



RESEARCH PAPER

Impact of natural menopause on multiple sclerosis: a multicentre study

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ABSTRACT

Objective To study the effect of natural menopause on multiple sclerosis clinical course.

Methods This was an observational, retrospective, multicentre, cohort study. Menopause onset was defined by the final menstrual period (FMP) beyond which no menses occurred for 12 months. We included multiple sclerosis (MS) patients with FMP occurred after 2005 and a recorded follow-up of at least 2 years pre-FMP and post-FMP. We excluded patients with primary progressive course, iatrogenic menopause and with other confounders that could mask menopause onset. We compared relapse-rate and expanded disability status scale (EDSS) scores pre-FMP and post-FMP, searching for possible interactions with age, disease duration, cigarette smoking and nulliparity status.

Results 148 patients were included (mean observation: 3.5 years pre-FMP and post-FMP). Most patients (92%) received disease-modifying therapies, mainly first-lines. After menopause the annualised relapse rate (ARR) significantly decreased (from 0.21 ± 0.31 to 0.13 ± 0.24 ; $p=0.005$), while disability worsened (increase of mean 0.4 vs 0.2 points after menopause; $p<0.001$). Older age and long-lasting disease were associated with ARR reduction ($p=0.013$), but not with disability worsening. Cigarette smokers showed a trend to a higher disability accumulation after menopause ($p=0.059$).

Conclusion Natural menopause seems to be a turning point to a more progressive phase of MS. Relapse rate is also reduced after menopause, but this effect could be driven most by ageing and shifting to progressive phase in patients with long-lasting disease. Cigarette smoking could speed up disability progression after menopause.

INTRODUCTION

Menopause is a physiological event during woman's life that marks the end of reproductive competence. It is associated with a permanent cessation of ovarian follicular activity,¹ leading to a stable reduction of oestrogen blood levels.¹ The onset of menopause is defined retrospectively by the final menstrual period (FMP), beyond which no menses occur for 12 months.²

In women, the course of multiple sclerosis (MS), an inflammatory and degenerative disease of central

nervous system (CNS), is deeply influenced by physiological milestones associated with oestrogen levels modifications. After puberty, females become much more susceptible than males in developing MS.³ During pregnancy, especially in the third trimester, women affected by MS are protected against disease activity, while in the postpartum there can be a rebound of CNS inflammation.⁴ These changes are probably due to the effect of oestrogens on the immune system and CNS cells. Indeed, oestrogens resulted to have an anti-inflammatory^{5–9} and probably, a direct neuroprotective role^{8–13} in animal model of MS.

Up to now few studies have addressed the role of menopause on the course of MS: some have shown a worsening of subjective symptoms after menopause,^{14–16} while one found a higher disability accumulation.¹⁷ There are no data regarding inflammatory activity during menopause transition.

OBJECTIVES

Aim of our study was to understand the impact of natural menopause on MS course, investigating both the inflammatory and progressive aspects of the disease.

MATERIALS AND METHODS

This was an observational, retrospective, multicentre, cohort study.

Data collection and menopause onset definition

We asked all MS centres in Lombardia, a region in the north of Italy with around 10 million inhabitants, to participate to this study. Women with MS aged >35 years afferent at MS centres were interviewed to know their menopause status. Menopause onset was defined by the FMP beyond which no menses occurred for 12 months. We asked patients for the year and month of their FMP; if patients recalled only the year and season (summer/autumn/winter/spring) we entered in the database a fixed month (July/October/January/April). Women who did not remember this information were not included.

During the interview we collected information about comorbidities (gynaecological, autoimmune and endocrinological diseases), cigarette smoking

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(considered positive if the patient was actively smoking up to 5 years before FMP), number of pregnancies, artificial insemination procedures, estroprogestinic therapy before FMP and hormone replace therapy (HRT) after FMP.

