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ORIGINAL RESEARCH ARTICLE

# Teriflunomide Concentrations in Cerebrospinal Fluid and Plasma in Patients with Multiple Sclerosis: A Pharmacokinetic Study

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## Abstract

**Background** Teriflunomide is a disease modifying treatment (DMT) approved for relapsing-remitting multiple sclerosis (RRMS) in adults and children. It reduces lymphocyte proliferation by inhibiting the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH) and thereby the pyrimidine synthesis. Although most DMTs in multiple sclerosis (MS) modulate or inhibit the immune system in the periphery, the efficacy may improve if the agent also targets immune activity within the central nervous system (CNS), acts as a neuro-protective and enhances neuro-regeneration. The objective of this study was to determine the passage of teriflunomide over the blood-cerebrospinal fluid barrier (BCSFB).

**Methods** Plasma and cerebrospinal fluid (CSF) teriflunomide concentrations were determined at steady state in 12 patients with RRMS, treated with oral teriflunomide 14 mg once daily. Included patients were all clinically stable without relapse or disability worsening within 6 months prior from baseline and were on no other immune modulating or immunosuppressive drugs.

**Results** The mean teriflunomide concentrations in plasma and CSF were 38775 (SEM ± 7256) ng/mL and 68 (SEM ± 15) ng/mL, respectively. The passage over the BCSFB was 0.17 % (SEM ± 0.01). While no correlation was found between the function of the BCSFB assessed with the albumin ratio and the CSF teriflunomide concentration, the CSF and plasma teriflunomide concentrations were highly correlated ($r_s = 0.90$, $< 0.0001$).

**Conclusions** Further studies are warranted to determine if the obtained CSF teriflunomide concentration reflects that in the CNS and is able to influence inflammatory and degenerative processes within the CNS.

**Key Points**

The passage of teriflunomide over the blood-cerebrospinal fluid barrier (BCSFB) is low, less than 1%.

The teriflunomide concentrations in cerebrospinal fluid (CSF) and plasma are highly correlated and stable at steady state.

## 1 Introduction

Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS), with both inflammatory and degenerative features [1, 2]. In 85–90% of patients the early phase is characterised by a relapsing-remitting (RR) course, i.e., relapses of transient new or aggravated neurological symptoms followed by complete or partial symptom remission and often extensive clinically silent periods, lasting from months to several years. Immune-mediated exacerbations of white matter demyelinating lesions cause relapses and new lesion formation on magnetic resonance imaging (MRI). Treatment with disease-modifying therapies (DMTs) reduce this activity as well as the rate of neurological disability. Although several of the approved drugs in MS show limited passage over the blood-brain barrier (BBB) they reduce disease activity by immunomodulation or immunosuppression in the periphery. However, during later stages of MS, chronic inflammatory lesions within the CNS may become more important in the pathogenesis of MS [3, 4]. Treatment of such compartmentalised

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inflammation may have beneficial effects and some agents may be neuroprotective as well as augment remyelination and promote neuro-restoration. To achieve these additional effects, the ability to pass the BBB for DMTs is probably crucial. The objective of this study was to determine teriflunomide concentrations in plasma and cerebrospinal fluid (CSF) and thereby the passage over the blood-CSF barrier (BCSFB) in patients with RRMS.

Oral teriflunomide, 7 mg and 14 mg is approved for the treatment of relapsing forms of MS [5, 6] . In two Phase III pivotal studies teriflunomide reduced the annual relapse rate, the risk of disability progression, and reduced the rate of lesion formation on magnetic resonance imaging (MRI) [7, 8]. Safety from four pivotal studies showed that the most common reason for discontinuation of teriflunomide treatment was increased liver function tests, diarrhoea, hair thinning, and nausea [7–10]. In a study of pooled safety data from these and their extension studies, no new or unexpected safety signals were detected [11].

Teriflunomide is the active metabolite of leflunomide, a drug that is used for the treatment of rheumatoid arthritis. The exact mode of action for teriflunomide is not completely known, but it most likely acts by inhibiting de novo pyrimidine synthesis via reversible non-competitive inhibition of the mitochondria's enzyme dihydroorotate dehydrogenase (DHODH), leading to reduced proliferation of B and T lymphocytes [12–14].

