Therapeutic lag in reducing disability progression in relapsing-remitting multiple sclerosis: 8-year follow-up of two randomized add-on trials with atorvastatin

HIGHLIGHTS

- Exposure to atorvastatin was associated with milder disease progression after 8 years
- There is a therapeutic lag in the effect of statins on disability progression
- Clinical trials should be extended in the long-term to evaluate any delayed or latent effect of the intervention

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Therapeutic lag in reducing disability progression in relapsing-remitting multiple sclerosis: 8-year follow-up of two randomized add-on trials with atorvastatin

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Abstract

Background. Current treatments for relapsing remitting multiple sclerosis (RRMS) reduce inflammation, but have a partial or modest effect on disability. This effect may require a much longer follow-up than standard trial design, in particular in RRMS with relatively-preserved functional reserve. We aimed to assess the long-term clinical evolution of RRMS patients exposed to atorvastatin in two trials (ACTIVE and ARIANNA).

Methods. We retrospectively looked at 69 participants randomized with atorvastatin or placebo as add-on therapy to interferon-beta for 24 months at a single MS centre. We recorded relapses, 1-point EDSS progression and progression to EDSS 4.0. Cox regression was performed for these three questions. A Poisson regression model was used to evaluate the association between atorvastatin treatment and annualized relapse rate (ARR).

Results. After 8.4±2.3 (3.7-11.9) years from trial, the use of atorvastatin was associated with reduced risk of 1-point EDSS progression (HR=0.440; 95%CI=0.225-0.861; p=0.017), and of EDSS 4.0 (HR=0.310; 95%CI=0.123-0.784; p=0.013). We found no significant association between atorvastatin and relapses.

Discussion. These data suggest that a delayed treatment effect maybe seen with atorvastatin added to interferon-beta, eight years after entering the clinical trials. Long-term follow-up of trial cohorts should be mandated.

Introduction

The central need in multiple sclerosis (MS) therapeutics is to delay or prevent progression. In relapsing-remitting MS (RRMS) over a dozen disease modifying treatments (DMT) are now available to reduce relapse rate, but with a partial or modest effect on disability progression.[1]

Statins, HMG-CoA reductase inhibitors, widely used to reduce serum cholesterol levels, have shown a variety of anti-inflammatory and neuroprotective properties in animal models, which are attractive when the biology of MS is considered. They can potentially inhibit antigen presentation and facilitate an anti-inflammatory T-lymphocyte response;[2] they can improve cerebral hemodynamics with up-regulation of endothelial nitric oxide synthase (eNOS) and inhibition of inducible NOS (iNOS); and finally counteract glutamate excitotoxicity.[3,4]

Eight trials with statins (simvastatin or atorvastatin) have been carried out in clinically isolated syndrome (CIS) and RRMS patients without yielding any reduction in the annualized relapse rate (ARR).[5] The largest double blind study conducted so far, SINCOMBIN (n=307), assessing the efficacy of a statin as add-on therapy to interferon-beta1a, failed to demonstrate any beneficial effect in reducing relapse activity. The authors also looked at the absolute change in brain parenchymal fraction, 12 months after randomization, and again there was no difference between the two groups.[6] On the other hand, in a (secondary) progressive environment, simvastatin 80mg/day reduced the annualized rate of whole-brain atrophy

compared with placebo by 43% (from 0.584%/year in placebo group to 0.288%/year in the active group).[7]

We hypothesized that, whilst statins have a limited anti-inflammatory activity in humans, nevertheless they could exert a 'long-range' neuroprotective effect on disability progression. This time lag might be some years after the end of the index trial, in particular in RRMS, where functional reserve capacity is generally preserved.[8] We therefore carried out a post-hoc analysis of two trials of atorvastatin in RRMS, where the patients had post-trial long-term follow-up from one centre to examine whether there was any evidence for any carry-over protective effect on disability.[9,10]

Methods

Study design and population

We included RRMS patients randomized in the ACTIVE and ARIANNA trials at the Federico II MS Centre of Naples, Italy from 2005 to 2008.[9,10] The "Federico II" ethical standards committee on human experimentation approved the study and written informed consent was obtained from all participants. All patients were assessed throughout by one assessor (VBM), according to clinical practice. Patients and assessor were blind to the use of atorvastatin.

