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Application of serum neurofilament light chain (sNfL) and glial fibrillary acidic protein (sGFAP) as biomarkers in the clinical management of multiple sclerosis (NeuroFilMS): a protocol for an observational cohort study

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ABSTRACT

Introduction Advances in ultrasensitive assay techniques have enabled precise quantification of serum neurofilament light chain (sNfL) and serum glial fibrillary acidic protein (sGFAP), highlighting their potential as dynamic biomarkers for detecting neuroaxonal injury, disease activity and progression in multiple sclerosis (MS). In the NeuroFilMS study, sNfL is being investigated prospectively as a prognostic biomarker for clinical and radiological disease activity in relapsing MS, while sGFAP is retrospectively explored as a marker of disease progression. The aim is to assess whether longitudinal monitoring of sNfL can inform diagnostic and therapeutic decisions in the treatment of people with MS (pwMS) and whether retrospective sGFAP measurements provide additional insights into disease progression. The study additionally aims to evaluate the comparability of different assay methods.

Methods and analysis NeuroFilMS is a prospective, multicentre study that will be conducted across multiple MS study centres throughout Germany, in collaboration with the German Multiple Sclerosis Registry set up by the German National Multiple Sclerosis Society (Deutsche Multiple Sklerose Gesellschaft). The study aims to enrol 1500 pwMS diagnosed with relapsing MS. Participants will be randomised in a 2:1 ratio (n=1000 vs n=500) to either immediate sNfL reporting or delayed sNfL reporting to treating physicians, in order to evaluate how sNfL availability influences therapeutic decision-making in routine healthcare regarding diagnostics and therapy decisions. Over a 2-year follow-up period, pwMS will attend three study visits integrated into routine care, including blood sampling for sNfL, clinical evaluations and routine MRI assessments; sGFAP will be measured retrospectively in batches, as it is not currently available for routine diagnostic use. The study follows the standardised protocols for biosample collection, performed both within clinical routine laboratory procedures and through research collaboration. Statistical analyses will involve both descriptive and inferential methods to evaluate biomarker performance and clinical associations.

Ethics and dissemination The study has received ethical approval from the Clinical Ethics Committee of Charité–Universitätsmedizin Berlin (EA4/136/24) and will be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Findings will be disseminated through peer-reviewed publications, conference presentations and engagement with patient organisations and clinical networks.

Trial registration number DRKS00034337.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Large, real-world cohort with robust design. The multicentre, registry-embedded, randomised-controlled (immediate vs delayed serum neurofilament light chain (sNfL) reporting) design with 1500 people with multiple sclerosis (MS) ensures diverse representation and allows pragmatic evaluation of biomarker impact on clinical decision-making.

⇒ Longitudinal assessment. Prospective sNfL measurements, retrospective serum glial fibrillary acidic protein (sGFAP) analysis, clinical and MRI outcomes and patient-reported measures over 18–24 months provide a multidimensional view of MS disease activity and progression.

⇒ Analytical rigour and translational potential. Standardised biosample collection, cross-platform assay comparison and quality control procedures strengthen analytical validity and inform pathways for biomarker integration into routine practice.

⇒ Challenges and constraints. Variability in assay results, reliance on routine MRI availability, estimated 15% attrition and retrospective sGFAP measurement may limit immediate applicability; as an observational study, residual confounding cannot be fully excluded.

INTRODUCTION

Multiple sclerosis (MS) is the most prevalent autoimmune disease of the central nervous system and the leading cause of non-traumatic disability in young adults.[1 2] Recent epidemiological studies report a steady rise in its prevalence, with some regions showing annual increases of up to 3% per year, likely due to improved diagnostic techniques but also evolving environmental risk factors.[1 3 4]

Fluid biomarkers play an increasingly important role in the management of neurological disorders, aiding in diagnosis, prognosis and therapeutic monitoring.[5] In MS, early initiation of high-efficacy disease-modifying therapy (DMT) is essential to minimise the risk of disability progression. Since the availability of the first DMTs in the 1990s, a broad range of DMT options with different efficacy profiles, mechanisms of action and adverse event profiles has become available. A growing body of evidence indicates the superiority of using highly effective treatments in MS as initial treatment after diagnosis in comparison to initiation of DMT with moderate efficacy.[6] Thus, there is a growing need for prognostic and predictive fluid biomarkers to timely assess both disease activity and progression, and to monitor treatment response.[5 7–10] Compared with cerebrospinal fluid, serum biomarkers are less invasive and more accessible, while offering comparable sensitivity for detecting disease activity or progression.[11]

