Supplementary Online Content

Shirani A, Zhao Y, Karim ME, et al. Association between use of interferon beta and progression of disability in patients with relapsing-remitting multiple sclerosis. *JAMA*. 2012;308(3):247-256.

eAppendix. Additional Statistical Analyses and Results

eFigure 1. Multivariable Time-Dependent Cox Regression Analysis of Potential Factors Affecting Time to Reach Confirmed and Sustained EDSS 4 for β-IFN Exposed Patients (n=840) and a Contemporary Control Cohort (n=795), With β-IFN Treatment as a Time-Varying Covariate

eFigure 2. Multivariable Time-Dependent Cox Regression Analysis of Potential Factors Affecting Time to Reach Confirmed and Sustained EDSS 4 for β-IFN Exposed Patients (n=840) and a Historical Control Cohort (n=909), With β-IFN Treatment as a Time-Varying Covariate

eFigure 3. Multivariable Time-Dependent Cox Regression Analysis of Potential Factors Affecting Time to Reach Confirmed and Sustained EDSS 6 for β-IFN Exposed Patients (n=868) and a Contemporary Control Cohort (n=829), With β-IFN Treatment as a Time-Varying Covariate While Assuming a Six-Month Delay in the Initiation of β-IFN Treatment Effect as Well as a Six-Month Carry-Over Effect After Stopping β-IFN Treatment

eFigure 4. Multivariable Time-Dependent Cox Regression Analysis of Potential Factors Affecting Time to Reach Confirmed and Sustained EDSS 6 for β-IFN Exposed Patients (n=868) and a Historical Control Cohort (n=959), With β-IFN Treatment as a Time-Varying Covariate While Assuming a Six-Month Delay in the Initiation of β-IFN Treatment Effect as Well as a Six-Month Carry-Over Effect After Stopping β-IFN Treatment

eFigure 5. Multivariable Time-Dependent Cox Regression Analysis of Potential Factors Affecting Time to Reach Confirmed and Sustained EDSS 6 for β-IFN Exposed Patients (n=868) and a Contemporary Control Cohort (n=829), With β-IFN Treatment as a Time-Varying Covariate With Three Levels: 1) Pre-treatment Unexposed Time; 2) β-IFN Exposed Time; and 3) Post-exposed Time After Stopping β-IFN Treatment

eFigure 6. Multivariable Time-Dependent Cox Regression Analysis of Potential Factors Affecting Time to Reach Confirmed and Sustained EDSS 6 for β-IFN Exposed Patients (n=868) and a Historical Control Cohort (n=959), With β-IFN Treatment as a Time-Varying Covariate With Three Levels: 1) Pre-treatment Unexposed Time; 2) β-IFN Exposed Time; and 3) Post-exposed Time After Stopping β-IFN Treatment

eFigure 7. Multivariable Time-Dependent Cox Regression Analysis of Potential Factors Affecting Time to Reach Confirmed and Sustained EDSS 6 for β-IFN Exposed Patients (n=1094) and a Historical Control Cohort (n=959), With β-IFN Treatment as a tIme-Varying Covariate

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix. Additional Statistical Analyses and Results

Additional Statistical Analyses

A series of complementary analyses were conducted to further explore the treatment effect and to examine for potential bias. Findings from these analyses are reported under 'Additional results' (page 4 onwards)

Additional analysis 1: influence of a delayed or carry-over effect of β-IFN treatment on findings

We explored how any potential delay for treatment to take effect or a possible carry-over effect[1] of β-IFN treatment could have influenced our findings. For this analysis, we assumed a six-month delay for β-IFN to take effect once treatment was initiated and a six-month 'carry-over' for the treatment effect to disappear once the patient stopped β-IFN. We adopted this somewhat arbitrary six-month cut-off based on findings from MS clinical trials and case reports,[2, 3] although we recognize that there is no consensus on the duration of the delay period or the carry-over effect related to β-IFN treatment.

Additional analysis 2: exploration of the unexposed time before and after β-IFN treatment

The time-dependent Cox model used in our study assumed that time-periods unexposed to β-IFN were equivalent - i.e. the time before starting β-IFN and the time after stopping β-IFN, or expressed another way, a patient who stopped treatment was considered to have the same risk of reaching EDSS 6 as a patient who had been followed an equivalent amount of time and had not yet been exposed to the treatment. In order to examine the potential for any bias arising from this assumption, we considered 3 levels for β-IFN treatment, with these 3 levels included as a time-dependent covariate: 1) pre-treatment unexposed time; 2) β-IFN exposed time; and 3) post-exposed time, after stopping β-IFN treatment.

Additional analysis 3: impact of patients potentially eligible for the historical control cohort who were later exposed to β-IFN and subsequently excluded

As shown in Figure 1 (in the main paper), 239 patients were considered for the unexposed historical control cohort (i.e. were eligible for β-IFN treatment between April/1985 and June/1995), but were exposed to β-IFN during the follow-up time and were excluded from the analyses. Of these patients, 233 were first exposed to β-IFN after July/1995 (when the drug was licensed in Canada), and 6 were first exposed prior to June/1995. In order to examine any potential bias created by excluding these 239 patients, we performed an additional analysis in which these patients were considered for inclusion in the time-dependent Cox model.