Teriflunomide has a molecular weight of 270.2 Da, a low solubility in water and a high permeability. The documented bioavailability is almost 100%. After oral administration, teriflunomide is rapidly absorbed, and a maximum concentration is reached at 1–4 h post-dose. With oral intake of teriflunomide doses of 7 and 14 mg daily, steady state is reached after approximately 3 months, and the accumulation is approximately 30-fold, giving rise to plasma concentrations of 20–60 mg/L [15]. Teriflunomide has very high protein binding (99.5%), mainly to albumin [16], is eliminated mostly through direct biliary excretion of unchanged drug, and has a long half-life of approximately 18–19 days, dependent upon teriflunomide re-uptake via the enterohepatic circulation [6]. Several immunotherapeutics used in MS, including teriflunomide, are substrates of the efflux transporter breast cancer receptor protein (BCRP) or ATP-binding cassette (ABC) G2, and BCRP/ABCG2 genotypes had pharmacokinetic effects on the teriflunomide concentration in healthy subjects [17, 18].

The passage of teriflunomide over the blood brain barrier (BBB) in humans is currently unknown. However, in naïve and experimental rats the concentration in the brain was 676–1108 ng/mL [19], which is approximately 2–4% of blood concentrations, exceeding the $IC_{50}$ values for teriflunomide inhibiting DHODH (270 ng/mL) [20].

## 2 Methods

### 2.1 Study Design, Patients, and Clinical Evaluation

This was a pharmacokinetic single-centre study performed at the MS Centre, Sahlgrenska University Hospital, Gothenburg, Sweden. Eligible patients were women and men, aged 18–65 years with RRMS, treated with teriflunomide 14 mg, once daily, for at least 6 months. They should have a normal complete blood cell count, normal liver function test, no severe renal disease, and fertile females should use safe contraceptive methods. Exclusion criteria were other CNS diseases, other diseases or treatments affecting absorption, metabolism or elimination of teriflunomide, and other immune-modulating or immunosuppressive drugs.

Participating subjects should be clinically stable without relapse or disability worsening within 6 months from baseline. Disability was assessed with Expanded Disability Status Scale (EDSS) [21], and all had an MRI scan of the brain. Patients were instructed to take their tablet of teriflunomide without food at 0800 h daily during a period of at least 20 days before sampling. In 50% of patients, the samples of peripheral blood and CSF were obtained before tablet intake (at approximately 0800 h) and in 50% at four hours after tablet intake (the expected peak plasma level at steady state). Lumbar puncture was performed, and plasma/serum and CSF was handled and stored according to the consensus protocol of the BioMS–EU network for CSF biomarker research in MS [22].

### 2.2 Determination of Teriflunomide in Plasma and CSF

Teriflunomide concentrations in plasma and CSF were determined with high performance liquid chromatography (HPLC). The analyses were performed at Labcorp, study number 8419351 in accordance with Guidelines on Good Clinical Practice, ICH E6 (R2) and Good Clinical Practice, CPMP/ICH/135/95 (June 2017). Sample analyses used two methods, HMRHPP for plasma and TERMHCP for CSF, which were validated under Labcorp study 8260414 and 8346832, respectively. For calibration standards and quality controls in sample analysis, accuracy acceptance criteria is ± 15.0% (± 20% for lower limit of quantification (LLOQ). Precision acceptance criteria is 15.0% (20.0% for LLOQ). The LLOQ is 10.0 ng/mL for both matrices. For incurred sample reanalysis, the reanalysis result should be within ±20% from original results to be acceptable.