Neurofilament light chain protein (*NfL*), a component of the neuronal cytoskeleton within the intermediate filament family,[12] has emerged as a robust biomarker of neuroaxonal damage in MS. Elevated serum NfL (sNfL) levels reflect active inflammation and correlate with increased disability, higher relapse rates and new MRI lesions.[13–24] Clinically, sNfL may serve as a prognostic indicator, predicting future relapse risk and the emergence of new MRI lesions.[22 25–34] Longitudinal studies demonstrate that rising sNfL levels are associated with disease worsening, brain atrophy and cognitive decline.[26 27 35–37] Importantly, sNfL levels decrease in response to DMTs, highlighting its potential as a surrogate marker for treatment response.[38–45] For instance, the RADIANCE trial demonstrated that ozanimod significantly reduced MRI activity and sNfL levels over 24 weeks.[46] Similarly, evobrutinib led to sustained sNfL reduction over 2.5 years,[47] and ofatumumab lowered relapse rates, MRI lesion counts and sNfL levels more effectively than teriflunomide, particularly when initiated early.[48–52] These findings underscore the utility of sNfL in tracking disease activity and guiding treatment decisions in MS.[25 53]

Glial fibrillary acidic protein (*GFAP*) is a well-established marker of astrocytic activation or damage and has been historically described in histopathological studies of MS lesions.[54] While its use as a serum biomarker is less validated compared with NfL, it has recently gained attention in neuromyelitis optica spectrum disorder (NMOSD)[55–59] and is now being increasingly investigated in MS.[60–63] Unlike NfL, GFAP reflects astroglial activation and potentially chronic disease processes, particularly progression independent of relapse activity (PIRA).[64 65] Elevated serum GFAP (sGFAP) levels have been linked to higher grey matter atrophy and confirmed disability worsening; in some studies, they also correlated with neurological disability in subsequent years, though not with acute disease exacerbations.[64] sGFAP levels also correlate with Expanded Disability Status Scale (EDSS), disease duration, brain atrophy and cognitive decline, indicating its potential as a marker of disease severity and progression.[56 60–64 66–74]

In collaboration with the German Multiple Sclerosis Registry (MS Registry),[75] we aim to investigate the prognostic value of sNfL in predicting disease activity and therapeutic decision-making in MS through a prospective, multicentre longitudinal cohort study across Germany. Leveraging the rich demographic and clinical data of the registry, we will examine sNfL's utility in forecasting clinical and radiological activity and evaluating its influence on real-world treatment decisions.

Given the current lack of serum biomarkers for MS monitoring, this study will also investigate the intra-individual variability and cross-assay comparability of sNfL across different laboratory tests. Additionally, sGFAP will be analysed retrospectively at the study's conclusion to explore its correlation with long-term outcomes and its complementary role alongside sNfL. These insights may contribute to a better understanding of biomarker-informed MS management and support more personalised treatment strategies.

METHODS AND ANALYSIS

Study design

The study is designed as a two-armed, observational, prospective, multicentre cohort study with randomised allocation of participants within the framework of the German MS Registry. It involves the longitudinal collection of the main biomarker of interest, sNfL, alongside clinical and imaging outcome measures in people with MS (pwMS), with the aim of evaluating the impact of sNfL on disease activity and treatment decision-making. Treating physicians in one arm will receive immediate sNfL biomarker reporting, while those in the other arm will receive delayed reporting (figure 1). Additionally, GFAP will be measured retrospectively at the end of the study for both groups.