Additional analysis 4: propensity score adjustment

Propensity scores can be calculated to represent the conditional probability of assignment to a treatment given a set of observed baseline (pre-treatment) characteristics.[4] Although propensity score-based methods are increasingly being used for selection bias adjustment in observational studies, there is little evidence that these methods control confounding more effectively or result in substantially different estimates of treatment effect compared to conventional multivariable models.[5] Moreover, a propensity score analysis alone cannot address immortal time bias.[6, 7] The presence of immortal time bias is a major issue and was shown to be '*sufficiently important to explain the apparent benefit of IFN in multiple sclerosis patients reported…*',[6] in Trojano et al's study of β-IFN treatment effect.[7] Re-analyses of the data taking into account immortal time bias resulted in an inability to find a beneficial association between β-IFN exposure and the risk of reaching EDSS 6 or secondary progressive MS.[6] Therefore, in this additional analysis, we maintained treatment exposure as being time-dependent in the Cox model (as in the main analysis), with the propensity score used to balance the model at baseline. The trade-off here is the 'one-

off' use of the propensity score at baseline. More complex models (where time-dependent propensity scores are considered)[8] require extensive risk-set matching, and systematic collection of observational data at each time interval under consideration (i.e. each treated and untreated time interval) which are not always available in observational datasets, such as ours. In addition, such models can rely on strong data-related assumptions which might not always be reasonable in the context of an observational study. Hence, a conventional propensity score approach was performed as a secondary, additional analysis only.

In the contemporary approach, propensity scores were calculated by constructing a logistic regression model to estimate the predicted probability of receiving β-IFN treatment for both the treated and contemporary untreated patients. Baseline variables included in the propensity score model were: sex, age, disease duration, EDSS, SES, Charlson comorbidities and the annualized relapse rate (based on the two years prior to baseline), and were all converted to categorical predictors. In addition, two-way interactions between disease duration and EDSS, disease duration and annualized relapse rate, and disease duration and age were considered. The goodness of fit for the propensity score model was assessed by the Hosmer-Lemeshow test, and the *c* statistic (area under the receiver operating characteristic curve).

For the historical approach, since the historical untreated patients never had a chance to be assigned to β-IFN treatment at baseline (i.e., a real-world predicted probability of zero), their propensity scores were calculated based on the coefficients derived from a new 'contemporary' logistic regression model in which all the baseline covariates and interaction terms listed above were included except for SES and Charlson comorbidities which were unavailable for the historical cohort.

In both approaches, patients in the non-overlapping tails of the propensity score distribution were excluded from the subsequent analyses. The propensity score was incorporated into the Cox model as a continuous covariate along with treatment as a time-dependent variable. A complementary analysis was also conducted with the propensity scores included as quintiles rather than as a continuous variable.

Additional Results

Proportion of patients reaching the outcome within 10 years after baseline

From life tables, the estimated cumulative proportion of patients who reached the main outcome within 10 years after baseline, was 25.0% for the treated cohort, 14.2% for the contemporary control cohort, and 27.5% for the historical control cohort.

β-IFN prescription patterns and reasons for right-censoring

In the β-IFN treated cohort, the first prescribed β-IFN was Avonex® for 135 (15.6%) patients, Rebif® for 410 (47.2%), and Betaseron® for 323 (37.2%). Participation in a clinical trial resulted in right-censoring for 25 (2.9%) patients in the β-IFN treated cohort, 47 (5.7%) in the contemporary control cohort, and 72 (7.5%) in the historical control cohort. The initiation of a non-β-IFN treatment accounted for right-censoring in 145 (16.7%) of the β-IFN treated cohort, 153 (18.5%) in the contemporary control cohort, and 38 (4.0%) in the historical control cohort.

Additional analysis 1: the influence of a delayed or carry-over effect of β-IFN treatment on the findings

When a delayed or carry-over effect of β-IFN treatment was considered in the Cox model, the direction of findings remained the same, albeit with a mild increase in the hazard of progression to EDSS 6 compared to the main analyses in which this effect was not considered. This was consistent for both the contemporary (eFigure 3) and historical control (eFigure 4) approaches.

Additional analysis 2: exploration of the unexposed time before and after β-IFN treatment

When the 3 levels for β-IFN treatment (pre-exposure, exposed and post-exposure time) were considered as a time-dependent covariate in the model, both the exposed and post-exposed time, compared to the pre-treatment unexposed time, were associated with a higher hazard of reaching the outcome when contemporary controls were included (eFigure 5). Expressed another way: when followed for a similar period of time, a patient either on treatment or post-cessation of treatment, compared to a treatment naïve, contemporary control patient, had a higher hazard of reaching the outcome. When historical controls were considered in the model, post-exposure compared to pre-exposure time was associated with a higher, though not statistically significant, hazard of reaching the outcome; and the β-IFN-exposed time compared to pre-exposed time was associated with a lower, though not statistically significant, hazard of progression to the outcome (eFigure 6).

Additional analysis 3: impact of patients potentially eligible for the historical control cohort who were later exposed to β-IFN and subsequently excluded

Including patients potentially eligible for the historical unexposed control cohort who were found to be exposed to β-IFN in the Cox model did not change findings; the hazard ratio was not statistically significant and moved closer to one (eFigure 7), compared to the main analysis in which these patients were not included (Figure 3).

In summary, none of the additional analyses showed a statistically significant lower hazard of reaching the outcome associated with β-IFN-exposed time. Therefore our main finding remained unchanged; i.e. we did not find strong evidence supporting the effectiveness of the β-IFNs on long-term disability in relapsing-onset MS.

Additional analysis 4: propensity score adjustment

In the contemporary approach, 19 patients in the non-overlapping tails of the propensity score distribution were excluded. The *c* statistic was 0.67 (95% CI: 0.64-0.69), and the Hosmer-Lemeshow goodness of fit test was not significant (χ^2=5.65, P=0.69). From the propensity score adjusted Cox model, exposure to β-IFN treatment was not associated with a statistically significant hazard of reaching EDSS 6 (HR=1.34; 95% CI: 0.93-1.92, P=0.12). Findings were virtually unchanged when the propensity score was considered as quintiles (HR=1.33; 95% CI: 0.93-1.92). In the historical approach, 7 patients in the non-overlapping tails of the propensity score distribution were excluded. The propensity score adjusted Cox model for the historical approach was also not statistically significant for the association between β-IFN exposure and progression to EDSS 6 (HR=0.84; 95% CI: 0.63-1.11, P=0.23) and findings were again virtually unchanged when the propensity score was considered as quintiles (HR=0.84; 95% CI: 0.63-1.11, P=0.23).