Details on the included population, trial design and results are fully reported elsewhere and tabulated in **Supplementary Table 1**.[9,10]

For this current analysis the inclusion criteria were: 1) participation in the ACTIVE or ARIANNA trials in Federico II MS Centre of Naples for the entire study duration; 2) follow-up visits at this centre after trial termination; 3) EDSS≤3.0 at inclusion (suggestive of relatively preserved functional reserve capacity).[8]

The patient flow is shown in **Figure 1**. Briefly, from the original ARIANNA trial (n=154), 80 patients (52%) were enrolled at the Federico II MS Centre of Naples. The ACTIVE trial, which was single centre, by definition, enrolled all patients (n=45). Therefore, in total, we had access to 125 patients, of which 16 patients (13%) were lost-to-follow-up after trial termination (as they were referred uniquely for trial participation and, afterwards, returned under the care of their previous physician),

14 (11%) had early trial termination, and 26 (20%) were excluded due to baseline EDSS>3.0, leaving 69 to be included in this study. Patients were followed-up for an average period of 8.4±2.3 (3.7-11.9) years (**Figure 1**).

Treatment exposure

In the ACTIVE trial, RRMS patients were randomized to either atorvastatin 20mg/day or placebo in addition to subcutaneous interferon-beta1a 44mcg three times/week. In the ARIANNA trial, RRMS patients were randomized to either atorvastatin 40mg/day or placebo, as an add-on to subcutaneous interferon-beta1b 250mcg every other day. After the two-year trial duration, patients discontinued the atorvastatin or placebo and were continued on the original interferon-beta treatment or subsequently discontinued or switched to another disease modifying treatments (DMTs) as clinically indicated; total number of DMTs after trial termination was calculated and patients were classified into (1) staying on 1^{st} line DMTs, or (2) requiring 2^{nd} line DMTs, in accordance with European and Italian regulatory agencies.

Clinical outcomes

During the follow-up period, the patients were evaluated every 3 months, or at the occurrence of a clinical relapse, by an Expanded Disability Status Scale (EDSS) qualified neurologist blinded to the use of atorvastatin. The following major clinical outcomes were recorded: occurrence of clinical relapse, time from randomization to the first relapse (time to first relapse) and annualized relapse rate (ARR); 1-point EDSS progression (confirmed after 12 months, independent of relapse), time to 1-

point EDSS progression; proportions reaching EDSS 4.0 (confirmed after 12 months), time to EDSS 4.0.

Statistical analyses

Our study population included patients fulfilling the inclusion/exclusion criteria above. We conducted a missing pattern analysis to compare those with and without follow-up to assess whether specific variables were associated with the probability of having missing data at follow-up. Mean, standard deviation, range and proportions were calculated.

Preliminary comparisons between treated and untreated patients were performed with t-test, chi-square test and Mann-Whitney test, as appropriate. Cox regression models were employed to assess differences in rates: of relapse occurrence (time to the first relapse); of 1-point EDSS progression; and of reaching of EDSS 4.0; results were reported as adjusted Hazard Ratios (HR) with 95% confidence interval (95%CI). A multivariable Poisson regression model was employed to evaluate the association between atorvastatin treatment and ARR; adjusted coefficient (Coef) and 95% CI were subsequently calculated. Covariates included in the multivariable models were age, sex, disease duration, baseline EDSS, relapses in previous 2 years, protocol (ACTIVE or ARIANNA), number of DMTs after trial termination, and need for 2^{nd} line DMT. Results were considered statistically significant if $p<0.05$. Stata 15.0 has been used for data processing and analysis.

Results

No demographic (age, sex) and clinical characteristics (disease duration, baseline EDSS, relapses in previous 2 years) were associated with the likelihood of having data lost at follow-up. Results are presented in **Supplementary Table 2**. Thus, the missing data pattern was completely at random.

In the original trial populations, atorvastatin treated and untreated patients were similar for potential confounder.[9,10] Similarly, in the included population at baseline, atorvastatin treated and untreated patients were similar for age, gender, disease duration (time from symptom onset to baseline), EDSS, number of relapses in previous 2 years, follow-up duration, protocol of original inclusion, and DMTs after trial termination (**Table 1**).

At follow up, the previous use of atorvastatin was associated with reduced rate of 1-point EDSS progression (HR=0.440; p=0.017), and of reaching of EDSS 4.0 (HR=0.310; p=0.013) (**Table 1; Figure 2**). No significant associations were found between atorvastatin treatment and time to first relapse and ARR during the observation period (**Table 1**). The clinical trial covariate (ACTIVE or ARIANNA, also accounting for different atorvastatin dose) did not affect the results.