Timeline and patient recruitment

Participants are recruited from the German National Multiple Sclerosis Society-certified MS centres participating in pharmacovigilance documentation and then in the NeuroFilMS study. Eligibility is assessed by qualified medical staff according to predefined inclusion and exclusion criteria, and patients are recruited consecutively at participating centres. Patients may already be enrolled in the MS Registry or may be

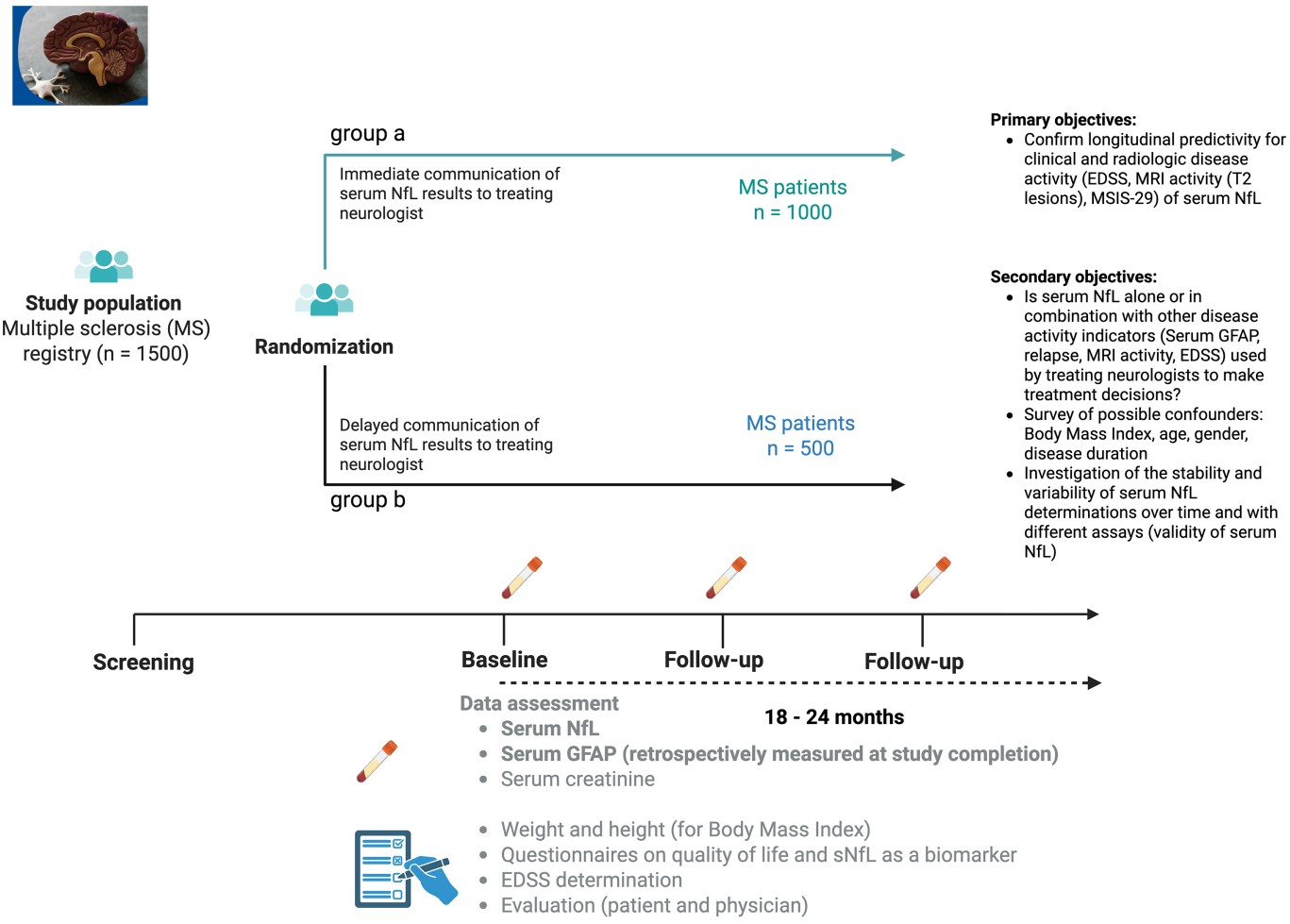

Figure 1 Study design of the NeuroFilMS study, a multicentre observational study investigating the role of sNfL as a biomarker in MS. The study includes 1500 patients with MS from the German MS Registry randomised into two groups: group A (treating physicians receive immediate sNfL result reporting, n=1000) and group B (treating physicians receive delayed reporting of sNfL results, n=500). The study aims to confirm the predictive value of sNfL for clinical and radiological disease activity and assess its stability and variability over time. Secondary endpoints explore whether sNfL is used alone or in combination with other disease activity indicators (relapse, MRI activity, EDSS) in treatment decision-making. Data collection includes clinical assessments, imaging, biomarker measurements and PROs (such as quality-of-life questionnaires) over an 18–24 months' follow-up period. sGFAP is measured retrospectively and results are not available to treating physicians during the course of study. EDSS, Expanded Disability Status Scale; MSIS-29, Multiple Sclerosis Impact Scale, 29-item version; MS Registry, Multiple Sclerosis Registry; PROs, patient-reported outcomes; sGFAP, serum glial fibrillary acidic protein; sNfL, serum neurofilament light chain.

newly included. All participants are informed about the study by the responsible medical staff. Ethical approval for the study was obtained from the Berlin Ethics Committee in January 2025. The first patient was enrolled in May 2025. Completion of enrolment of the planned 1500 patients is expected by mid-2027, with last patient out anticipated around mid-2029.

Outcomes and endpoints

Primary endpoint

The baseline and longitudinal association between sNfL levels and disease activity over the follow-up period of 18 months, defined by:

- Changes in EDSS.
- Occurrence of new or enlarging T2-hyperintense lesions on MRI.
- Occurrence of clinical relapses.

Secondary endpoints

The secondary endpoints include:

Clinical use of sNfL in routine care, defined as:

- The association between baseline and longitudinal association of sNfL levels (alone or in combination with other disease activity indicators, including sGFAP, relapse occurrence, MRI activity and EDSS) and clinical decision-making, such as treatment initiation, escalation or modification.

Influence of potential confounders on sNfL levels and clinical outcomes, defined as:

- The association of sNfL concentrations and disease activity measures with body mass index (BMI), age, sex and disease duration.

Longitudinal stability and intraindividual variability of serum sNfL, defined as:

- Variability of sNfL measurements over time within individuals and analytical comparability of sNfL across assay platforms.