In summary, model adjustment using propensity scores resulted in similar estimates for the hazard of progression to EDSS 6 compared to the corresponding findings from the main analysis for both the contemporary and historical approaches.

References

1. Cleophas TJ. A simple method for the estimation of interaction bias in crossover studies. *J Clin Pharmacol.* 1990;30:1036-40.

2. Johnson KP. The historical development of interferons as multiple sclerosis therapies. *J Mol Med (Berl).* 1997;75:89-94.

3. Richert ND, Zierak MC, Bash CN, Lewis BK, McFarland HF, Frank JA. MRI and clinical activity in MS patients after terminating treatment with interferon beta-1b. *Mult Scler.* 2000;6:86-90.

4. D'Agostino RB, Jr. Propensity score methods for bias reduction in the comparison of a treatment to a non-randomized control group. *Stat Med.* 1998;17:2265-81.

5. Sturmer T, Joshi M, Glynn RJ, Avorn J, Rothman KJ, Schneeweiss S. A review of the application of propensity score methods yielded increasing use, advantages in specific settings, but not substantially different estimates compared with conventional multivariable methods. *J Clin Epidemiol.* 2006;59:437-47.

6. Renoux C, Suissa S. Immortal time bias in the study of effectiveness of interferon-beta in multiple sclerosis. *Ann Neurol.* 2008;64:109-10; author reply 10.

7. Trojano M, Pellegrini F, Fuiani A, et al. New natural history of interferon-beta-treated relapsing multiple sclerosis. *Ann Neurol.* 2007;61:300-6.

8. Lu B. Propensity score matching with time-dependent covariates. *Biometrics.* 2005;61:721-8.

eFigure 1. Multivariable Time-Dependent Cox Regression Analysis of Potential Factors Affecting Time to Reach Confirmed and Sustained EDSS 4 for β-IFN Exposed Patients (n=840) and a Contemporary Control Cohort (n=795), With β-IFN Treatment as a Time-Varying Covariate
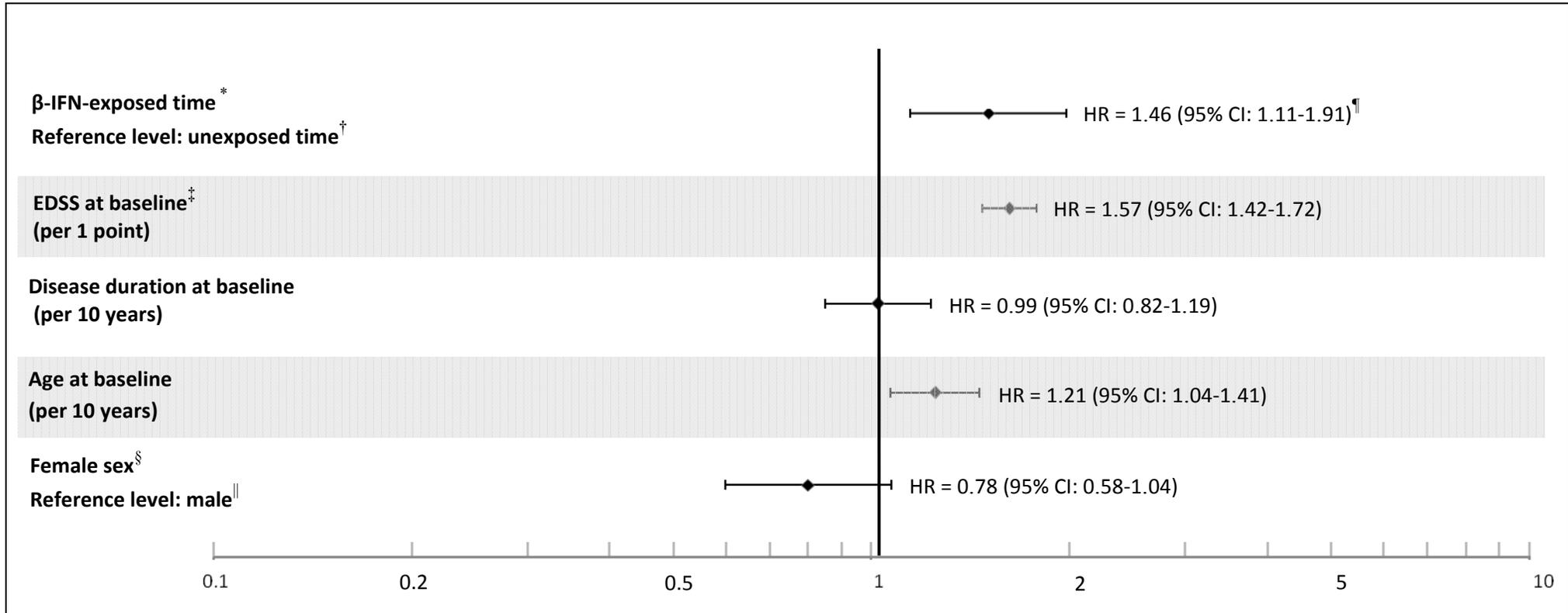
Findings are expressed as adjusted hazard ratios (HRs) with 95% CIs. Covariates included in the model were sex, age at baseline, disease duration at baseline, EDSS at baseline, and β-IFN exposure as a time-varying covariate. Sixty two patients from the β-IFN treated and contemporary control cohorts had already reached EDSS 4 by the baseline and therefore were not included in the analysis. Forty one patients who were censored before the earliest event did not contribute to the analysis.