### 2.3 Determination of Inflammatory and Degenerative Biomarkers in Peripheral Blood and CSF

Licensed laboratory technicians in the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital performed the biomarker analyses. Cerebrospinal fluid and serum albumin and immunoglobulin G (IgG) levels were analysed using the IGG-2 and ALBT2 reagent cassettes on a Cobas C module instrument (Roche). The CSF/serum albumin ratio was calculated as (CSF albumin [mg/L]/serum albumin [g/L]). Serum and CSF concentrations of kappa free light chain (KFLC) were measured using the N Latex FLC kappa kit, on an Atellica NEPH 630 instrument (Siemens), following the instructions by the manufacturers. The KFLC index was calculated using the equation ([CSF KFLC/serum KFLC]/[CSF albumin/serum albumin]). CSF neurofilament light (NfL) concentration was measured with a sandwich ELISA method (NF-light® ELISA kit, UmanDiagnostics AB, Umeå, Sweden). Intra- and inter-assay coefficients of variation were below 10%. The LLOQ of the assay was 31 ng/L. Plasma NfL concentration was measured using the Simoa® NF-light™ Advantage Kit on an HD-X Analyzer (Quanterix, Billerica, MA). The intra-assay and inter-assay coefficients of variation were below 10%.

### 2.4 Statistics

Spearman's Rho correlation was used to analyse bivariate correlations and the analyses was carried out in SPSS 23.0 for Windows software. A $p$-value $\leq 0.05$ was considered significant.

## 3 Results

In this pharmacokinetic study we included 12 RRMS patients, treated with teriflunomide for 1.4–5.6, mean 3.4 years. No patient had a recent relapse or new or enlarging lesion formation on brain MRI. However, two patients had NfL concentrations above the age-adjusted reference level in CSF [23]. All, except one patient had a moderate to high T2 lesion load on MRI and one patient had no signs of increased intrathecal immunoglobulin production (Table 1).

### 3.1 Teriflunomide Concentrations in Plasma and Cerebrospinal Fluid

The concentration of teriflunomide in paired plasma and CSF samples at 0 compared with 4 h after tablet intake of teriflunomide 14 mg was essentially unchanged, indicating stable teriflunomide concentrations at steady state (Fig. 1). The teriflunomide ratio (CSF/plasma teriflunomide concentration) was calculated for 0 h, 4 h and for 0 + 4 h combined, and used as a measurement of teriflunomide BCSFB passage into CNS (Fig. 2). The mean CSF/plasma teriflunomide concentration ratio was calculated to 0.17% at 0 h, 4 h and 0 + 4 h. However, in a post hoc analysis we assumed that 99.5% of teriflunomide is protein bound in blood allowing only 0.5% to cross over the BCSFB. Thus, of approximately 195 ng/mL (0.5% of 39.000 ng/mL, the mean concentration in plasma)

**Table 1** Baseline demographic and clinical characteristics

| | |
|---|---|
| Age: mean years (range) | 52 (36–65) |
| Gender: F/M | 5/7 |
| Disease duration: mean years (range) | 17 (2–31) |
| Teriflunomide treatment: mean years (range) | 3.4 (1.4–5.6) |
| Previous DMT: INF-β/GA/not treated | 9/1/2 |
| Number of relapses: median (range) | 2 (1–10) |
| Time to previous relapse: mean years (range) | 12 (2–22) |
| Cerebral MRI, number of T2 lesions: > 20/10–20/< 10 | 6/5/1 |
| Cerebral MRI, number of new or enlarging T2 lesions/Gd+ T1 lesions: (range) | 0/0 |
| EDSS: median (range) | 3 (0–3.5) |
| CSF OCB: no/yes | 4/8 |
| Kappa index: mean (range), $n = > 3.43$ | 34.4 (3.3–104), 11 |
| Albumin ratio: mean (range) | 5.8 (3.7–9.9) |
| Plasma NfL concentration (pg/mL): mean ± SEM (range) | 18.3 ± 4.5 (6.1–61.8) |
| CSF NfL concentration (ng/mL): mean ± SEM (range) | 770 ± 128 (300–1180) |

*CSF* cerebrospinal fluid, *DMT* disease-modifying therapy, *EDSS* expanded disability status scale, *GA* glatiramer acetate, *Gd+* gadolinium enhancing, *INF-β* interferon beta, *MRI* magnetic resonance imaging, *NfL* neurofilament light chain, *OCB* oligoclonal IgG bands, *SEM* standard error of the mean