Ethics and dissemination

The study has received ethical approval from the Clinical Ethics Committee Charité–Universitätsmedizin Berlin (EA4/136/24; approval date: 1 October 2024) and will be conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization–Good Clinical Practice guidelines.[76] The study has been prospectively registered at Germany Registry for Clinical Studies (DRKS00034337) on 11 April 2025. All participants will provide written informed consent prior to inclusion in the study, including consent for participation in the German MS Registry and for the collection, storage and analysis of biological samples. All study data will be pseudonymised, and data handling will comply with applicable data protection regulations, including the EU General Data Protection Regulation (GDPR), the German Federal Data Protection Act (BDSG) and the Berlin Data Protection Act (BlnDSG).

Study findings will be disseminated through publication in peer-reviewed journals and presentation at national and international scientific conferences. In addition, results will be communicated to relevant stakeholders, including clinicians, researchers and patient organisations, through established professional networks such as the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) and the Deutsche Gesellschaft für Neurologie (DGN). Efforts will also be made to provide accessible summaries of findings for pwMS and the broader public.

Study population

A total of 1500 patients diagnosed with relapsing MS, including both relapsing-remitting MS (RRMS) and relapsing secondary progressive MS (SPMS), will be enrolled from multiple MS Registry centres across Germany, ensuring a diverse representation in terms of age, sex and disease characteristics as well as academic and non-academic study centres. The currently included study centres are listed in table 1. Further initiations of centres that are already participating in the MS Registry are planned. Following confirmation of diagnosis according to the latest revised McDonald criteria,[77] participants will be randomised in a 2:1 ratio: group A (n=1000) will receive immediate sNfL reporting to the treating physician, while group B (n=500) will receive delayed reporting (after completion of the study). Eligibility includes both newly diagnosed (de novo) and previously diagnosed patients currently receiving DMT for at least 6 months.

Inclusion and exclusion criteria

Eligible participants are adults aged 18–60 with a confirmed diagnosis of relapsing MS (RRMS or SPMS)[77] who provide written informed consent for both the sNfL study and the German MS Registry. They must be on a stable DMT for at least 6 months or treatment naïve during that period, including de novo patients. Exclusion criteria include non-relapsing MS, recent DMT changes, significant comorbidities, traumatic brain injury or severe accidents in the last 3 months, pregnancy planning, pregnancy or breastfeeding and participation in interventional trials. For full details, please refer to table 2. Attending neurologists at the MS centres will screen potential study participants, review medical histories and current health status and obtain informed consent from suitable pwMS before enrolment.