Key: The following numbers reflect the actual number of patients entered into the Cox model, including 41 patients who were censored before the earliest event.

[*] 2344.8 person-years (112 patients reached the outcome)

[†] 3940.9 person-years (112 patients reached the outcome with 44 during the unexposed time from the β-IFN-exposed cohort and 68 from the contemporary control cohort)

[‡] EDSS at baseline ranged from 0 to 6.5

[§] 1254 patients (67 reached the outcome with 45 from the β-IFN-exposed cohort and 22 from the contemporary control cohort)

[||] 381 patients (157 reached the outcome with 111 from the β-IFN-exposed cohort and 46 from the contemporary control cohort)

[¶] The statistically significant higher hazard of reaching the outcome associated with treatment when the contemporary controls were considered could suggest the presence of an 'indication bias.'

β-IFN=beta-interferon; EDSS=Expanded Disability Status Scale

eFigure 2. Multivariable Time-Dependent Cox Regression Analysis of Potential Factors Affecting Time to Reach Confirmed and Sustained EDSS 4 for β-IFN Exposed Patients (n=840) and a Historical Control Cohort (n=909), With β-IFN Treatment as a Time-Varying Covariate
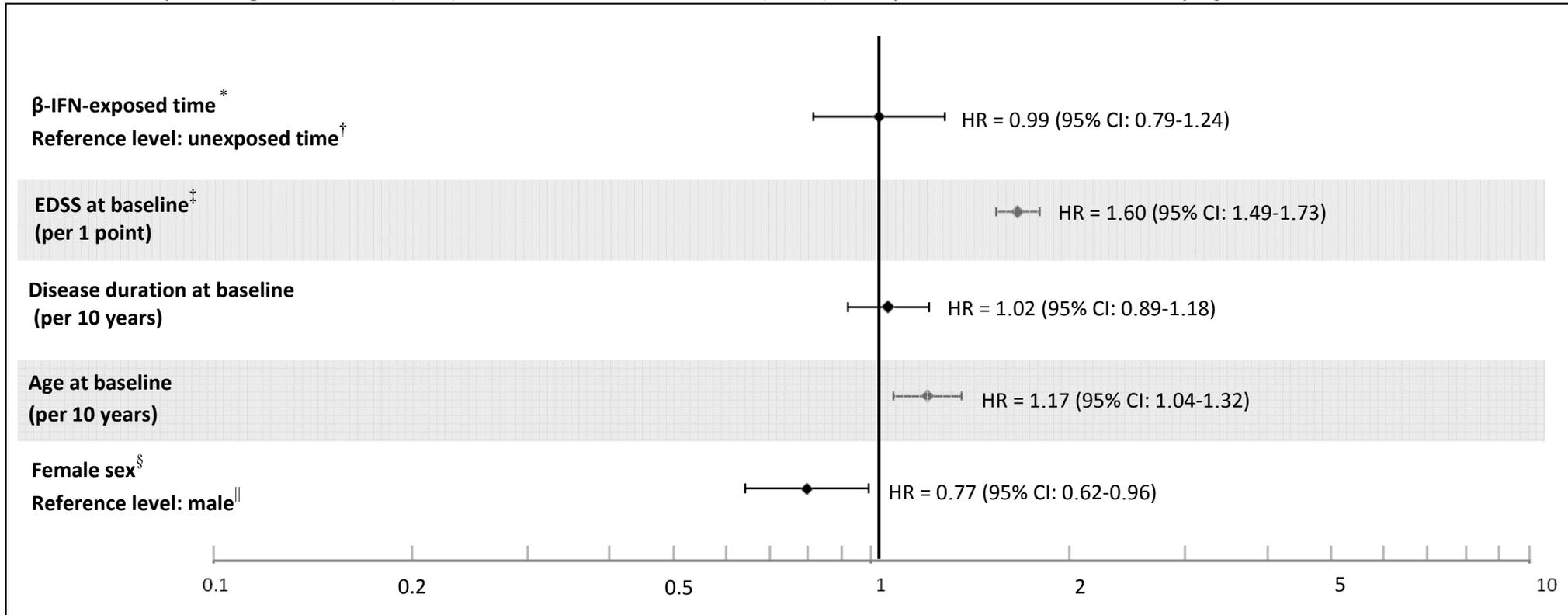
Findings are expressed as adjusted hazard ratios (HRs) with 95% CIs. Covariates included in the model were sex, age at baseline, disease duration at baseline, EDSS at baseline, and β-IFN exposure as a time-varying covariate. Seventy eight patients from the β-IFN treated and historical control cohorts had already reached EDSS 4 by the baseline and therefore were not included in the analysis. Thirty five patients who were censored before the earliest event did not contribute to the analysis.

Key: The following numbers reflect the actual number of patients entered into the Cox model, including 35 patients who were censored before the earliest event.

