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ORIGINAL RESEARCH ARTICLE

Effect on Cognition of Estroprogestins Combined with Interferon Beta in Multiple Sclerosis: Analysis of Secondary Outcomes from a Randomised Controlled Trial

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Abstract

Introduction Cognitive impairment is a disabling symptom in multiple sclerosis (MS). While its management remains challenging, beneficial effects on cognition of interferon beta (IFN-β) have been reported and a positive effect from estroprogestins has been hypothesised, suggesting that the combination of the two medications in women with MS could offer a promising treatment strategy.

Objectives We investigated whether a combination of estroprogestins and IFN-β can improve cognition in women with MS.

Methods Women with relapsing-remitting (RR) MS were randomly assigned (1:1:1) to receive subcutaneous IFN-β-1a (Rebif®, Merck Serono, Geneva, Switzerland) 44 mcg three times a week (tiw) (group 1), subcutaneous IFN-β-1a 44 mcg tiw plus ethinyl estradiol 20 mcg and desogestrel 150 mcg (Mercilon®, MSD Italia SRL, Rome, Italy) (group 2) or subcutaneous IFN-β-1a 44 mcg tiw plus ethinyl estradiol 40 mcg and desogestrel 125 mcg (Gracial®, Organon Italia S.p.A., Rome, Italy) (group 3) in a randomised controlled trial, for which we report the analysis of secondary outcomes. At baseline and at 24 months, all patients underwent magnetic resonance imaging (MRI) and a comprehensive cognitive assessment, including Rao's Brief Repeatable Battery (RBRB) and questionnaires for depression, fatigue and quality of life. Failure in at least two of the RBRB tests defined 'cognitive impairment'.

Results At baseline, there was no difference in the proportion of cognitively impaired patients. At month 24, the proportion of patients with cognitive impairment was lower in group 3 (34.8%) than in group 1 (47.6%) ($p = 0.03$). The risk of developing cognitive impairment over 24 months was lower in group 3 ($p = 0.02$). Mood and fatigue scores were comparable across the groups over time at both time points. However, at month 24, group 3 showed worsening on the sexual function subscale of the 54-item MS quality-of-life questionnaire ($p = 0.03$).

Conclusions This study suggests that the combination of high-dose estroprogestins and IFN-β may have positive effects on cognition. However, the effect of this treatment on sexual function requires caution to be exercised.

Protocol Number NCT00151801, registered in ClinicalTrials.gov

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Key Points

We found the combination of high-dose estroprogestins with interferon beta may have positive effects on cognition in women with multiple sclerosis (MS).

Therefore, we suggest estrogens may be a viable therapeutic option to preserve or restore cognitive function in patients with MS.

A possible negative effect of estroprogestin on sexual function on patients with MS requires caution to be exercised.

1 Introduction

Cognitive impairment is a common and disabling symptom in multiple sclerosis (MS), and it particularly affects domains such as memory, attention and executive functions [1, 2]. It is frequently associated with depression and fatigue, resulting in reduced quality of life that is independent of physical disability [3].

Effective treatments for cognitive impairment in MS are lacking. However, a beneficial effect from interferon beta (IFN-β) on information processing and learning/memory domains has been reported, along with a sustained effect in reducing the risk of cognitive deterioration [4–8]. Women receiving IFN-β seem to be more protected than men against the development of cognitive impairment. This could suggest a greater response to therapy in women or could be a consequence of the better prognosis associated with the female sex in MS [8, 9].

Gender and sex hormones have a recognised role in MS clinical characteristics and in pathology [10]. The prevalence and expression of symptoms differ between the sexes, and these differences can be explained, at least partially, by sex differences in the characteristics of tissue damage [11, 12]. Experimental data suggest that sex hormones can exert anti-inflammatory and protective effects on brain tissue. Estrogens can also enhance brain plasticity by increasing neurogenesis, connectivity and synaptic transmission, particularly in areas sub-serving complex cognitive tasks, and by boosting the brain's energy supply and utilization [13]. In the healthy brain, as well as in neurodegenerative diseases, these effects of estrogens result in a reduced risk of cognitive decline with hormonal-replacement therapies for menopause [14].