Table 1 Overview of participating study sites, including institution names and corresponding addresses

Institution	Address
Universitätsklinikum Heidelberg	Im Neuenheimer Feld 400 69 120 Heidelberg
St. Josefs-Krankenhaus Potsdam	Allee nach Sanssouci 7 14 471 Potsdam
meinmedicum MVZ	Marienbader Platz 22 61 348 Bad Homburg v. d. Höhe
Praxis Dr Andreas Kowalik	Leuschnerstraße 12 70 174 Stuttgart
Krankenhaus der Barmherzigen Brüder Trier	Nordallee 1 54 292 Trier
Charité – Universitätsmedizin Berlin, HSA Mitte	Charitéplatz 1 10 117 Berlin
Charité – Universitätsmedizin Berlin, ECRC HSA Berlin-Buch	Lindenberger Weg 80 13 353 Berlin
Klinikum Ibbenbüren, Klinik für Neurologie	Große Str 41 49 477 Ibbenbüren
Uniklinikum der MHB, Neurologie & Schmerztherapie	Seebad 82/83 15562 Rüdersdorf bei Berlin
Praxis für Neurologie und Psychiatrie Andreas Stockert / Dr. med. Claudia Rettenmayr	Mühlstr 8 75 172 Pforzheim
NeuroMed Campus Nelles, Scharpegge, Haupt, Scharwat – Fachärzte für Neurologie, Schmerztherapie	Werthmannstr 1 c 50935 Köln
Universitätsklinikum Mannheim GmbH, Klinik für Neurologie	Theodor-Kutzer-Ufer 1–3 68 167 Mannheim
MVZ Leipziger Land GmbH	Rudolf-Virchow-Str. 2 04552 Borna

Randomisation

Participants will be randomised into two groups:

Table 2 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
► Ages between 18 and 60 years.	► Non-relapsing MS course.
► Confirmed diagnosis of relapsing MS (RRMS or SPMS).	► Ages below 18 or above 60 years.
► Written informed consent for participation in both the sNfL study and the MS Registry.	► Inability or unwillingness to provide written informed consent.
► Stable disease-modifying therapy for at least 6 months or no DMT in the past 6 months.	► Unstable treatment (recent DMT change within the past 6 months).
► Inclusion of de novo patients (directly after diagnosis) is permissible.	► Significant comorbid conditions that may interfere with study assessments or biomarker measurements.*
	► Pregnant or breastfeeding women.†
	► Traumatic brain injury or severe accident in the last 3 months before inclusion.
	► Current desire to have children.
	► Participation in interventional clinical trials.‡

*Exclusion is limited to comorbid conditions that significantly impair study assessments or biomarker interpretation. Common comorbidities are not excluded; they will be systematically recorded and, where feasible, incorporated into statistical analyses as potential confounders and/or effect modifiers, or otherwise reported descriptively.
†Pregnancy: women who are pregnant or breastfeeding at the time of enrolment will be excluded to avoid potential confounding effects on biomarker levels.
‡Concurrent participation in other studies: while participation in multiple observational studies is permitted, patients enrolled in interventional clinical trials will be excluded to prevent overlapping effects of different treatments on study outcomes.
DMT, disease-modifying therapy; MS, multiple sclerosis; MS Registry, Multiple Sclerosis Registry; RRMS, relapsing-remitting multiple sclerosis; sNfL, serum neurofilament light chain; SPMS, secondary progressive multiple sclerosis.

- Group A, n=1000. Treating physicians receive immediate sNfL results to inform further diagnostics and treatment decisions.
- Group B, n=500. Treating physicians receive delayed sNfL reports after study completion; therapeutic decisions follow current standard practices and are not influenced by sNfL results.

Approximately 90% of participants are expected to have RRMS and 10% SPMS. Within group A, this corresponds to roughly 900 RRMS participants (approximately 300 newly diagnosed (de novo) and 600 established cases) and 100 SPMS participants (around 33 newly diagnosed and 67 established cases). In group B, about 450 participants may have RRMS (approximately 150 de novo and 300 established cases) and 50 will have SPMS (roughly 17 de novo and 33 established cases).