[*] 2344.8 person-years (112 patients reached the outcome)

[†] 8085.4 person-years (312 patients reached the outcome with 44 during the unexposed time from the β-IFN-exposed cohort and 268 from the historical control cohort)
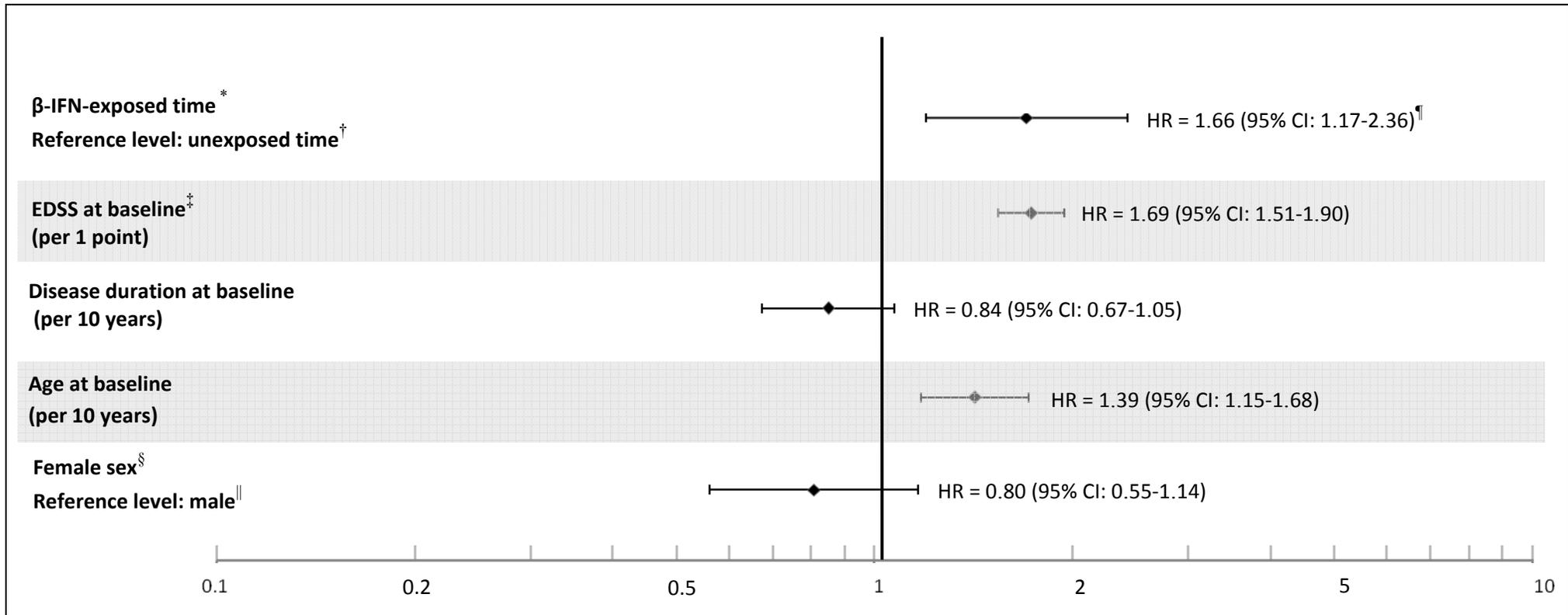
[‡] EDSS at baseline ranged from 0 to 6.5

[§] 1333 patients (306 reached the outcome with 111 from the β-IFN-exposed cohort and 195 from the historical control cohort)

[||] 416 patients (118 reached the outcome with 45 from the β-IFN-exposed cohort and 73 from the historical control cohort)

β-IFN=beta-interferon; EDSS=Expanded Disability Status Scale

eFigure 3. Multivariable Time-Dependent Cox Regression Analysis of Potential Factors Affecting Time to Reach Confirmed and Sustained EDSS 6 for β-IFN Exposed Patients (n=868) and a Contemporary Control Cohort (n=829), With β-IFN Treatment as a Time-Varying Covariate While Assuming a Six-Month Delay in the Initiation of β-IFN Treatment Effect as Well as a Six-Month Carry-Over Effect After Stopping β-IFN Treatment

Findings are expressed as adjusted hazard ratios (HRs) with 95% CIs. Covariates included in the model were sex, age at baseline, disease duration at baseline, EDSS at baseline, and β-IFN exposure as a time-varying covariate. Fifty four patients who were censored before the earliest event did not contribute to the analysis.

Key: The following numbers reflect the actual number of patients entered into the Cox model, including 44 patients who were censored before the earliest event.

[*] 2556.9 person-years (65 patients reached the outcome)

[†] 4262.6 person-years (73 patients reached the outcome with 29 during the unexposed time from the β-IFN-exposed cohort and 44 from the contemporary control cohort)
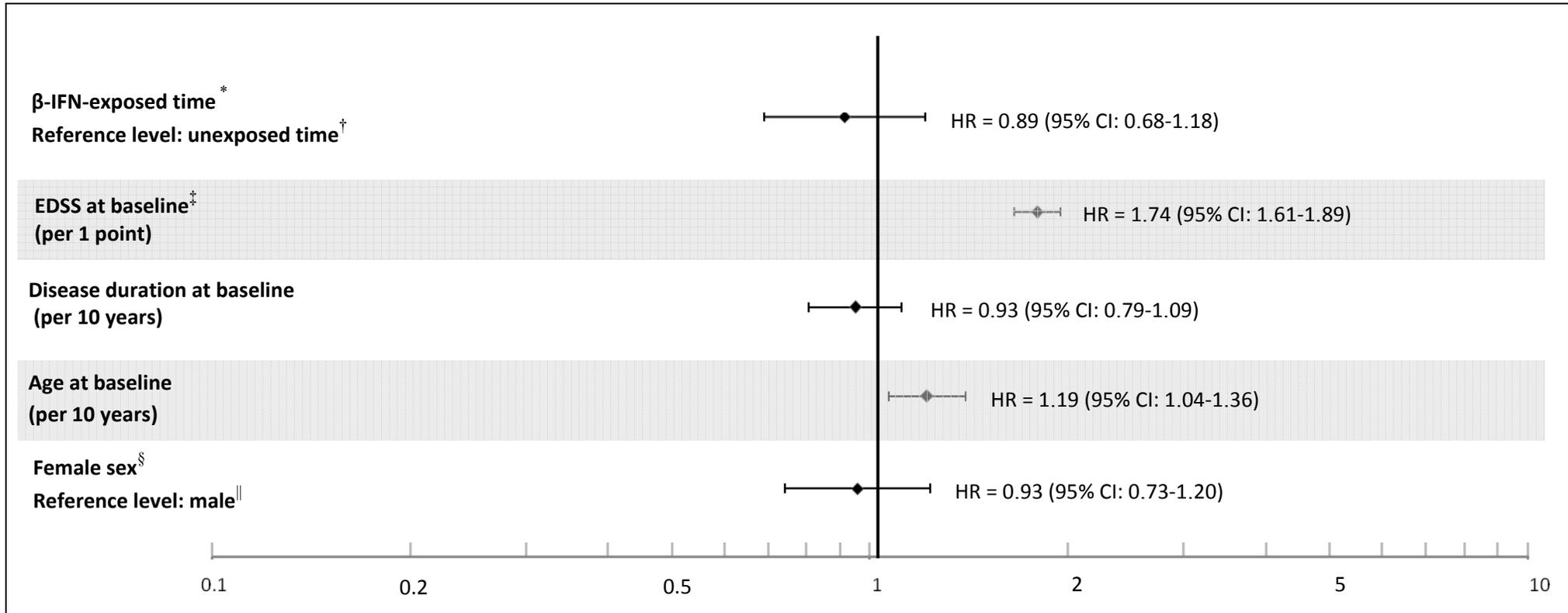
[‡] EDSS at baseline ranged from 0 to 6.5