MS clinical data such as age of MS onset, number and dates of relapses, disability measured annually with expanded disability status scale (EDSS), disease-modifying therapies (DMTs) received and type of MS were collected from paper/digital clinical records. We defined the disease course at the beginning and at the end of follow-up according to the last classification criteria.¹⁸ Those patients who presented a non-active, but progressive disease in the last year were considered to have a secondary progressive (SP) course. The observational period in this study was fixed up to 4 years before and after the FMP.

Inclusion and exclusion criteria

Inclusion criteria were:

- ▶ FMP occurred after 2005 (this year was arbitrarily chosen to reduce recall bias).
- ▶ FMP occurred after >3 years from MS onset.
- ▶ ≥2 years of recorded follow-up in MS centres before and after FMP.

Exclusion criteria were:

- ▶ Primary progressive MS.
- ▶ Previous immunosuppressive therapy (cyclophosphamide, mitoxantrone).
- ▶ Previous pelvic radiotherapy.
- ▶ Previous hysterectomy/endometrial ablation.
- ▶ Polycystic ovary syndrome.
- ▶ Previous history of neoplasm/HIV.
- ▶ Use of estroprogestinic therapy (oral contraceptives) up to 3 years before FMP.
- ▶ Pregnancy up to 5 years before FMP.

Outcomes

Primary endpoint was to compare the annualised relapse rate (ARR) over the whole period of observation before and after the FMP. Secondary, we performed analyses of variance of the number of relapses recorded every year during the peri-menopause period (2 years pre-FMP and post-FMP) and of the annual EDSS scores during the whole period of observation.

Statistical analysis

We used descriptive statistics (mean, median, SD, range) for continuous variables, while we described categorical variables as percentage of subjects falling in each group.

We analysed ARR, mean number of relapses and EDSS scores variation pre-FMP and post-FMP using parametric and non-parametric tests for paired samples as needed. In case of three or more measurements we used post-hoc pairwise comparisons corrected by Bonferroni.

Every analysis was performed both on the whole sample and after removal of patients who discontinued natalizumab or fingolimod during the observation because the interruption of such therapies can be associated with severe disease reactivation^{19 20} that could bias the results.

Thereafter, we set a repeated measure analysis of variance (ANOVA) mixed model searching for any significant interaction within and between the following factors: age at menopause, MS duration, smoking status (yes/no), nulliparity status (yes/no) and HRT use (yes/no). Age and disease duration were transformed in categorical variables. Univariate and multivariate analyses were performed.

Table 1 Clinical and demographic characteristics of the cohort

Clinical and demographic characteristics	Cohort (148 pts)
Age of MS onset (years), mean±SD (range)	35.4±8.6 (15–53)
MS duration since menopause onset (years), mean±SD (range)	14.9±8.1 (3–40)
SP course at the beginning/end of follow-up, pts (%)	7 (5%)/14 (10%)
Age of menopause onset (years), mean±SD (range)	50.3±3.8 (32–58)
Nulliparity, pts (%)	39 (26%)
Hormonal replacement therapy post-menopause, pts (%)	5 (3%)
Gynaecological diseases, pts (%)	16 (11%)
Autoimmune diseases, pts (%)	25 (17%)
Endocrinological diseases, pts (%)	18 (13%)
Cigarette smokers, pts (%)	43 (29%)

MS, multiple sclerosis; SP, secondary progressive; pts, patients.

Finally, we used parametric and non-parametric tests for independent samples to compare groups identified by categorical variables.

All p values reported are two-tailed and a $p \leq 0.05$ was considered statistically significant. All p values < 0.1 were considered as a possible statistical trend. All analyses were performed using IBM SPSS Statistic Release V.21.0 for Mac.

Sample size calculation

Sample size calculation was based on primary endpoint. We chose Wilcoxon signed rank test, assuming that ARR would have a non-normal distribution. Considering a 0.05 α , 90% of statistical power and detection of an effect size $\geq 30\%$ we calculated a sample size of 125 subjects (G*Power V.3.1 software for Mac). We set sample size to 140 subjects considering that at least 10% could be excluded in sensitivity analysis.