Study assessments

Participants will be recruited from various MS Registry centres across Germany to ensure diversity in age, sex and disease profiles. At baseline, comprehensive evaluations will document demographic information, weight and height, disease history, treatment regimens and clinical outcomes, including the EDSS,[78] MRI findings (T2 lesion count) and patient-reported outcomes (PROs) (eg, Multiple Sclerosis Impact Scale, 29-item version (MSIS-29); Hamburg Quality of Life Questionnaire for Multiple Sclerosis (HAQUAMS); patient evaluation; other quality-of-life questionnaires). In addition, up to 20 mL of blood (serum) will be collected for biomarker analyses, including sNfL, serum creatinine, retrospective sGFAP analysis and additional sNfL assays (figure 1).

Study data will be integrated with the German MS Registry, operated by MS Research and Project Development, a non-profit company (MS Forschungs-und Projektentwicklungs-gGmbH). The MS register annually records key clinical parameters such as patient characteristics, treatment details, care utilisation, outcomes and pharmacovigilance data, thereby enriching the study's dataset.

Participants will have two additional flexible follow-up visits over a period of 1.5–2 years, which will be scheduled according to individual patient needs in routine care and disease activity. At each follow-up, up to 20 mL of blood will be collected for ongoing biomarker analysis, and routine clinical assessments will be performed. PROs (eg, MSIS-29,[79] HAQUAMS[80] and other patient evaluations) will also be gathered through validated and custom-developed questionnaires. Evaluation questionnaires on the acceptance of incorporating sNfL into healthcare will be completed by patients and treating physicians.

Laboratory assessments

At baseline and during all follow-up visits, up to 20 mL of serum will be collected for biomarker analysis. A separate focus is placed on the technical and methodological aspects of sNfL measurement, including the assessment of its intraindividual longitudinal variability and its intra-assay and interassay variability across different analytical platforms (eg, the Siemens Healthineers assay in

collaboration with the Medical Faculty Mannheim of Heidelberg University and the Roche Elecsys platform at the University of Basel), as well as other assays used in routine healthcare.

According to randomisation:

Group A

- Eight millilitres of serum will be immediately analysed for sNfL and serum creatinine in the study centre's partner laboratory, and the results will be communicated to the treating physicians.
- An additional 8 mL of serum will be cryopreserved for retrospective analysis of sGFAP and sNfL, along with additional assays.

Group B

- Eight millilitres of serum will be cryopreserved for retrospective analysis of sGFAP and sNfL, along with additional assays.

Serum creatinine will be measured alongside sNfL, as kidney function is associated with circulating sNfL levels.[81]

Blood samples will be collected by venipuncture at all study visits using standard operating procedures. Serum will be obtained using serum separator tubes and allowed to clot at room temperature and sent to the collaborating laboratories of the study centre and to the laboratory of Charité–Universitätsmedizin Berlin. Samples that will be shipped to Charité laboratory use light-protected, temperature-controlled insulated packaging, in accordance with the standard operating procedures for biological specimen transport.

At the Charité site, samples will be centrifuged at 1500×g for 15 min at room temperature. The resulting serum will be aliquoted into prelabelled polypropylene tubes to avoid repeated freeze–thaw cycles and stored at −80°C until analysis.

To minimise preanalytical variability, all samples will be processed within a standardised time window after collection (within 7 days of blood withdrawal), and handling procedures are harmonised across study sites.

Serum concentrations of sNfL and sGFAP will be quantified using ultrasensitive immunoassay technologies, including single molecule array (Simoa; Quanterix, Billerica, Massachusetts, USA), Elecsys (Roche Diagnostics), chemiluminescent immunoassay (CLIA)-based assays (Fujirebio) and Atellica/ADVIA Centaur (Siemens Healthineers), depending on group allocation (group A vs group B) and the collaborating laboratory of the respective study centre.

Cryopreserved samples will be used for retrospective analyses of sNfL in combination with sGFAP. Measurements will be performed in collaboration with the University of Basel using the Elecsys platform (Roche Diagnostics) for sNfL and sGFAP and at the University of Mannheim using the Atellica and ADVIA Centaur systems (Siemens Healthineers) for sNfL only, to enable cross-assay validation and interplatform comparison of results.