[§] 1297 patients (95 reached the outcome with 67 from the β-IFN-exposed cohort and 28 from the contemporary control cohort)

[‖] 400 patients (43 reached the outcome with 27 from the β-IFN-exposed cohort and 16 from the contemporary control cohort)

[¶] The statistically significant higher hazard of reaching the outcome associated with treatment when the contemporary controls were considered could suggest the presence of an 'indication bias.'

β-IFN=beta-interferon; EDSS=Expanded Disability Status Scale

eFigure 4. Multivariable Time-Dependent Cox Regression Analysis of Potential Factors Affecting Time to Reach Confirmed and Sustained EDSS 6 for β-IFN Exposed Patients (n=868) and a Historical Control Cohort (n=959), With β-IFN Treatment as a Time-Varying Covariate While Assuming a Six-Month Delay in the Initiation of β-IFN Treatment Effect as Well as a Six-Month Carry-Over Effect After Stopping β-IFN Treatment

Findings are expressed as adjusted hazard ratios (HRs) with 95% CIs. Covariates included in the model were sex, age at baseline, disease duration at baseline, EDSS at baseline, and β-IFN exposure as a time-varying covariate. Twenty patients who were censored before the earliest event did not contribute to the analysis.

Key: The following numbers reflect the actual number of patients entered into the Cox model, including 20 patients who were censored before the earliest event.

[*] 2556.9 person-years (65 patients reached the outcome)

[†] 8921.3 person-years (251 patients reached the outcome with 29 during the unexposed time from the β-IFN-exposed cohort and 222 from the historical control cohort)
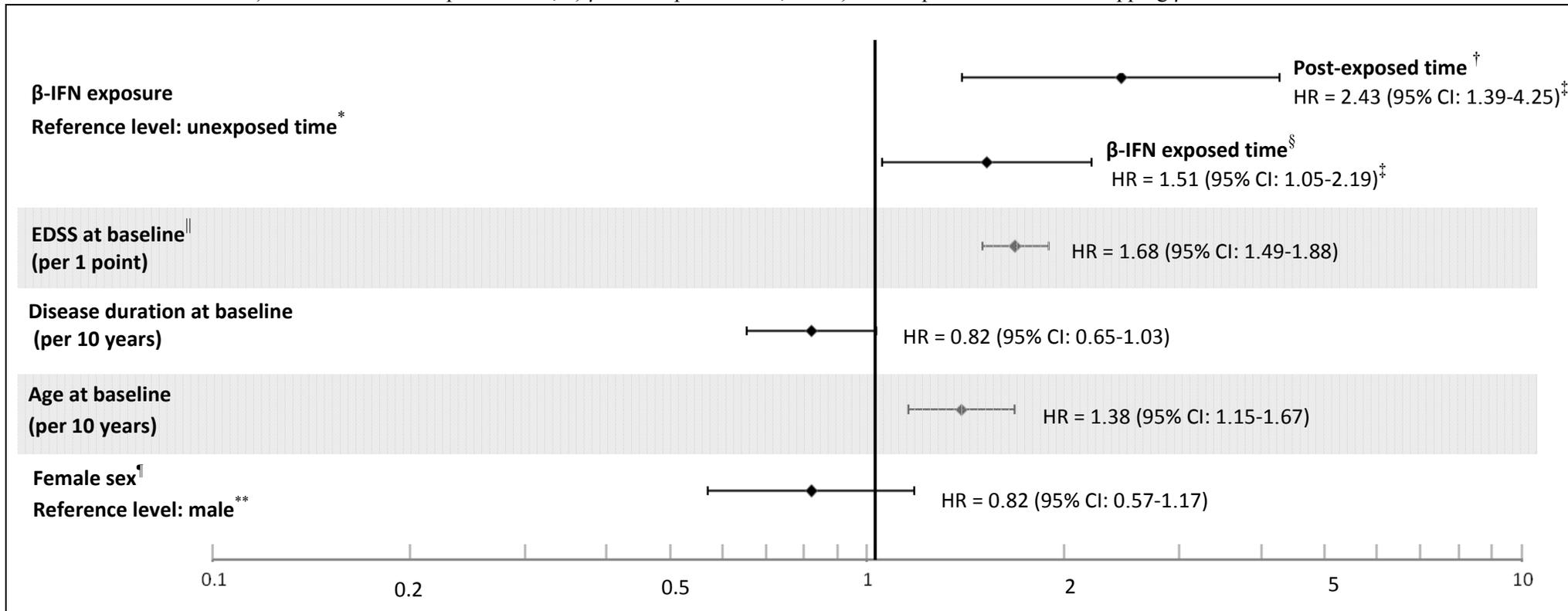
[‡] EDSS at baseline ranged from 0 to 6.5