Previously, we demonstrated that combining IFN-β with high-dose estroprogestins has a pronounced anti-inflammatory effect in patients with relapsing-remitting MS (RRMS) [15]. In the present study, we test the hypothesis that, in women with RRMS, the effect of this combination therapy can specifically benefit cognition and aspects of quality of life related to cognition.

2 Material and Methods

2.1 Participants

Data from patients participating in a multicentre trial on the effect of estroprogestins in combination with IFN-β in women with MS (ClinicalTrials.gov number NCT00151801) are included in this study, where we analyse the secondary outcomes of the trial [15]. Recruitment from five Italian MS centres started in September 2004; the last visit was in November 2009. The investigators obtained approval for the study protocol from the local ethics committee, and each patient provided written informed consent. We enrolled women with RRMS [16] aged between 18 and 45 years with an entry score $\leq$5.0 in the Expanded Disability Status Scale (EDSS) [17] and at least two relapses in the previous 48 months or one relapse during the previous 12 months. Exclusion criteria included relapses or steroid intake in the previous 60 days; pathologies of the reproductive system; pregnancy or interruption of pregnancy in the previous 12 months; receipt of prior immunosuppressive therapy, glatiramer acetate, IFN-β or any experimental drugs before entry into the study; estroprogestins in the previous 3 months; and severe psychiatric illnesses, including severe depression, alcohol or substance abuse.

2.2 Study Design

After a screening phase, women with RRMS were randomly assigned (1:1:1) to receive subcutaneous IFN-β-1a (Rebif®, Merck Serono, Geneva, Switzerland) 44 mcg three times a week (tiw) (group 1) or subcutaneous IFN-β-1a 44 mcg tiw plus ethinyl estradiol 20 mcg and desogestrel 150 mcg (Mercilon®, MSD Italia SRL, Rome, Italy) (group 2) or subcutaneous IFN-β-1a 44 mcg tiw plus ethinyl estradiol 40 mcg and desogestrel 125 mcg (Gracial®, Organon Italia S.p.A., Rome, Italy) (group 3). The randomization list was computer generated, with a dynamic allocation provided by an independent national research organization (Istituto Superiore Sanità, Rome, Italy).

A two-physician (treating and assessing) model was used to assist with study masking. At each site, the treating physician was responsible for evaluating patient eligibility, supervising study drug administration and recording and managing adverse events and monitoring safety

assessments. The treating physician was unblinded to treatment arm. The assessing physician was exclusively responsible for all neurological assessments, beginning with the screening assessment, and for cognitive testing. Patients underwent clinical assessments, cognitive evaluations and magnetic resonance imaging (MRI) scanning at baseline, month 12 and month 24 and completed the self-reported questionnaires for quality of life, fatigue and depression at the same time points.

2.3 Behavioural Assessments

To assess cognitive functions we used the Rao's Brief Repeatable Battery [18], which includes the following:

- the selective Reminding Test—Long-Term Storage (SRT-LTS), SRT – Consistent Long-Term Retrieval (SRT-CLTR) and SRT-delayed recall (SRT-D) for verbal memory acquisition and delayed recall;
- the 10/36 Spatial Recall Test (10/36-SPART) and the 10/36-SPART-delayed recall (10/36-SPART-D) for visuospatial memory acquisition and delayed recall;
- the Paced Auditory Serial Addition Test at 3 (PASAT-3) and 2 (PASAT-2) seconds, and the Symbol Digit Modalities Test (SDMT) for concentration, sustained attention and information processing speed;
- the Word List Generation (WLG) for verbal fluency on semantic stimulus.

A test failure was defined as performance one standard deviation (SD) below the mean Italian normative values [19]. Cognitive impairment was defined as failure in at least two tests [20].