Discussion

This 8-year follow-up of two clinical trials of atorvastatin, demonstrated that 2-year exposure was associated with milder disease progression in RRMS patients after a mean follow-up of 8.4 years, with delayed risk of a 1-point EDSS progression and of reaching of EDSS 4.0. No effect was detected on relapse frequency. The analyses were run on a subset (69/125, 56%) of the original trial population, however the lost-at-follow-up status was completely random and we do not think affects our overall study results.

Previous trials with statins in RRMS were run for 9-24 months and were designed to show early anti-inflammatory properties of HMG-CoA reductase inhibitors.[2] The ACTIVE trial had EDSS progression as a secondary outcome and the group difference in time to 1-point increase of EDSS score, sustained for at least 3 months, reached borderline significance (p=0.053).[10]

It has been postulated that clinical trials in RRMS are too short and that 'long-range' effects might exist, particularly on neuronal pathways with relatively preserved functional reserve.[8] Supportive evidence for this comes from a recent meta-analysis looking at all the published observational studies for glatiramer acetate and interferon-beta (n=14) in which the long term effects were examined.[11] Time to reach EDSS 4 and 6 and time to progression to SPMS were all significant at a median follow-up time of 8.5 years. We feel that our analysis of atorvastatin long-term

effects adds weight to this hypothesis and is in line with previous studies showing delayed effects of DMTs on outcomes of disability progression.[8,12]

Of note, we did not detect any association between statins and relapses. This could be due to use of DMTs, that are mainly designed to prevent relapses and were used accordingly after trial termination, "masking" any possible effect of statins on relapses; however, we cannot exclude this result could point towards a primary neuroprotective role of statins.

This study has a number of potential limitations, which we have tried to mitigate. Firstly, a significant number were not included in the analysis, either because they were lost at follow-up (13%), or not finish the study (11%), or had an EDSS >3.0 (20%), but they were largely similar in their demographic and clinical characteristics. We deliberately decided to include patients we felt would have more functional reserve available to demonstrate a delayed effect from the atorvastatin (i.e., an enriched population). Secondly, the study is retrospective, though being performed at a single center should reduce the variability, for example in terms of DMT choice after trial termination. The inclusion of patients recruited in other centres of the ARIANNA trial would have increased post-trial clinical follow-up and treatment heterogeneity, also affecting our time varying statistical models. Thirdly, the exact atorvastatin and interferon-beta formulations were different between the trials, though this seems to be less likely to influence the 8-year outcomes. The impact of specific DMTs used after trial termination was not considered due to sample size constraints, but treatment groups were similar, also considering this is a single-

centre study, with one single assessor responsible for medical decisions. However, DMTs used after trial termination along with other possible confounders (e.g., neutralizing antibodies) are generally not included in long-term extension studies.[12,13]

In conclusion, our exploratory, hypothesis-generating study would suggest that it would be worth extending long-term observation to clinical trials, to look for any delayed or latent effect of the intervention on disability, particularly where there is relatively preserved functional reserve at lower EDSS levels.

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Declaration of conflicting interests

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CVR, AC, AN, MP and RP report nothing to disclose.

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VBM has received honoraria from Almirall, Bayer, Biogen Idec, Genzyme, Merck Serono, TEVA, Mylan, Novartis, and Roche.

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Figure 1. Patient Flow.

Figure 2. Kaplan-Meier curves for the probability of 1-point EDSS progression and of reaching of EDSS 4.0.

Kaplan-Meier plots estimating the probability of experiencing 1-point EDSS progression (**A**), and of reaching of EDSS 4.0 (**B**) in relation to the exposure to atorvastatin during the 2-year duration trial (red) or placebo (blue). P-values and hazard ratios (HR) are shown from Cox regression models.

