (20.2)
Isolated Spinal Syndrome	33 (14.2)	33 (11.7)
Isolated Supratentorial Syndrome	50 (21.6)	74 (26.2)
Multifocal	38 (16.4)	50 (17.7)
First DMD prescription before MS Diagnosis, <i>n</i> (%)	22 (9.2)	24 (8.4)
First DMD prescription before the first EDSS worsening event, <i>n</i> (%)	119 (50.0)	214 (74.6)
Brain MRI T2 lesions within the first year and before the first EDSS worsening event, <i>n</i> (%)		
0 - 2	13 (16.0)	17 (8.9)
> 2	68 (84.0)	174 (91.1)

OB Positive, <i>n</i> (%)	133 (83.7)	168 (85.7)
First EDSS Evaluation, mean (SD)	1.5 (1.0 - 2.0)	2.0 (1.5 – 3.0)
Relapse/s before EDSS Worsening, <i>n</i> (%)	165 (69.3)	220 (76.7)

Abbreviations: pCIS = Pediatric Clinically Isolated Syndrome; EDSS = Expanded Disability Status Scale; DMD = Disease Modifying Drug; OB = Oligoclonal Bands.

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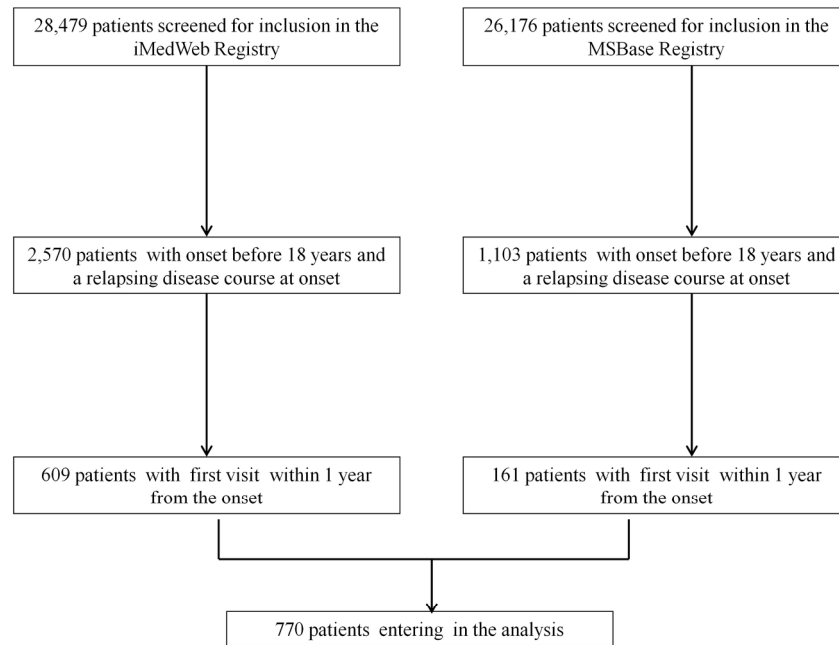
**Figure 1. Patients disposition.**

Figure 1. Patients disposition.

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**Figure 2. Risk of a 2nd clinical attack during the follow-up in pCIS patients. Univariate (A) and multivariate (B) Cox proportional hazard regression models**

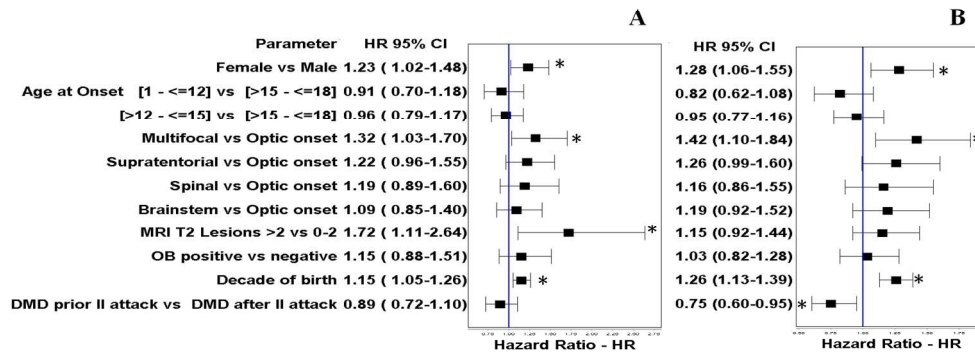


Figure 2

190x142mm (300 x 300 DPI)