Mood, fatigue and quality of life were evaluated with the Hamilton Depression Scale (HAM-D) [21], the Fatigue Severity Scale (FSS) questionnaire [22] and the 54-item MS quality of life questionnaire (MSQoL-54) [23].

2.4 Magnetic Resonance Imaging (MRI) Data Acquisition and Analysis

All MRI scans were performed on a 1.5 Tesla magnet (Philips Gyroscan NT 15, The Netherlands) at baseline, month 12 and month 24. The MRI protocol included proton density- and T2-weighted spin echo images (TR 2000 ms, TE 20/90 ms, matrix size 256 × 256, field of view 24 × 24 cm, slice thickness 3 mm, gap 0 mm, 48 axial slices) and T1-weighted images (TR 600 ms, TE 15 ms, matrix size 256 × 256, field of view 24 × 24 cm, slice thickness 3 mm, gap 0, 48 axial slices) obtained before and 5 min after an intravenous injection of gadolinium-diethylenetriamine penta-acetic acid (Gd) 0.1 mmol/kg.

Volumes of hyperintense lesions on T2-weighted images (T2-LL), hypointense lesions on T1-weighted post-contrast images (T1-LL) and hyperintense lesions on T1-weighted post-contrast images (T1-Gd-LL) were quantified using a semi-automated method (Jim 4.0, Xinapse System, Leicester, UK). The number of patients with Gd-enhancing lesions at baseline and at month 24 was calculated for each group. The cumulative number of combined unique active (CUA) lesions, defined as new Gd-enhancing lesions or new T2-weighted lesions (non-enhancing on post-contrast T1-weighted acquisitions, to avoid double-counting), was also quantified at month 24.

2.5 Statistical Analysis

For data analysis, normalized values of cognitive scores were obtained using Italian normative data [14]. Balancing of treatment groups after randomization was tested with the Kruskal–Wallis test or the Chi-squared test, as appropriate. Differences between cognitively impaired patients and non-cognitively impaired patients at baseline were tested with the Mann–Whitney test.

The between-group comparison in the proportion of cognitively impaired patients at month 24 was assessed with a proportion logistic-regression model with adjustment for baseline covariates: study group, age, years in formal education and number of impaired cognitive tests at baseline.

Differences in the MSQoL subscale scores at different time points and between the groups were tested with an analysis of variance (ANOVA) model with repeated measures.

Risk factors for cognitive impairment at 2 years were identified using a logistic multivariate model: age, disease duration, years of formal education, EDSS score, baseline number of impaired tests, baseline MRI parameter (T1-LL, T2-LL, T1-Gd-LL) and cumulative number of CUA lesions at month 24 were entered into a univariate model. Variables that were significant in the univariate model were included in the multivariate model that identified risk factors for cognitive impairment at month 24.

Between-group comparisons were carried out for the scores of cognitive tests, HAM-D and FSS at different time points using a *t*-test or the Wilcoxon Signed Rank test, if the criteria for a dependent samples *t*-test were not met.

All p values <0.05 (two-tailed) were considered as significant. All statistical analyses were performed using SAS 9.1 (SAS Institute, Inc., Cary, NC, USA). Data were analysed by an independent research organization (Trial Form Support [TFS], S.L., Rome, Italy) that had no role in the study design and data collection.

3 Results

3.1 Demographic, Clinical and MRI Characteristics at Baseline

Of 150 randomized women, 149 took at least one dose of the study drug. Of these 149 patients, 142 had a complete baseline neuropsychological assessment (Fig. 1).

There were no significant differences among the three groups in baseline demographic, clinical and MRI characteristics (Table 1). Mean scores of cognitive tests, Ham-D and FSS were also similar across groups [Table S1 in the Electronic Supplementary Material (ESM)]. We did not find significant differences in the MSQoL-54 domains, except for the subscale 'role limitations due to emotional problems', which was significantly higher in group 3 (mean ± SD score, group 1: 55 ± 40; group 2: 43 ± 39; group 3: 76 ± 36; $p = 0.014$) (Table S2 in the ESM).