The Insubria Ethic committee approved this research.

RESULTS

Sample characteristics

A total of 148 women with natural menopause were included in the study. Sixteen MS centres participated to the study. The mean observation period was 3.7 ± 0.6 years before and 3.5 ± 0.7 years after menopause onset.

Table 1 reports the demographic and clinical characteristics of our sample. No one of the women included performed an artificial insemination.

Gynaecological diseases were uterus fibroids (6), uterus polyps (2), ovarian cysts (5), cervical dysplasia (2) and endometriosis (1). Endocrinological diseases were thyroid diseases (22), pheochromocytoma (1) and type 1 diabetes (2). Autoimmune diseases were autoimmune thyroiditis (9), antiphospholipid syndrome (1), psoriatic arthritis (1), asymptomatic connectivitis (2), unspecified arthritis (1), type 1 diabetes (2), psoriasis (1) and vitiligo (1). We transformed age of menopause from continuous to categorical, splitting it into two percentiles (mean values: 47.4 ± 2.9 and 53.2 ± 2.0 years). We did the same for MS duration (mean values: 8.4 ± 3.3 and 21.0 ± 5.6 years).

Most patients (136, 92%) received DMTs during the observation period: 102 (69%) were treated only with first-line therapies, six (4%) only with second-line therapies, 28 (19%) with both. First-line DMTs were interferons-beta, glatiramer acetate, teriflunomide and dimethyl fumarate. Second-line DMTs were natalizumab, alemtuzumab and fingolimod. This classification is in line with Italian Medicines Agency prescription rules. In the group of first-line DMTs we included three patients treated with azathioprine, which was the only drug received for two of them.

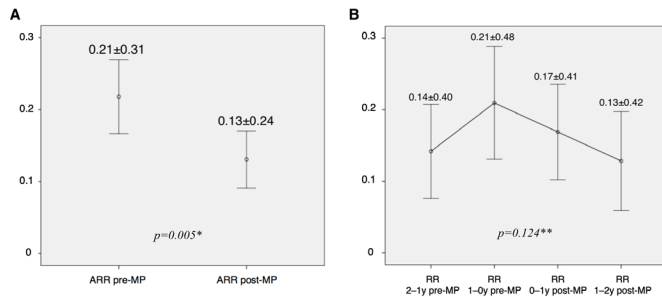


Figure 1 (A) Comparison of ARR (mean±SD) over the whole observation period before and after menopause onset. (B) Analysis of variance of number of relapses (mean±SD) recorded every year in the peri-menopause period. ARR, annualised relapse rate; MP, menopause; RR, relapse rate; y, year. *Wilcoxon signed rank test. **Friedman test.

For the majority (116), the ongoing DMT during the observation period was started before the FMP (mean 6.6 ± 3.9 years). Twenty patients started a DMT after the FMP (mean 2.0 ± 1.6 years). During the observation period 12 patients suspended natalizumab or fingolimod. As previously stated, these women were excluded in sensitivity analyses (see further).

ANNUALISED RELAPSE RATE

Considering the whole period of observation, ARR significantly decreased after menopause, from 0.21 ± 0.31 to 0.13 ± 0.24 (reduction of 38%, $p=0.005$) (figure 1A). We did not observe any significant change in the mean number of relapses recorded every year in the peri-menopause period (figure 1B).

Disability

Mean EDSS score at menopause onset was 2.3 ± 1.4 (median 2.0, range 0–7.5) for the whole cohort. Annual EDSS score was not available in every time points for all patients. Therefore, EDSS scores variation analysis was performed on 108 subjects who had a complete dataset over 3 years before and after the FMP (6 years of follow-up). Baseline characteristics and ARR variation of the 40 women excluded in this analysis were similar with those of the whole cohort (data not showed).