Figure 1
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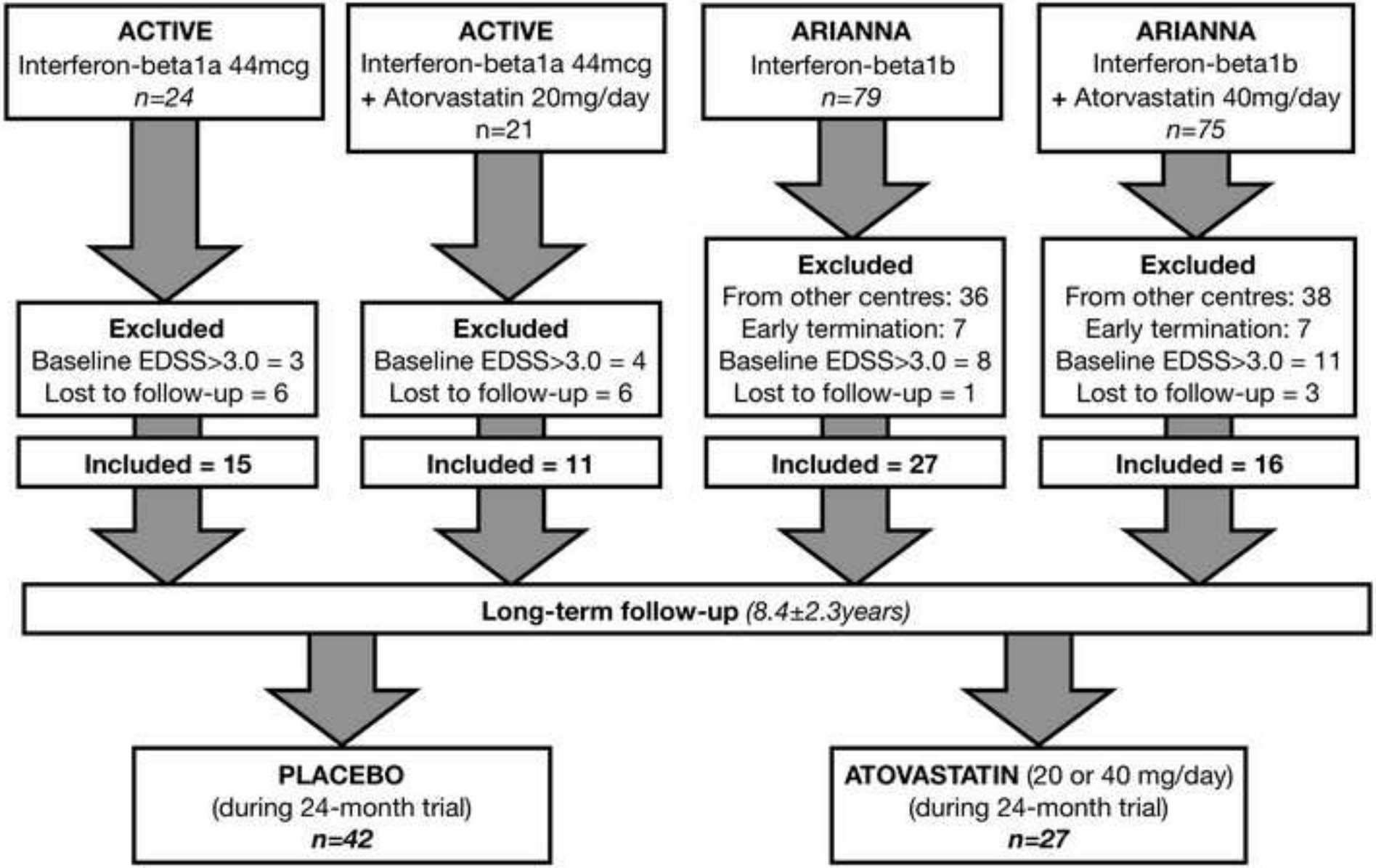