At baseline, 89 (59.9%) patients met the criteria for cognitive impairment. There was no significant difference between groups in the number of cognitively impaired patients (Table 1). Patients with cognitive impairment were significantly older (mean ± SD age 30.9 ± 6.3 vs. 27.5 ± 6.3 years; $p = 0.007$) and had longer disease duration (4.3 ± 4.3 vs. 2.6 ± 2.8 years; $p = 0.03$) than cognitively preserved patients (Table S3 in the ESM).

3.2 Clinical and MRI Characteristics at Follow-Up

Of 142 patients with a baseline assessment, one was lost at follow-up and 13 did not complete the cognitive battery at month 24. Therefore, follow-up data at month 24 were available for 128 patients: 42 in group 1; 40 in group 2; and 46 in group 3 (Fig. 1).

Figure 2 shows the number of patients with cognitive impairment in the three groups at different time points. At month 12, we did not find a significant difference in the proportion of patients with cognitive impairment across groups (23 [55%] in group 1; 20 [46%] in group 2; and 21 [45%] in group 3; $p = 0.24$]). At month 24, cognitive impairment was found in 20 (48%) patients in group 1, 21 (53%) in group 2 and 16 (35%) in group 3, and the proportion of cognitively impaired patients was significantly lower in group 3 than in group 1 ($p = 0.03$) (Fig. 2).

The logistic multivariate model revealed that protective factors for cognitive impairment at month 24 were the