All measurements will be performed in duplicate, and laboratory personnel will be blinded to clinical data.

Quality control procedures will include the use of internal controls, calibration standards and assessment of intra-assay and interassay coefficients of variation. Samples from the same participant will be analysed within the same assay run whenever possible to reduce batch effects.

Quality control

To ensure interlaboratory comparability of sNfL measurements across different assay platforms, rigorous quality control procedures will be implemented. These include the use of shared reference samples distributed across sites, blinded duplicate testing and participation in external quality assessment schemes where available. Interassay and intra-assay variability will be regularly assessed, and data harmonisation strategies (eg, Z-score normalisation) will be applied where necessary to enable pooled analyses. Furthermore, the clinical laboratories will participate in the NfL External Quality Assessment programme provided by the Reference Institute for Bioanalytics (www.rfb.bio) of the German Society of Clinical Chemistry on a regular basis throughout the study.

Data collection

The study will involve three visits over approximately 2 years: a baseline visit (visit 1), followed by two follow-up visits (visits 2 and 3) scheduled at flexible rather than fixed intervals based on standard clinical appointments and patient need. This pragmatic approach allows study integration into routine care, accommodates individual disease activity and enhances feasibility across participating study centres. Each participant's data are recorded through the MS Registry of the German Multiple Sclerosis Society. In addition to routine clinical and laboratory assessments, up to 20 mL of blood will be collected at each time point. This extra sampling is performed during routine blood draws, avoiding the need for separate venipuncture. Table 3 provides an overview of the data collected at each visit.

During routine ambulatory visits, standard MS parameters such as relapses, current and past DMTs and any adverse events (pharmacovigilance), as well as comorbid conditions, are documented in the MS Registry. MRI data will be collected as part of routine clinical care, and no standardised imaging protocol is mandated within this study. T2 lesion counts and, where available, information on new or enlarging lesions will be derived from radiological reports and/or structured entries within the German MS Registry. MRI assessments are performed according to local clinical practice at participating centres and are documented if available; no additional MRI scans or centralised image analyses are required by the study protocol. Additional questions regarding quality of life (eg, MSIS-29[79]), functional status (eg, Multiple Sclerosis Functional Composite,[82] Symbol Digit Modalities Test) and daily life impact (eg, HAQUAMS[80]) are administered. The physician evaluation questionnaire specifically gauges whether sNfL results have influenced any

Table 3 Timeline of key assessments and measurements throughout the study period

Parameter/assessment	Visit 1 (baseline)	Visit 2 (follow-up)	Visit 3 (follow-up)
Demographic and sociodemographic data Age; sex; weight and height (body mass index); education	X	X (if applicable)	X (if applicable)
Clinical/medical history (*including MS diagnosis details*) Diagnosis; disease onset; attack/relapse history (number of relapse(s)*); current symptoms and complaints; comorbidities; previous infections; fertility history (pregnancies); other exclusion criteria	X	X	X
Therapies Treatment status; relapse therapy; symptomatic therapy; full drug list; prescription of assistive devices	X	X	X
Pharmacovigilance Side effects of MS medications	X	X	X
EDSS score	X	X	X
SDMT	X (if routine)	X (if routine)	X (if routine)
MSFC	X (if routine)	X (if routine)	X (if routine)
PROs (eg, MSIS-29, HAQUAMS/HALEMS)	X	X	X
Cranial MRI (hyperintense lesion on T2-weighted MRI count)†	X (if routine)	X (if routine)	X (if routine)
Blood sampling (20 mL extra)	X	X	X
► sNfL	X	X	X
► Serum creatinine	X	X	X
► Retrospectively assessed (after the last patient out in frozen sera) sGFAP	X	X	X
Physician questionnaire (influence of sNfL on treatment decisions)	X	X	X

*Number of relapse(s) (a new or worsening acute neurological symptom lasting ≥24 hours and not explained by fever or infection).
†MRI data are documented if performed as part of the regular clinical routine. No additional MRI scans are mandated by this study protocol.
EDSS, Expanded Disability Status Scale; HALEMS, Hamburger Lebensqualitätsfragebogen für MS (German version of HAQUAMS); HAQUAMS, Hamburg Quality of Life Questionnaire for Multiple Sclerosis; MS, multiple sclerosis; MSFC, Multiple Sclerosis Functional Composite; MSIS-29, Multiple Sclerosis Impact Scale, 29-item version; PROs, patient-reported outcomes; SDMT, Symbol Digit Modalities Test; sGFAP, serum glial fibrillary acidic protein; sNfL, serum neurofilament light chain.

therapeutic decisions, such as initiating, escalating or changing DMTs.