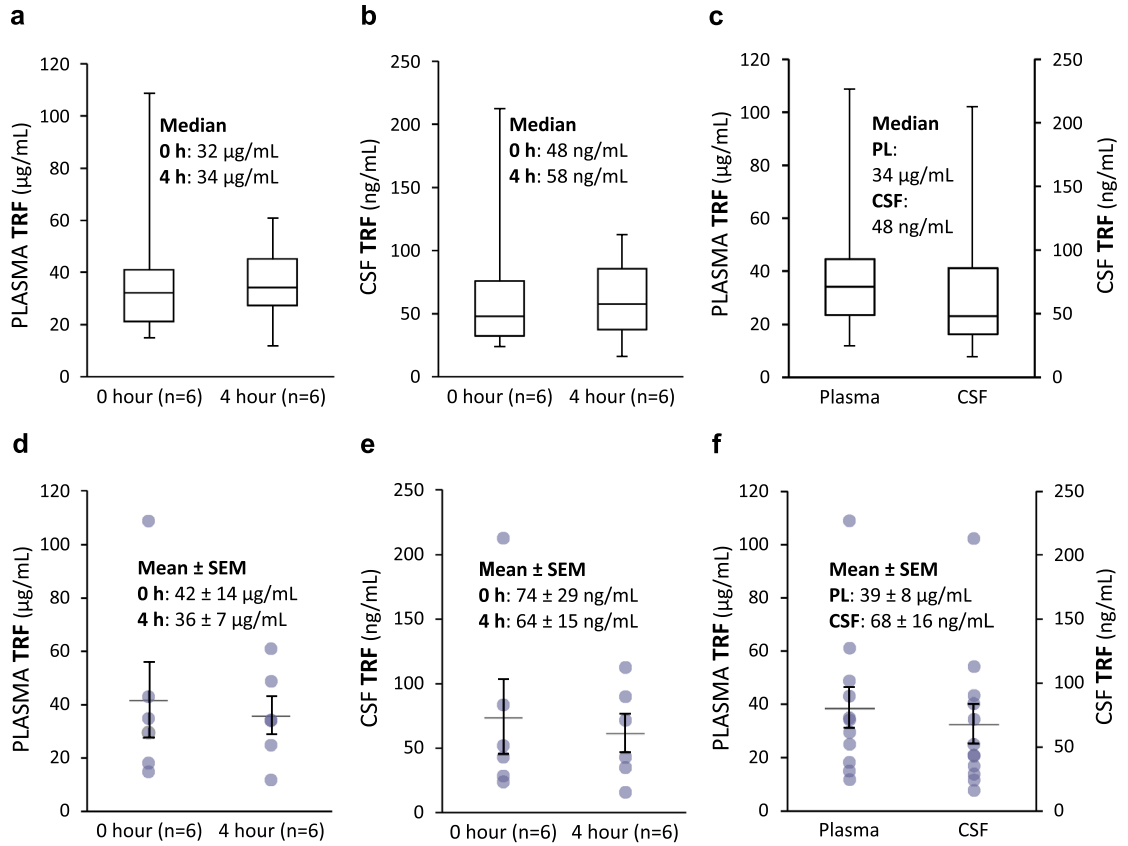


**Fig. 1** Teriflunomide concentrations in plasma and CSF, at hour 0, 4 hours and 0 + 4 hours combined. **a–c** Box plot distributions. **d–f** Dot plots of individual values

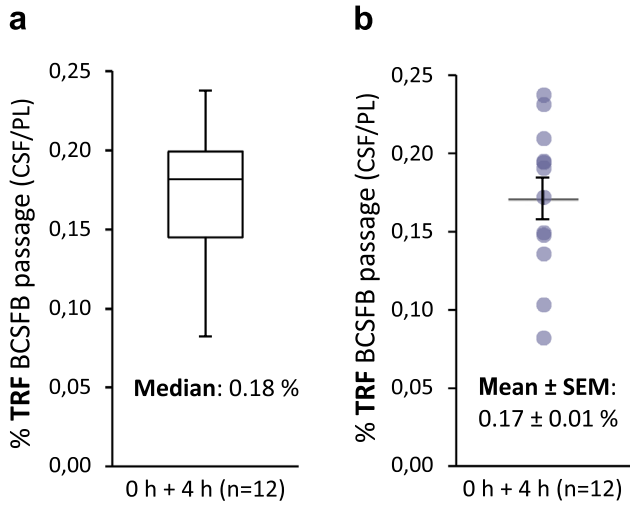


**Fig. 2** Percent teriflunomide BCSFB passages (CSF/plasma teriflunomide concentrations), at 0 + 4 h combined. **a** Box plot distribution. **b** Dot plot of individual values

teriflunomide, the estimated penetrans of free teriflunomide from blood to CSF was 35%.