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**Figure 3. Risk of attaining a 3-months confirmed EDSS worsening event during the follow-up. Univariate (A) and multivariate (B) Cox proportional hazard regression models in POMS patients.**

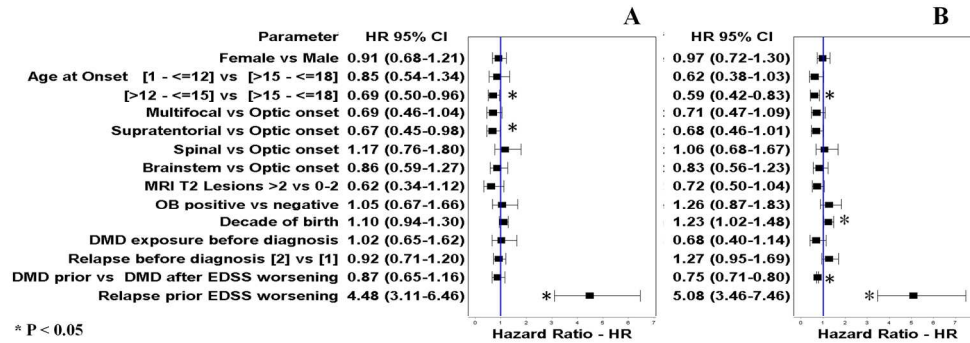


Figure 3

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**Figure 4. RECPAM - Risk classes from a "pruned" tree: 3-months confirmed EDSS worsening in POMS patients.**

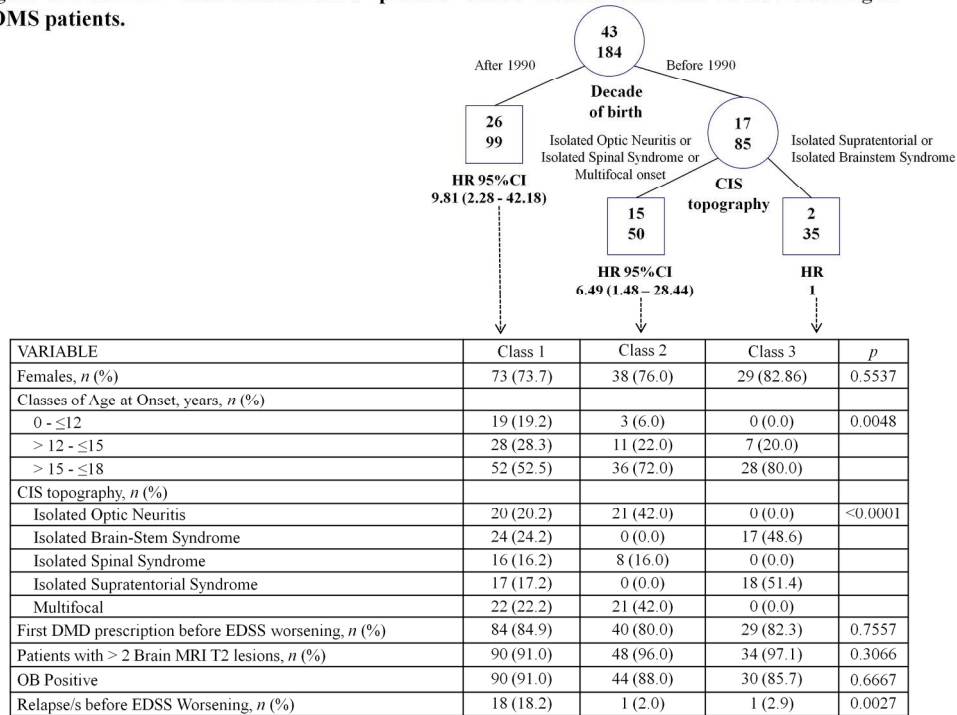


Figure 4

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