[§] 1381 patients (231 reached the outcome with 67 from the β-IFN-exposed cohort and 164 from the historical control cohort)

[||] 446 patients (85 reached the outcome with 27 from the β-IFN-exposed cohort and 58 from the historical control cohort)

β-IFN=beta-interferon; EDSS=Expanded Disability Status Scale

Findings are expressed as adjusted hazard ratios (HRs) with 95% CIs. Covariates included in the model were sex, age at baseline, disease duration at baseline, EDSS at baseline, and β-IFN exposure as a time-varying covariate. Fifty four patients who were censored before the earliest event did not contribute to the analysis.

Key: The following numbers reflect the actual number of patients entered into the Cox model, including 54 patients who were censored before the earliest event.

[*]3943.3 person-years (56 patients reached the outcome with 12 during the pre-treatment unexposed time from the β-IFN-exposed cohort and 44 from the contemporary control cohort)

[†]319.3 person-years (17 patients reached the outcome)

[‡] The statistically significant higher hazard of reaching the outcome associated with treatment when the contemporary controls were considered could suggest the presence of an 'indication bias.'
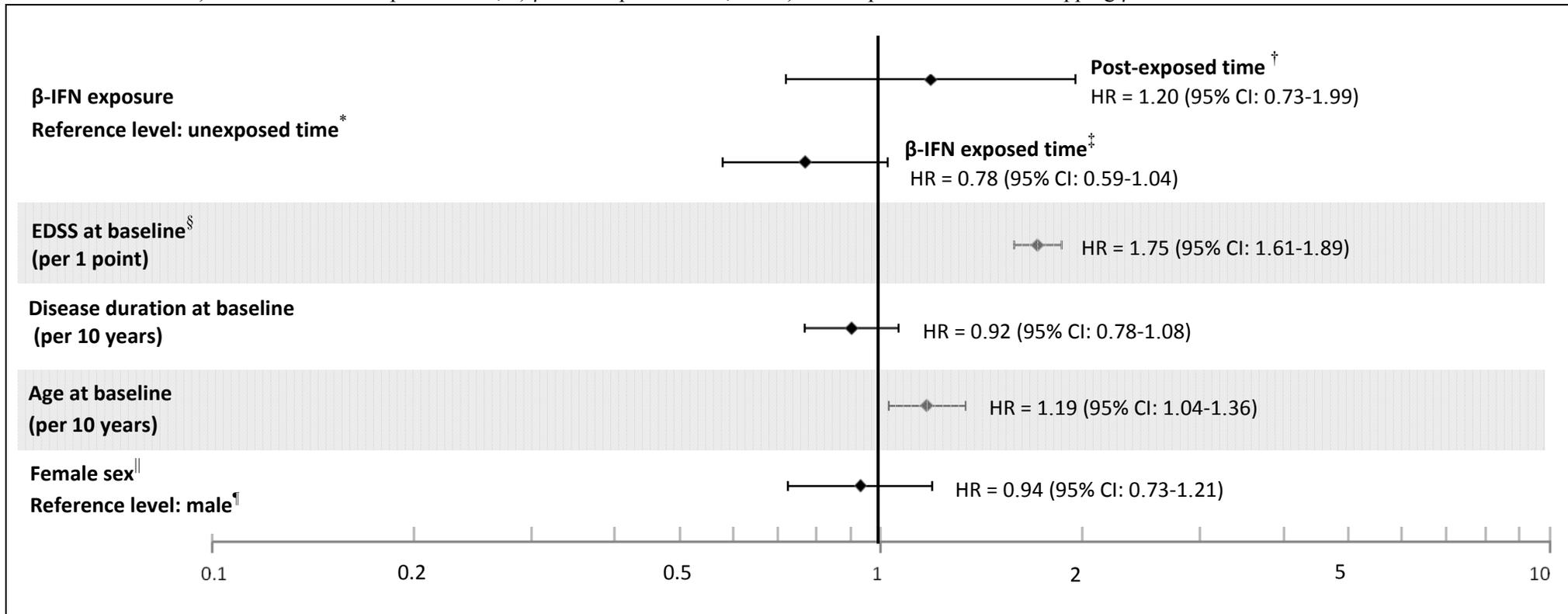
[§]2556.9 person-years (65 reached the outcome)

[‖] EDSS at baseline ranged from 0 to 6.5

[¶]1297 patients (95 reached the outcome with 67 from the β-IFN-exposed cohort and 28 from the contemporary control cohort)

[**] 400 patients (43 reached the outcome with 27 from the β-IFN-exposed cohort and 16 from the contemporary control cohort)

β-IFN=beta-interferon; EDSS=Expanded Disability Status Scale

eFigure 6. Multivariable Time-Dependent Cox Regression Analysis of Potential Factors Affecting Time to Reach Confirmed and Sustained EDSS 6 for β-IFN Exposed Patients (n=868) and a Historical Control Cohort (n=959), With β-IFN Treatment as a Time-Varying Covariate With Three Levels: 1) Pre-treatment Unexposed Time; 2) β-IFN Exposed Time; and 3) Post-exposed Time After Stopping β-IFN Treatment

Findings are expressed as adjusted hazard ratios (HRs) with 95% CIs. Covariates included in the model were sex, age at baseline, disease duration at baseline, EDSS at baseline, and β-IFN exposure as a time-varying covariate. Twenty patients who were censored before the earliest event did not contribute to the analysis.

