Protocol: **Analysis of risk of Tuberculosis in individuals using selected drugs in a cohort of multiple sclerosis patients.**

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1) Background

Multiple sclerosis (MS) is a chronic neurological condition characterised by inflammation and neurodegeneration, often necessitating long-term treatment with immunosuppressive therapies. While these treatments effectively manage MS symptoms and slow disease progression, they also compromise the immune system, increasing the risk of infections such as tuberculosis (TB). TB remains a significant global health concern, particularly in regions with high prevalence and limited healthcare resources.[1] This overlap between MS treatments and TB prevalence raises important questions about the heightened vulnerability of MS patients to TB.

The immunosuppressive therapies commonly used in MS, such as corticosteroids, disease-modifying therapies (DMTs), and monoclonal antibodies, can significantly alter the immune system's ability to combat infections. These therapies create a potential vulnerability to latent TB reactivation or new TB infections, a risk that is particularly concerning in endemic regions or patients with other predisposing factors. However, current evidence is sparse and fragmented, with most studies concentrating on the broader infectious risks of immunosuppression rather than TB-specific outcomes in MS patients.[2,3]

This lack of targeted research creates a critical gap in knowledge, limiting clinicians' ability to identify high-risk patients, implement appropriate screening protocols, and develop preventive strategies tailored to this unique population. Given the potentially severe consequences of TB in immunosuppressed individuals, understanding the risk of TB in MS patients is essential for informing clinical practice and improving patient outcomes.

The present study will evaluate the risk of TB in patients with MS using specific immunosuppressive drugs.

2) Data sources

This retrospective observational study utilised de-identified electronic medical records (EHR) data of the TriNetX Research Network (Cambridge, MA), which contains de-identified EHR data of more than 80 million patients from over 80 participating healthcare organisations predominantly from the U.S. Data in the TriNetX database has undergone extensive curation and mapping to common clinical entities and terminologies to ensure referential integrity and reliability,[4,5] consistent with the Reporting of studies Conducted using Observational Routinely collected Data (RECORD)

guidelines.[6] Because TriNetX data used in the study did not contain any protected health information, this research was determined exempt from the Institutional Review Board oversight.

3) Analytic approaches

Two analytic approaches to assess the risk of TB. A self-controlled case series and retrospective cohort analysis. The SCCS analysis implicitly controls for any non-time varying factors not included in the cohort analysis that are associated with both drug use and TB.

4) Exposures

The drugs of interest will be classified as:

- Beta interferons (Beta 1a Interferon, Beta Interferon, and Beta 1b Interferon)
- Anti CD-20 (Ofatumumab, Ocrelizumab, and Rituximab)
- Fumarates (Dimethyl, Monomethyl, and Diroximel)
- Siponimod-Fingolimod (Siponimod and Fingolimod)

Additionally, the specific drugs will be evaluated:

- Methylprednisolone
- Glatiramer acetate
- Teriflunomide
- Natalizumab (anti-cell adhesion molecule α4-integrin)
- Ocrelizumab
- Rituximab

5) Cohort analysis

a. Study population

We will include all individuals diagnosed with MS between Jan 1, 2010 and Nov 01, 2022. The end of follow-up is January 31, 2023. This timeline allows for at least 90 days of follow-up for individuals diagnosed until November 01, 2022.

We will exclude individuals: (i) who were diagnosed with TB before the diagnosis of multiple sclerosis or within 30 days; (ii) who were aged 100 years or older at diagnosis of MS; (iii) inconsistent data, i.e., diagnosis of TB after date of death; (iv) missing data on birthdate or sex.

b. Statistical analysis

Each individual will be followed up from the MS diagnosis date until the earliest of the following events: diagnosis of TB, death, or Jan 31, 2023 (final data collection date). Calendar time was used as the model's time scale.
We will estimate the time-dependent Cox model adjuster for time-fixed variates, such as age at MS diagnosis, race (Asian, Black, Native, White and others), Hispanic (yes or no), sex, and location (outside USA, midwest, northeast, south, west, missing); and time-varying variates: HIV infection, primary immunodeficiencies, solid-organ transplantation, hematopoietic stem cell transplantation, solid-organ malignancy, hematologic malignancy, chronic obstructive pulmonary disease, asthma, bronchiectasis, diabetes, chronic kidney disease, chronic liver disease, alcohol use disorder, diagnosis of latent tuberculosis, and treatment of latent tuberculosis. In addition, to account for potential confounding effects of the socioeconomic condition, we included diagnoses which may indicate increased risk due to socioeconomic and psychosocial circumstances (education and literacy, employment, housing, lack of adequate food or water, or exposure to occupational hazards). They were based on ICD-10 codes, Z55-Z65.
The exposure levels are:

- (i) no use of the drug
- (ii) 1- 30 days after starting to use the drug
- (iii) 31 days to stop the drug
- (iv) 1 - 180 days after ending the use of the drug
- (v) 181 - 360 after ending the use of the drug
- (vi) 361 days after the ending was considered equal to no drug use.

We will use robust standard error to estimate the 95% confidence intervals.