The influence from the albumin ratio (CSF/serum albumin concentration × 1000), a standardised measure of the BCSFB permeability, on the CSF teriflunomide concentrations and the teriflunomide CSF/plasma ratios was determined (Fig. 3). While no significant correlation was found between the integrity of the BCSFB and the CSF teriflunomide concentration or the teriflunomide CSF/plasma ratio, the plasma and the CSF teriflunomide concentrations were highly correlated ($r_s = 0.90$, $p < 0.0001$). Thus, a clear dependence of the CSF teriflunomide concentration from that in plasma was revealed but this correlation seemed independent of the albumin ratio.

## 3.2 Neurofilament Concentrations in Plasma and Cerebrospinal Fluid

The mean (± SEM) plasma and CSF NfL concentrations were **18.3** ± 4.5 pg/mL and **770** ± 128 ng/mL, respectively.

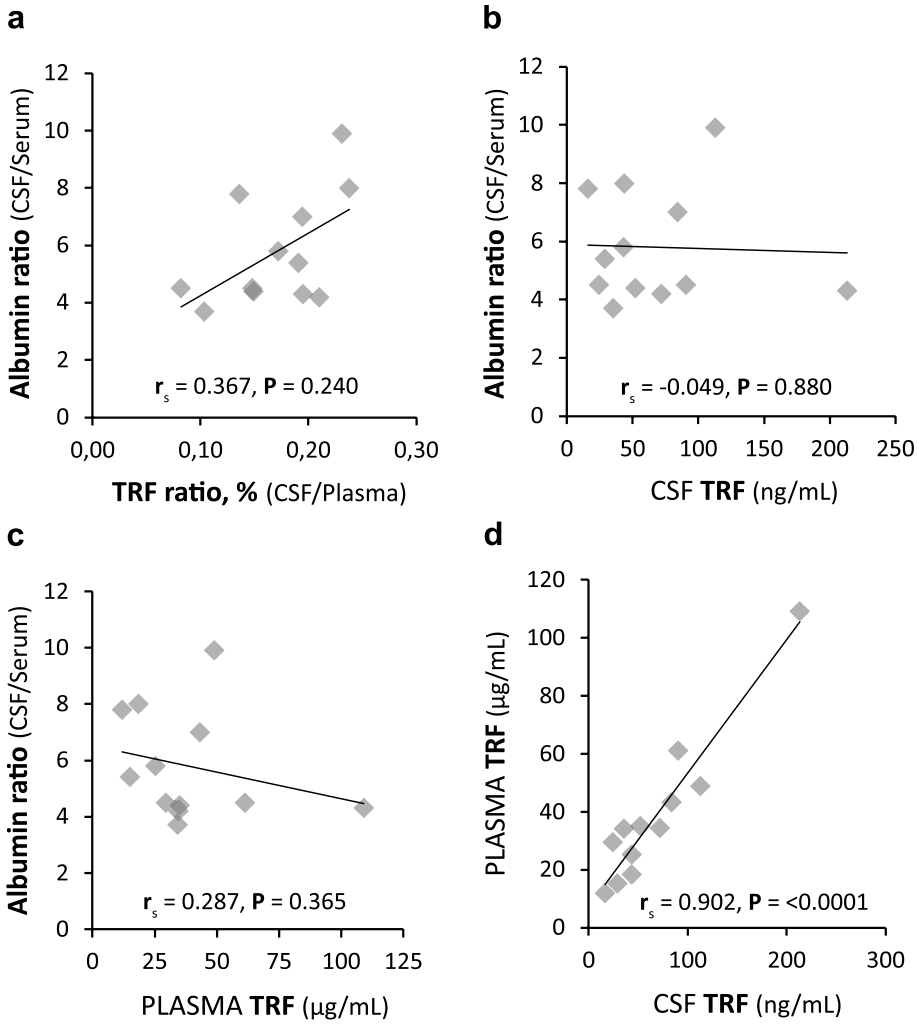


**Fig. 3** Spearman's Rho correlations. The influence of the albumin ratio (CSF/serum × 1000) on **a** the teriflunomide CSF/plasma ratio, **b** the teriflunomide CSF concentration and **c** the teriflunomide plasma concentration. **d** The mutual influence between CSF and plasma teriflunomide concentrations. For all analyses, a *p*-value of ≤ 0.05 was considered significant