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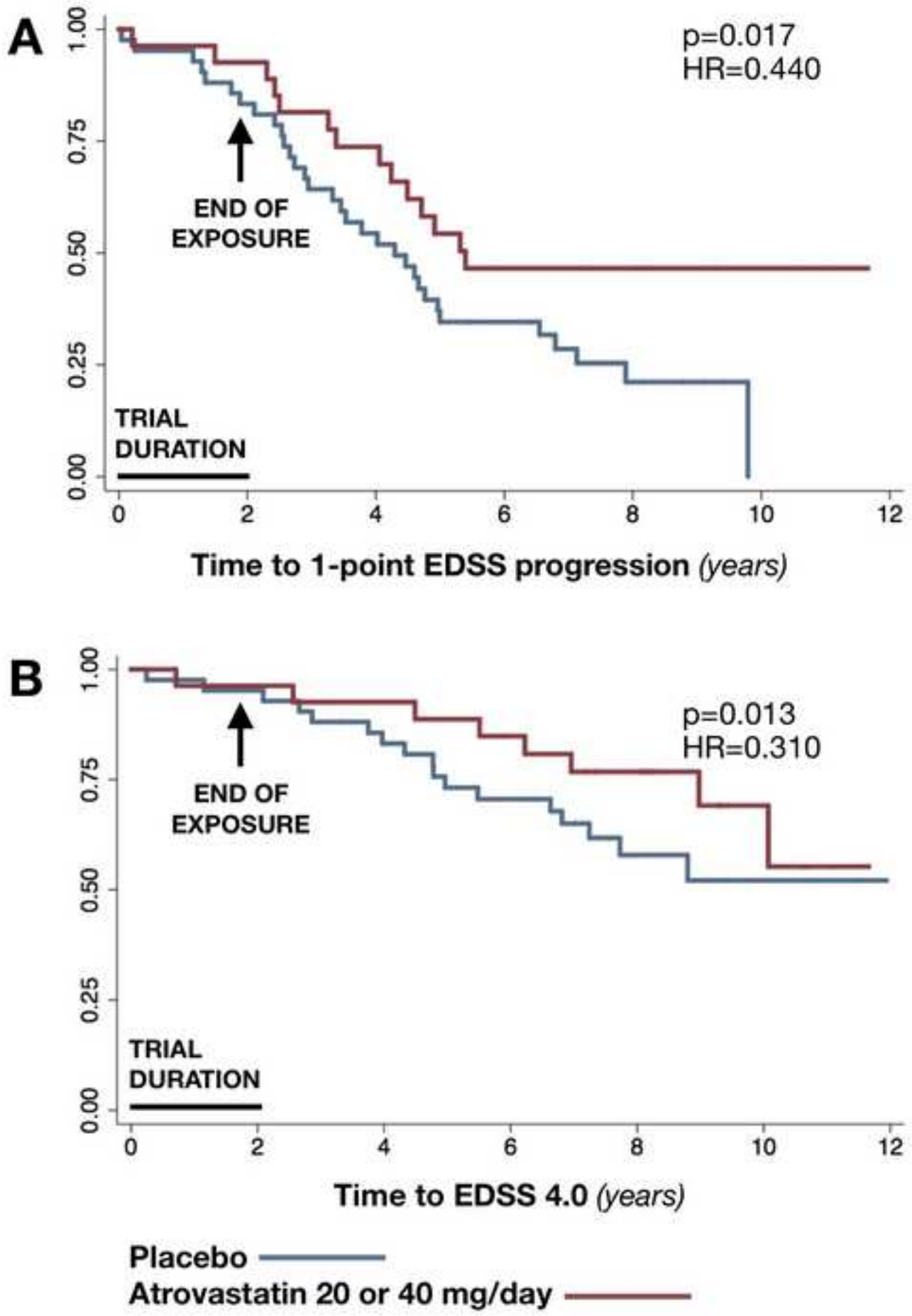

Table 1. Demographic and clinical features.

Table shows demographic and clinical features of included RRMS patients. P-values are reported from t-test and chi-square test; p-value, coefficient (Coeff) and 95% confidence intervals (95%CI) are reported from Poisson regression model; p-values, hazard ratio (HR) and 95%CI are reported from Cox regression models. Covariates were age, gender, disease duration, baseline EDSS, relapses in previous 2 years, protocol, number of DMTs after trial termination, and need for 2nd line DMT.

	Placebo *(n=42)*	**Atorvastatin** *(n=27)*	**p-values**	**Coeff/HR**	**95%CI** *Lower*	*Upper*
Age, *years*	33.1±6.1	34.7±8.3	0.387			
Gender, *female (%)*	23 (54.7%)	21 (77.7)	0.073			
Disease duration, *years*	7.3±4.3	6.5±4.3	0.457			
Baseline EDSS, *median (range)*	2.0 (1.5-3.0)	2.0 (1.5-3.0)	0.825			
Relapses *(in previous 2 years)*	0.7±0.9	0.9±1.1	0.398			
Follow-up duration, *years*	8.4±2.2	8.2±2.4	0.699			
DMTs after trial termination, *number*	1.8±0.9	1.8±0.8	0.725			
DMTs after trial termination, *1st/2nd line*	26/16	15/12	0.600			
Protocol, *ACTIVE/ARIANNA*	15 / 27	11 / 16	0.674			
Relapse occurrence	27 (64.2%)	17 (62.9%)	0.150	0.601	0.301	1.202
Time to first relapse, *years*	4.1±3.4	5.3±3.7				
ARR *(during study period)*	0.28±0.3	0.24±1.2	0.633	-0.250	-1.277	0.776
1-point EDSS progression	32 (76.1%)	14 (51.8%)	0.017*	0.440	0.225	0.861
Time to 1-point EDSS progression, *years*	4.5±2.6	5.8±3.1				
EDSS 4.0	17 (40.4%)	8 (29.6%)	0.013*	0.310	0.123	0.784
Time to EDSS 4.0, *years*	6.7±2.8	7.6±2.6				