6) Self-controlled case series analysis (SCCS)

The self-controlled case series (SCCS) method is an efficient and robust study design to evaluate the risk of developing TB after starting an immunosuppressive drug, even for patients undergoing long-term treatment. This design compares the rate of events (e.g., TB diagnosis) during exposed and unexposed periods within the same individuals. Using each individual as their control, the SCCS inherently adjusts for time-invariant confounders, such as genetic predispositions, socioeconomic factors, and baseline health characteristics. While immunosuppressive exposure may span years,

the SCCS can account for time-varying risks by incorporating flexible definitions of exposure periods, such as induction phases, maintenance therapy, and post-treatment windows. This allows researchers to examine dynamic changes in risk over time and assess whether TB risk peaks during specific intervals, such as shortly after treatment initiation. The SCCS design is particularly advantageous in this context because it minimizes confounding and is highly efficient. It requires data only from individuals who develop the outcome of interest while accommodating complex exposure patterns.[7]

In this study, we will use the date of the MS diagnosis as the beginning of the observation period and January 31, 2023, or death as the end of follow-up.

a. Study population

We will include only individuals who started the use of the drug after the diagnosis of MS. This will ensure that the period of 30 days to the end of follow-up will be more similar across all patients and that the prescription of the drug was related to the MS and not a previous condition.

b. Statistical analysis

The self-controlled case series method uses conditional Poisson regression to compare intra-person relative incidence rates in different study periods relative to the date of drug prescription. We will compare TB incidence rate in the period following the prescription of the drug until the end of data collection (31 January 2023 or death). The exposure periods will be similar to those of the cohort:

- (i) no use of the drug
- (ii) 0 day
- (iii) 1- 30 days after starting to use the drug
- (iv) 31 days to stop the drug
- (v) 0 - 180 days after ending the use of the drug
- (vi) 181 - 360 after ending the use of the drug
- (vii) 361 days after the ending was considered equal to no drug use.

The need to adjust for age (in quintiles) will be assessed through a likelihood test. Relative incidence estimates will not be calculated where the number of events in the period is ≤ 1, and periods with a number of events between 2 and 3 will not be regarded as strong evidence irrespective of statistical significance.

7) Choice of models

The primary model of this study will be the self-controlled case series (SCCS) due to its ability to adjust for all time-invariant and unmeasured confounders. This design is advantageous for controlling factors such as genetic predispositions or baseline comorbidities that remain constant within individuals. However, the SCCS method has some limitations, especially in the presence of time-varying covariates such as newly diagnosed comorbidities. Given the medium follow-up period of up to 12 years, we do not anticipate a significant degree of bias from changes in the risk of TB due to the development of new comorbidities. Furthermore, the exposure periods in this study are carefully defined, including a short period following drug initiation to mitigate reverse causation (e.g., cases where the drug may have been started due to the diagnosis of TB infection). Increased risks on day zero greatly indicate this type of problem. The model also includes a post-exposure period of up to 360 days to account for residual drug effects and to detect potential bias. Notably, if an increased TB risk is only observed in the late post-exposure period (181 to 360 days), it likely indicates bias due to time-varying covariates rather than a true residual drug effect.

The secondary model in the study will be a time-dependent Cox proportional hazards model, using the same exposure periods as the SCCS. This consistency allows the Cox model to account for reverse causation similarly. Unlike the SCCS, the Cox model will allow individuals who were using the drug before the diagnosis of multiple sclerosis (MS) to contribute person-years to the analysis. However, the time-dependent Cox approach has its limitations, particularly the potential for bias toward the null in the presence of time-varying confounders if drug use influences the evolution of these confounders over time.[8]

An alternative approach, such as marginal structural models, could be used to estimate the drug's effects on TB risk while accounting for time-varying confounders. However, due to the study's focus on multiple time points following drug initiation, including the post-exposure period, the time-dependent Cox model is better suited for estimating short-term effects. Importantly, any bias introduced by the Cox model in this scenario will likely attenuate the association (i.e., bias toward the null). Therefore, if an association between the drug and TB is detected using this approach, it would indicate a true underlying relationship.[8]

Lastly, all models will only contain one exposure (drug) each time. MS patients rarely use more than one immunosuppressant concomitantly. Therefore we will not explore synergistic effects on the risk of tuberculosis.

We will regard strong evidence of association if a particular drug has statistically significant effects and in the same direction in both models (SCCS and Cox), regardless of the magnitude of the point estimate.

8) References

1 Kyu HH, Maddison ER, Henry NJ, *et al.* The global burden of tuberculosis: results from the Global Burden of Disease Study 2015. *Lancet Infect Dis* 2018; **18**: 261–84.

2 Dantas LA, Pereira MS, Gauza A de M, *et al.* Latent tuberculosis infection reactivation in patients with multiple sclerosis in use of disease-modifying therapies: A systematic review. *Mult Scler Relat Disord* 2021; **55**: 103184.

3 Luna G, Alping P, Burman J, *et al.* Infection Risks Among Patients With Multiple Sclerosis Treated With Fingolimod, Natalizumab, Rituximab, and Injectable Therapies. *JAMA Neurol* 2020; **77**: 184–91.

4 Palchuk MB, London JW, Perez-Rey D, *et al.* A global federated real-world data and analytics platform for research. *JAMIA Open* 2023; **6**: ooad035.

5 Topaloglu U, Palchuk MB. Using a Federated Network of Real-World Data to Optimize Clinical Trials Operations. *JCO Clin Cancer Inform* 2018; : 1–10.

6 Benchimol EI, Smeeth L, Guttmann A, *et al.* The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement. *PLOS Med* 2015; **12**: e1001885.

7 Farrington P, Whitaker H, Weldeselassie YG. Self-controlled case series studies: a modelling guide with R. CRC Press, 2018.

8 Xie D, Yang W, Jepson C, *et al.* Statistical Methods for Modeling Time-Updated Exposures in Cohort Studies of Chronic Kidney Disease. *Clin J Am Soc Nephrol CJASN* 2017; **12**: 1892–9.