There was no significant correlation between plasma and CSF NfL concentrations and the plasma and CSF NfL concentrations seemed independent to the albumin ratio.

## 4 Discussion

We showed that there was no significant change in the teriflunomide concentration in plasma or CSF between the expected peak and trough levels of teriflunomide in RRMS patients treated with teriflunomide 14 mg once daily at steady state. The passage over the BCSFB of teriflunomide was estimated to 0.17%. This low penetrans of teriflunomide across the BCSFB was probably due to its high protein binding in blood of ≥ 99.5%, and a consequence of a small volume of distribution [16]. However, if we assume that only free teriflunomide had the potential to cross from blood to CSF the penetrans over the BCSFB was considerably higher. With a 0.5% free fraction of teriflunomide in blood the passage across the BCSFB was estimated to 35%.

Impairment of the BBB and BCSFB may influence the passage of compounds from blood to the CNS and CSF compartments. In our study cohort no patients had clinical or MRI signs of disease activity or abnormal albumin ratio indicating a preserved BBB and BCSFB [24]. This may partly explain why we found no correlation between the teriflunomide concentration in CSF or the teriflunomide CSF/plasma ratio and the albumin ratio. In contrast, a high correlation was revealed between plasma and CSF teriflunomide concentrations. Thus, in our clinically and neuroradiologically stable RRMS cohort, the teriflunomide concentration in CSF seemed independent of the albumin ratio and mostly influenced by the teriflunomide concentrations in plasma. However, it should be noted that albumin is a considerably larger molecule than teriflunomide and may not reflect the BBB/BCSFB passage of small compounds.

There is a renewed interest in investigating whether DMTs can cross into the CNS and reduce compartmentalised inflammation and degeneration and enhance the restorative processes in MS. We found median and mean (SD)

concentrations of teriflunomide in CSF of 48 and 68 (15) ng/mL, respectively. The biodistribution of teriflunomide has previously been investigated in naïve as well as in experimental autoimmune encephalomyelitis (EAE) rats after a single oral dose of [$^{14}$C]-teriflunomide [19]. The brain and spinal cord concentrations were relatively low compared to other tissues with higher concentrations in spinal cord than in brain. The overall concentrations of teriflunomide in the CNS were 676–1108 ng/mL, i.e., approximately 2–4% of blood concentrations [19]. Thus, the concentration we found in CSF was only 10% of that found in the CNS of experimental animals. However, teriflunomide inhibits DHODH activity in a competitive manner, with a half maximal inhibitory concentration ($IC_{50}$) of 270 ng/mL [20] and the $IC_{50}$ for teriflunomide inhibition of proliferation of mitogen-stimulated rat lymphocytes was 23.2 ng/mL in vitro [13]. Thus, although the concentrations of teriflunomide in CSF was considerably lower than in plasma, it might be sufficient to inhibit pathological processes occurring in the CNS in RRMS. This assumption is supported by the possible effect of teriflunomide on degeneration shown in the ASCLEPIOS I and II trials [25]. While a limited effect of teriflunomide was demonstrated on disease activity, the rate of brain atrophy development was not significantly different from that found for ofatumumab, which might indicate a neuroprotective effect of teriflunomide in MS.