We observed a significant disability accumulation during the period of study (figure 2), above all after menopause: EDSS score increased by 0.2 ± 0.6 points before FMP and by 0.4 ± 0.7 points after FMP ($p=0.014$, Wilcoxon signed rank test).

Sensitivity analysis

After removing women who suspended natalizumab or fingolimod (12 patients) the results of all analyses did not change significantly (online supplementary sTable-1).

Interactions

Use of HRT was not added to the model because of the very low number of subjects (3%).

In univariate analyses we did find two possible trends of interaction: within disease duration for ARR ($p=0.077$) and within cigarette smoking status for disability ($p=0.059$) (online supplementary sFigures 1 and 2). In the first case patients with long-lasting disease had higher reduction of ARR after menopause. In the latter, cigarette smokers had higher disability worsening after menopause. Age of menopause between cigarette smokers and non-smokers was similar.

In multivariate analyses the ARR variation pre-menopause and post-menopause was no more significant ($p=0.332$). It

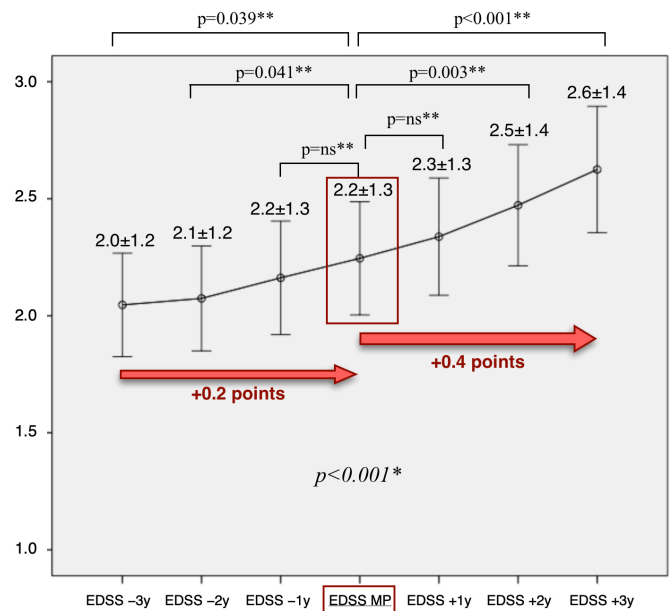


Figure 2 Analysis of variance of annual EDSS score (mean±SD) in the 3 years before and after menopause (total 6 years, 108 patients), with post-hoc pairwise comparisons. MP, menopause; y, year; *Friedman test=ANOVA repeated measure test. **Post-hoc pairwise comparison t-test for dependent samples corrected by Bonferroni.

was confirmed the interaction trend within disease duration ($p=0.060$) and we found an interaction trend within nulliparity status ($p=0.083$). We also found a statistically significant interaction with age+disease duration (bivariate interaction, $p=0.013$). After menopause nulliparous patients and younger patients with short-lasting disease had no significant reduction of ARR (online supplementary sFigures 3 and 4). Age of menopause was similar in nulliparous and multiparous women.

In multivariate analysis EDSS score variation remained significant ($p<0.001$). It was confirmed an interaction trend within smoking status ($p=0.071$) and we found a statistically significant interaction within age+disease duration+cigarette smoking (trivariate interaction, $p=0.015$): older, non-smokers patients with long-lasting disease (17 in total) had a stable and globally higher EDSS scores during the observation (EDSS ≥ 4 in 18% vs 5% at baseline) (online supplementary sFigure 5).