Key: The following numbers reflect the actual number of patients entered into the Cox model, including 20 patients who were censored before the earliest event.

[*]8602.0 person-years (234 patients reached the outcome with 12 during the pre-treatment unexposed time from the β-IFN-exposed cohort and 222 from the historical control cohort)

[†]2556.9 person-years (65 patients reached the outcome)

[‡]319.3 person-years (17 patients reached the outcome)

[§]EDSS at baseline ranged from 0 to 6.5

[‖]1381 patients (231 reached the outcome with 67 from the β-IFN-exposed cohort and 164 from the historical control cohort)

[¶]445 patients (85 reached the outcome with 27 from the β-IFN-exposed cohort and 58 from the historical control cohort)

β-IFN=beta-interferon; EDSS=Expanded Disability Status Scale

eFigure 7. Multivariable Time-Dependent Cox Regression Analysis of Potential Factors Affecting Time to Reach Confirmed and Sustained EDSS 6 for β-IFN Exposed Patients (n=1094) and a Historical Control Cohort (n=959), With β-IFN Treatment as a tIme-Varying Covariate
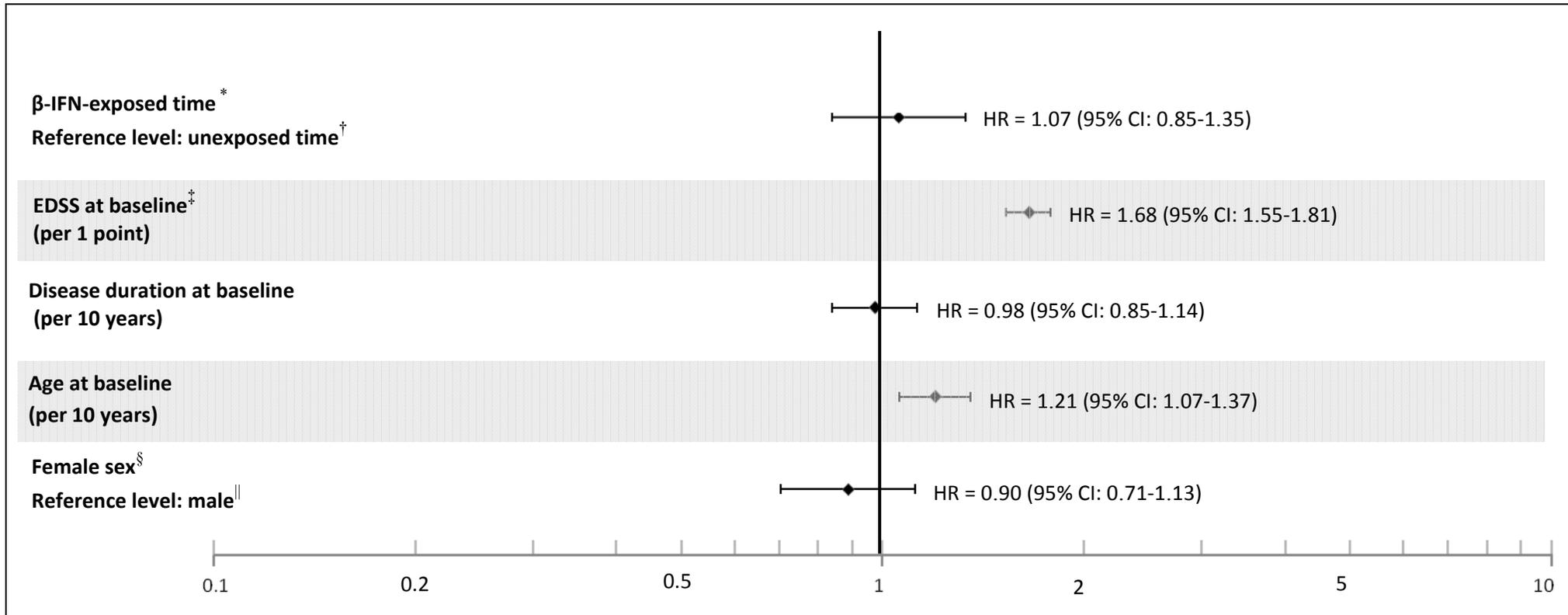
Historical patients who became eligible for β-IFN treatment between April/1985 and June/1995 (when the drug was not yet licensed in Canada), and were exposed to β-IFN during the follow-up time were not excluded from the model. Findings are expressed as adjusted hazard ratios (HRs) with 95% CIs. Covariates included in the model were sex, age at baseline, disease duration at baseline, EDSS at baseline, and β-IFN exposure as a time-varying covariate. Twenty patients who were censored before the earliest event did not contribute to the analysis.

Key: The following numbers reflect the actual number of patients entered into the Cox model, including 20 patients who were censored before the earliest event.

[*] 3393.4 person-years (105 patients reached the outcome)

[†] 10842.2 person-years (264 patients reached the outcome with 42 during the unexposed time from the β-IFN-exposed cohort and 222 from the historical control cohort)

[‡] EDSS at baseline ranged from 0 to 6.5

[§] 1555 patients (270 reached the outcome with 106 from the β-IFN-exposed cohort and 164 from the historical control cohort)

[‖] 498 patients (99 reached the outcome with 41 from the β-IFN-exposed cohort and 58 from the historical control cohort)

β-IFN=beta-interferon; EDSS=Expanded Disability Status Scale