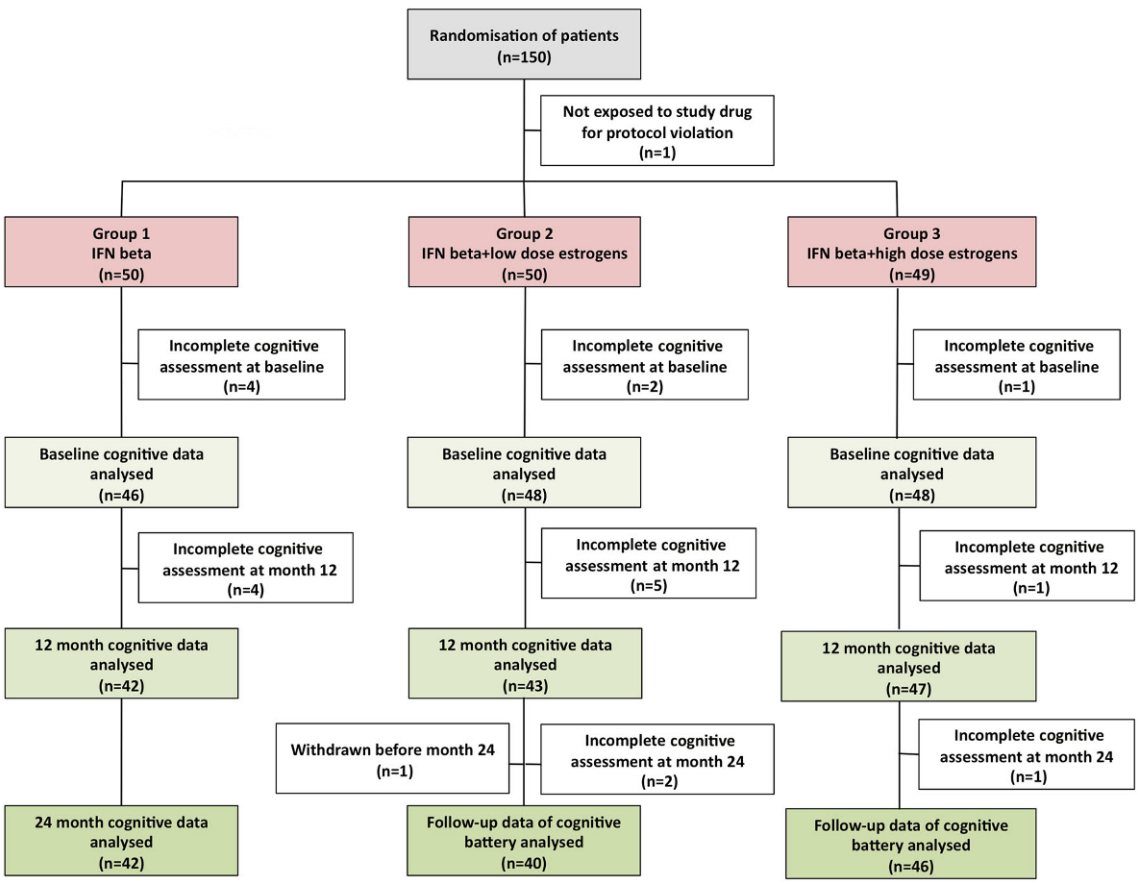

Fig. 1 Study flow-chart. *IFN* interferon

Table 1 Patient characteristics at baseline

Characteristics	Group 1 ($n = 46$)	Group 2 ($n = 48$)	Group 3 ($n = 48$)
Age (years)	30.4 ± 7.0	29.1 ± 6.4	30.6 ± 5.9
Education (years)	14.3 ± 5.1	15.1 ± 2.9	15.2 ± 3.1
Disease duration (years)	4.2 ± 4.5	3.3 ± 3.0	3.5 ± 3.9
Annualised relapse rate in the previous 2 years	0.8 ± 0.5	0.8 ± 0.3	0.8 ± 0.4
EDSS score	1.7 (0.7)	1.8 (0.9)	1.6 (1.0)
Cognitively impaired patients	31 (67)	27 (56)	31 (64)
Patients with Gd-enhancing lesions	26 (57)	31 (65)	28 (59)
Gd-enhancing lesions	29 ± 51	38 ± 78	39 ± 10
T2 hyperintense lesion volume (mm^3)	4771 (649–32,724)	4284 (762–61,428)	4378 (1142–51,298)
T1 hypointense lesion volume (mm^3)	549.5 (0–9964)	478 (0–15,435)	600 (0–15,501)

All values are expressed as mean ± standard deviation, *n* (%) or median (range) unless otherwise indicated. None of the between-group comparisons was significant (Kruskal–Wallis test, all nominal $p > 0.05$)

EDSS Expanded Disability Status Scale, *Gd* gadolinium-diethylenetriamine penta-acetic acid

absence of cognitive impairment at baseline (odds ratio [OR] 0.04; 95% confidence interval [CI] 0.01–0.14; $p < 0.001$) and belonging to group 3 (OR 0.27; 95% CI 0.08–0.93; $p = 0.02$). No other factors (i.e. age, years from disease onset, years of formal education, EDSS score and MRI measures) were associated with the development of cognitive impairment at follow-up.

Testing the specific changes in cognitive domains over time (Fig. 3), the Wilcoxon Signed Rank test showed significant improvements in the following tests for the following groups: STR-LTS in all three groups; STR-CLTR in groups 1 and 3; STRD in group 2; SPART-D in group 1; PASAT 3 and 2 in all three groups; and SDMT in group 2. No significant between-group differences were found in WLG and SPART-D.