DISCUSSION

Aim of our study was to understand how the natural menopause impacts on the course of MS. Until now very little attention has been given to this issue. Thus, considering that oestrogens are so important in modifying MS course during different milestones of women reproductive life,^{3,4} it is obvious to think that some changes should occur after menopause too, when a permanent cessation of ovarian follicular activity leads to a persistent reduction of oestrogen levels.¹

Results of our study show that after menopause disability increases, while relapse-rate decreases. Our findings are in line with a previous work showing a mild worsening of disability after menopause,¹⁷ and suggest that disability accumulation is not due to a higher inflammatory activity, but probably to neurodegenerative processes. Mean age of menopause onset was not different from healthy women.²

Before opening the discussion, it is important to highlight main limitations of our study, first the lack of MRI data. However, in retrospective observational studies it is very difficult to obtain

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reliable information from neuroimaging, because patients perform MRI scan in different timepoints, with no standardised protocol and no centralised reading. Another important issue was the impossibility to perform a true 'natural history study'. Nowadays the majority of women with MS are on DMTs that could alter the disease course, making more difficult to detect the modifications due to physiological events like menopause. However, we chose not to exclude treated patients (or include patients treated only, eg, with first-line drugs) to avoid serious selection bias (ie, inclusion of benign or stable MS only). For the same reason we did not include in the ANOVA model variables on DMTs. Surprisingly, in our population there were few patients with a clinically defined SP MS and the median EDSS score was low (2.0), although mean age (50 years) and disease duration (15 years) were quite high. Probably, inclusion of patients mostly treated with DMTs was a selection bias. Finally, retrospective design precluded us to measure body mass index and to dose blood hormonal values, the latter still considered only supportive diagnostic criteria.²

Despite the above limitations, our study has strength points: the strict inclusion criteria avoided important confounders. Outcome recording was ensured by inclusion of MS patients with a regular and complete follow-up at specialised MS centres. Main results were confirmed after the exclusion of patients who discontinued DMTs associated with a 'iatrogenic' reactivation/rebound of the disease.^{19 20} Finally, not being able to use a suitable control group we used an ANOVA mixed model with possible confounders (age, disease duration, cigarette smoking and nulliparity status) to better understand our observation.

Actually, oestrogens fluctuations have a deep impact on MS course. At the onset of puberty the female:male ratios of MS incidence rises from 1:1 to 3:1.³ During pregnancy, in particular in the third trimester, the inflammatory activity is suppressed, with a rebound of relapses in the postpartum period.⁴ This is probably caused by high oestrogen levels that lead to a systemic shift in the immune response from a T helper 1 to T helper 2 profile,²¹ the latter known to be protective against MS.²² In postpartum period the abrupt fall of oestrogens would lead to a rapid return to a T helper 1 imbalance, causing a rebound of the disease.⁴ In experimental autoimmune encephalomyelitis (EAE), oestrogens resulted to be protective by reducing pro-inflammatory cytokines produced by T lymphocytes and macrophages, and by decreasing the recruitment of inflammatory cells into the CNS.⁵⁻⁹ Also, oestrogens probably have an active neuroprotective role over oligodendrocyte and neurons,⁸⁻¹³ and maybe on microglia too.²³ All these experimental data have been somewhat confirmed in some small clinical studies that observed a reduction of new lesions formation²⁴⁻²⁶ and relapses²⁴ in women taking oestrogens. However, another study did not find any beneficial effect in women taking progesterone and oestradiol for preventing postpartum reactivation.²⁷

Menopause corresponds to a permanent cessation of ovarian follicular activity, with a stable and persistent reduction of oestrogen levels. Although its onset is arbitrary defined by the FMP beyond which no menses occur for 12 months,² the process of reproductive ageing is gradual and begins much earlier than the FMP. Oestrogen levels may fluctuate up and down drastically over the 2-5 years of the menopausal transition, with high variability between and within subjects.¹ After the FMP the reduction in oestradiol is gradual too, reaching its nadir after 1 or 2 years¹. This progressive decline in oestrogen levels and their previous fluctuations could explain why we did not observe significant variations in the number of relapses recorded every year in the peri-menopause period. Moreover, we did not observe a higher

inflammatory activity right after the FMP, as it happens in the postpartum,⁴ when oestrogen levels are low too. Instead, we did observe a significant ARR reduction after menopause onset. This could be explained by the reduction in function and number of CD4 T and B lymphocytes (immunosenescence), secondary both to ageing²⁸ and to menopause itself.²⁹ However, multivariate analysis detected a significant interaction with age and disease duration: younger patients with a short-lasting disease did not have any significant decrease in ARR after menopause. This finding could mean that main drivers of ARR reduction in our population were ageing and disease duration, both known to be associated with a decrease of disease activity.^{28 30} We did not find in literature any explanation for the possible influence of parity on relapse rate after menopause.