The concentration of a drug in CSF is believed to reflect its unbound concentration in the CNS, and the CSF concentration of a drug is therefore often used as a surrogate for the CNS concentration. However, there are a number of factors that may influence the CSF concentration such as sampling site, sampling conditions and transporters at the BBB and BCSFB [26]. Although, we obtained the samples at the expected peak and trough concentration of teriflunomide, we cannot demonstrate evidence for these pharmacokinetic timepoints, since a sample of only one pair of plasma and CSF was obtained from each patient. The observed variability of plasma and CSF concentrations might be due to pharmacokinetic differences between patients. However, repeated lumbar punctures are usually not accepted by patients and ethically questionable for research. Moreover, repeated lumbar punctures would most likely further damage the BCSFB. Another limitation of the study design is the possibility of non-adherence to teriflunomide dosing. However, these limitations seemed not to impact the overall results of the study. Since we obtained plasma and CSF at steady state at only two distinct timepoints that showed essentially similar mean and median teriflunomide concentrations, sampling is less likely to be a matter of concern. Furthermore, we did not find signs of a disrupted BCSFB in our patients and the teriflunomide concentration in CSF seemed not to be influenced by the albumin ratio. Teriflunomide is the active metabolite of leflunomide and both agents are high affinity substrates for the efflux transporter ABCG2/BCRP [27]. The expression of these transporters is different at the BBB and the BCSFB, which may influence their concentration in CSF and CNS [26]. Thus, we cannot rule out that the concentration of teriflunomide in the CSF may not predict the accurate unbound concentration in the CNS.

We used an assay that determined the total plasma teriflunomide concentration. No assay for analysis of unbound teriflunomide was available, the fraction considered to be the source for passage across the BCSFB. However, we found that plasma and CSF teriflunomide concentrations were highly correlated. Thus, if the CSF teriflunomide concentration is dependent on the free fraction of teriflunomide in plasma, our data suggest that this fraction is not constant but is a function of the total plasma teriflunomide concentration and imply that a higher dose of teriflunomide most likely give rise to higher teriflunomide concentrations in CSF. Another possibility to increase teriflunomide concentration in the CSF/CNS compartment would be modulation of the ABCG2/BCRP transporters.

Neurofilament light in CSF and blood is a biomarker of axonal damage and reflects disease activity and treatment response in MS [28]. The NfL concentration in blood and CSF has previously shown to be highly correlated [28, 29]. However, in the present study there was unexpectedly no association between plasma and CSF levels of NfL. While other DMTs have shown convincing decreases of plasma/serum NfL, the effect has been modest with teriflunomide [25, 30]. Although, our study population was limited to only 12 patients, who were of older age where the variability of plasma NfL is higher [31], our results warrant further investigations to explore if teriflunomide affects the penetrans of NfL from CSF to blood.

## 5 Conclusion

In conclusion, we show for the first time the teriflunomide concentration at steady state in human CSF obtained from RRMS patients treated with teriflunomide. Although the passage over the BCSFB was only 0.17%, the calculated passage of free teriflunomide in plasma to CSF was 35%. According to previous investigations, this concentration might be sufficient to interfere with pathological or restorative processes in the CNS of RRMS. However, further studies are warranted to compare the CSF and CNS concentrations of teriflunomide.

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## Declarations

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**Conflict of interest** JL has received lecture honoraria from Biogen, BMS, Celgene, Janssen, Merck, Novartis, Sanofi, Roche and Alexion; has served on scientific advisory boards for Biogen, BMS, Merck, Novartis, Sanofi, Roche and Alexion; serves on the editorial board of the Acta Neurologica Scandinavica; and has received unconditional research grants from Biogen and Novartis. HF and AN: no conflict of interest.

**Availability of data and material** On request, through email contact, original data can be provided by the corresponding author.

**Ethics approval** This study was approved by the regional ethical review board of Gothenburg, Sweden (diary number 773-17) and registered in ClinicalTrials.gov (Identifier: NCT04129736 (https://clinicaltrials.gov/ct2/show/ NCT04129736)). The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

**Consent to participate** All patients participated voluntary in this study and informed consent was obtained from all subjects.

**Code availability** Not applicable.

**Author contributions** JL conceptualised and planned the study, collected data, wrote and contributed intellectually to the manuscript. HF analysed biomarkers in plasma and cerebrospinal fluid, reviewed and contributed intellectually to the manuscript. AN collected the data, performed the analytic calculations and compiled the figures, reviewed and contributed intellectually to the manuscript.

**Consent to publish** Not applicable.



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