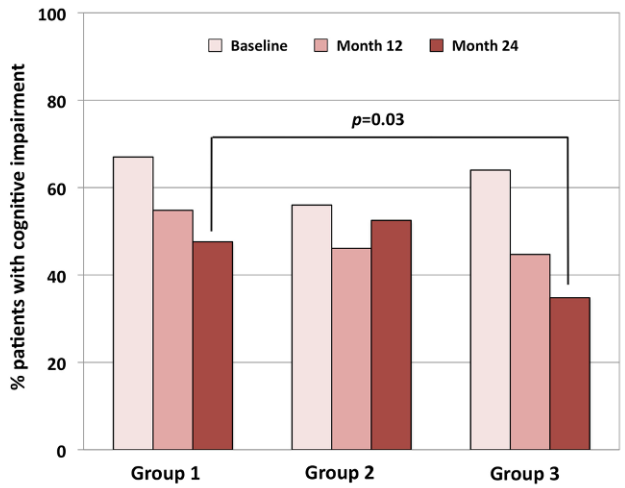

Fig. 2 Number of patients with cognitive impairment at baseline and at month 24. Only significant *p* values, which refer to the logistic-regression model with adjustment for study group, age, years in formal education and number of impaired cognitive test at baseline, are reported

During the follow-up, we did not observe significant differences across groups in relapse rate, EDSS score, Ham-D and FSS. ANOVA with repeated measures showed only a significant difference in 'satisfaction with sexual function' subscales at month 24 in the group treated with estroprogestins, with a significant difference between groups 1 and 3 ($F = 5.07$, $df = 1.0$, $p = 0.03$). No significant differences were found in the other MSQoL subscales (Fig. 4).

4 Discussion

This study suggests that, over 2 years, high doses of estrogen in combination with IFN-β may exert protective and reparative effects against the development of cognitive decline in women with RRMS.

During the follow-up, we observed an improvement in cognitive function in all treatment groups, which supports previous findings of a positive effect of IFN-β on cognition [6–8]. However, a significant proportion of patients in the group treated with high-dose estrogens improved their cognitive status compared with those treated with IFN-β alone, supporting the hypothesis that estrogens contribute to the preservation of cognition in MS through specific direct or indirect effects. Indeed, inflammation has a deleterious effect on cognitive function, as demonstrated by the worsening of neuropsychological performance during relapses [24, 25]. As previously suggested, high doses of estrogens may enhance the anti-inflammatory effects of IFN-β and thus exert their beneficial effects on cognition [15]. Estrogens are also known as potent and efficacious mediators of synaptic transmission, promoting neural plasticity and neurogenesis and sustaining the energetic demand by increasing glucose transport, aerobic glycolysis and mitochondrial function [13, 26]. In experimental