On the other hand, we found a significant higher disability progression after menopause, especially in cigarette smokers. Instead, age and disease duration were not associated with disability accumulation. On the contrary, we found that older and non-smokers patients with long-lasting disease had a stable EDSS scores over the whole observation. This excludes the possibility that disability accumulation was driven mainly by the shift to the progressive phase, because we would have observed the opposite. This finding could be explained by a lower sensitivity to disability change of EDSS in patients with higher scores.³¹ However, it cannot be excluded that it was due by chance only because of the low number of patients in this group (17 subjects).

Therefore, disability progression after menopause could be secondary to the gradual reduction of oestrogens and their neuroprotective role. In fact, oestrogens can bind to two different nuclear receptors: Estrogen Receptor (ER) α and ER β . Both are expressed in immune cells, but only ER β is widely expressed in the brain and endothelial cells. Although the majority of the effect of oestrogens on the immune system are linked to ER α , the ER β plays a pivotal role in the repair of myelin and axons in EAE. ER β acts directly on oligodendrocytes^{11 12} and indirectly through CD11c+macrophages and dendritic cells in the CNS that are able to remove local immune mediated block in oligodendrocyte maturation.¹¹ Moreover, in EAE oestrogens preserve synaptic transmission¹³ and have a role in sparing myelin and axons in spinal cord, neurons and synapses in the brain.¹⁰ Furthermore, the reduction of the anti-inflammatory role of oestrogens after menopause⁵⁻⁹ could cause a progressive, inflammatory-driven damage of axons and myelin sheet, contributing to a subtle disability accumulation.

Cigarette smoking has been identified as a significant, but not powerful, risk factor of MS susceptibility and of a more progressive course.³² Toxic compound contained in cigarette smoke, especially free radicals, could contribute to axonal degeneration in MS, above all after menopause when the protective effect of oestrogen is lost.³² Moreover, cigarette smoking increases the catabolism of oestrogen, and probably, reduces their end-organ effects.³³ Indeed, women who are cigarette smokers undergo menopause earlier than non-smokers,³⁴ but not in our sample. In conclusion, smoke intrinsic toxicity combined with its anti-oestrogenic effects could speed up the conversion to a progressive phase of the disease in women with MS after menopause.

CONCLUSION

Natural menopause seems to be a turning point to a more progressive phase of MS, probably caused by the loss of oestrogens and their anti-inflammatory, neuroprotective properties. Relapse rate is also reduced after menopause, but this effect could be driven most by ageing and shifting to progressive phase

in long-lasting diseases. Actually, the impact of menopause on MS course seems to be gradual and moderate, especially in patients receiving DMTs (the majority in our study). Cigarette smoking could speed up disability progression after menopause because of its neurotoxicity and anti-oestrogenic effects.

Studies on the effect of HRT in patients with MS are mandatory, especially during menopause transition. It has been reported that HRT ameliorates physical quality of life in post-menopausal women with MS,³⁵ and it cannot be excluded that it could have some effect on disease course too.

When available, adding a neuroprotective therapy in this period could be important. Finally, it seems reasonable to advise patients with MS to reduce or stop cigarette smoking, especially near menopause onset.

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Contributors DB designed the study, which was reviewed and then approved by all the authors through collegial meetings. POA organised the meetings and the multicentre working group. All the authors enrolled the patients in each centre, collected the data and entered them into a database. DB did the statistical analyses, that were revised by POA, GM and OF. DB drafted the work, that was first revised by MZ and POA, then by all authors. All the authors gave the final approval of the version to be published.

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