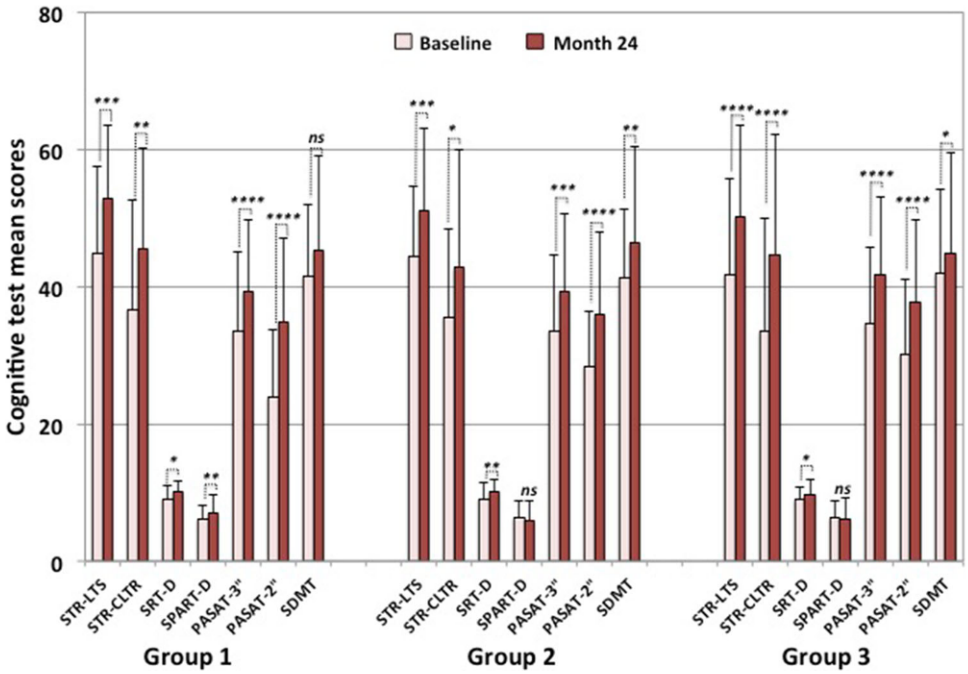

Fig. 3 Mean scores of cognitive tests at baseline and at 24 months. Values for cognitive tests are expressed as mean ± standard deviation scores. *p* values refer to Wilcoxon Signed Rank test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$). *ns* not significant, *PASAT-2* paced auditory serial addition test-2 seconds, *PASAT-3* paced auditory serial addition test-3 seconds, *SDMT* symbol digit modalities test, *SPART-D* spatial recall test-delayed, *SRT-CLTR* selective reminding test-consistent long term retrieval, *SRT-D* selective reminding test-delayed, *SRT-LTS* selective reminding test-long term storage

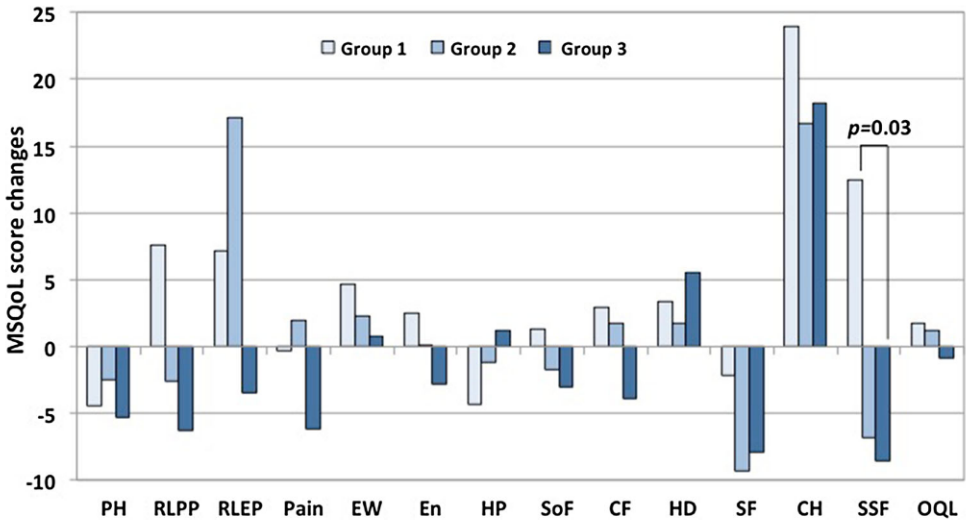

Fig. 4 Changes over time (month 24 vs. baseline) in the 54-item MS quality of life questionnaire (MSQoL-54) subscales according to study group. Increasing scores indicate improved aspects of quality of life. *p* value refers to repeated measures analysis of variance (ANOVA). *CF* cognitive function, *CH* change in health, *En* energy, *EW* emotional well-being, *HD* health distress, *HP* health perception, *OQL* overall quality of life, *PH* physical health, *RLEP* role limitation due to emotional problems, *RLPP* role limitation due to physical problems, *SF* sexual function, *SoF* social function, *SSF* satisfaction with sexual function

models, estrogens help to increase neurogenesis in the dentate gyrus of the hippocampus and promote a rapid increase of dendritic spine numbers or contacts in the hippocampus, the prefrontal cortex, the medial amygdala and the hypothalamus [27]. In these specific regions, neuronal plasticity helps to maintain cognitive function and protects women from cognitive decline with aging [14, 27]. Although both IFN-β and estrogens may improve cognitive function in MS [6, 13], the lack of a group of patients treated only with estroprogestins in this study leaves uncertainty over the individual contributions of these drugs on preserving/restoring cognition, i.e. whether estroprogestins with high-dose estrogens enhance the IFN-β effect or whether IFN-β predisposes the immune system to the beneficial effects of estroprogestins.

When we investigated the effects of the combination therapy on specific aspects of cognition domains, we could not identify specific cognitive domains that benefitted more from this treatment, observing instead a global advantage for cognitive function. The size of the sample and the

relatively mild cognitive impairment in our population may have limited our ability to detect specific effects of the combination therapy on cognitive domains.

Consistent with previous reports, which have demonstrated a beneficial effect of IFN-β on quality of life [28, 29], we also observed some benefit in the 'change in health' score of the MSQoL in all treatment groups. However, we also found a decrease in the 'satisfaction with sexual function' in the groups treated with estroprogestins. This effect could be due to a drug-related decrease in the total and free testosterone levels and an increase in the sex hormones binding protein concentrations [30]. The presence of androgens is crucial for the maintenance of sexual desire in women [31]. In MS, estroprogestins could alter a balance that is already precarious because of the presence of increased risk factors for sexual dysfunction [32, 33]. We previously showed that typical adverse events related with estroprogesntins and IFN-β, inculding flu-like syndrome and deep vein thrombosis, may occur also in combination therapy [15], therefore also this aspects, should be taken into account before prescription.

This study is not without limitations. We previously mentioned the lack of a group receiving estroprogestins only. While this may have limited our ability to disentangle the effect of IFN-β on cognition from that of estroprogestins, we could not withhold disease-modifying treatments in eligible patients, as this is unethical. Although we did not test specific domains with measures of frontal executive function and interference tests, this study used one of the most diffusely accepted cognitive batteries for cognition in MS, making our results more easily interpretable [18].

5 Conclusions

Overall, our results, observed over a period of 24 months, suggest that high doses of estrogens may be a viable therapeutic option to preserve or restore cognitive functions in patients with MS. However, since estroprogestins carry side effects that may affect quality of life and are risk factors for cardiovascular disorders and breast cancer, this strategy would require a careful risk–benefit assessment in individual cases.

Contributions LDG coordinated the study procedures, acted as treating physician and drafted the manuscript. FM and FDA acted as assessing physicians. VTB performed the MRI data collection and analysis. VAP undertook the statistical plan and the data analysis. FF and NP contributed to the discussion of results and writing up of the manuscript. PP supervised MRI data collection and analysis and contributed to the discussion of the results and the revision of the manuscript. VT generated the hypotheses of the study, designed the study with CP and revised the manuscript. CP designed the study with VT, was responsible for recruitment and data analysis, and contributed to writing up and subsequent revision of the manuscript.

Study investigators Prof. C. Pozzilli (Chief Investigator), Department of Neurology and Psychiatry, Sapienza University of Rome, Italy; Dr S. Cottone, Villa Sofia Hospital, Palermo, Italy; D.G. Di Battista and Dr E. Ferraro, S. Filippo Neri Hospital, Rome, Italy; Dr N. Falcone, Belcolle Hospital, Viterbo, Italy; Dr C. Gasperini, S. Camillo Hospital, Rome, Italy; Prof M. R. Tola, S. Anna Hospital, Ferrara, Italy.

Compliance with Ethical Standards

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Conflict of interest Nikolaos Petsas has received a lecture fee from Biogen Idec-Portugal. Patrizia Pantano has received funding for travel from Novartis, Genzyme and Bracco and speaker honoraria from Byogen. Carlo Pozzilli has received consulting and/or lecture fees and/or research funding and travel grants from Almirall, Bayer Schering, Biogen, Genzyme, Merck Serono, Novartis, Roche and Teva. Laura De Giglio, Fabiana Marinelli, Valeria T. Barletta, Veronica A. Pagano, Floriana De Angelis, Fulvia Fanelli and Valentina Tomassini have no conflicts of interest.

Ethical Approval The local ethics committees approved the protocol. Participants provided written informed consent.

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