Data management

All clinical information and laboratory results are collected under pseudonyms, ensuring no personal identifiers (such as name or date of birth) are present in the study dataset. Each participant is assigned a unique ID code, which is used to label all samples, questionnaires and electronic files. This pseudonymisation process prevents unauthorised individuals from linking any specific data point or sample back to an individual.

Electronic data entry occurs via the MS Registry's secure web infrastructure. Routine clinical data (EDSS, relapse history, treatment changes and PROs) are recorded during visits and transferred in pseudonymised form to the MS Registry. Study-specific data, including sNfL and serum creatinine, are also entered into this system, maintaining the same ID code. The laboratory receives tubes labelled only with the ID code, processes the samples immediately or stores them in −80°C freezers for retrospective analysis and returns the results to the clinical team under the same pseudonymised code.

All documents related to informed consent are kept in locked, access-controlled cabinets, separate from the ID list. Only study personnel and authorised MS Registry staff have access to pseudonymised data. The password-protected servers hosting these data comply with institutional and national data protection regulations, including the EU GDPR, the BlnDSG and the BDSG.

Study data and biomaterial are stored for a minimum of 10 years in accordance with legal requirements. Participants may withdraw their consent at any point, prompting deletion or disposal of their data and samples unless already analysed or published in anonymised aggregated form.

Sample size considerations

The sample size of 1500 participants is determined to provide adequate statistical power (≥80%) to detect significant associations between sNfL and sGFAP levels and primary and secondary endpoints, accounting for variability in biomarker measurements and clinical outcomes. This calculation is based on an anticipated medium effect size (Cohen's r≈0.3) and a significance level of α=0.05, which initially suggested that approximately 90 participants would be necessary to achieve the desired power. However, given the study's multiple primary endpoints and the requirement for robust subgroup analyses, the sample size was substantially increased to 1500. This larger cohort not only accommodates the complexity of analysing multiple outcomes and subgroups but also accounts for an estimated 15% dropout rate over the 2-year study period. This estimation is based on similar longitudinal MS studies, which typically report dropout rates ranging from 10% to 20% due to factors such as loss to follow-up, withdrawal of consent or adverse events unrelated to the study procedures.

Statistical analysis

The statistical analysis plan for this study encompasses both descriptive and inferential methodologies to comprehensively address the primary and secondary objectives. Descriptive statistics will summarise baseline demographic and clinical characteristics, including age, sex, BMI, MS subtype and disease duration, using means and SDs for continuous variables and frequencies and percentages for categorical variables. sNfL concentration will also be reported using appropriate summary statistics.

For the primary objective, the predictive value of sNfL will be assessed through correlation analyses using Pearson or Spearman correlation coefficients to evaluate the relationship between baseline sNfL levels and primary endpoints. Multiple linear, logistic and, where appropriate, count-based regression models (eg, for lesion counts) will be employed to determine the predictive power of sNfL for continuous outcomes and binary outcomes, adjusting for potential confounders such as age, sex, BMI, serum creatinine, disease duration, MS subtype and comorbidities.

Clinical disease activity (relapse occurrence and EDSS progression) will be analysed using logistic and linear regression models, respectively, while radiological disease activity (MRI T2 lesion count) will be analysed using count-based or linear models, as appropriate. The predictive performance of sNfL across these outcomes will be further evaluated using multivariable regression models and receiver operating characteristic (ROC) analyses. ROC curves will be generated to determine the area under the curve for sNfL in predicting primary endpoints, and sensitivity, specificity and optimal cut-off values will be calculated.

The variability of sNfL measurements will be examined by conducting Bland-Altman plots and intraclass correlation coefficients to assess agreement between different assay platforms. Longitudinal changes and intraindividual variability in sNfL levels, as well as their association with clinical and radiological outcomes, will be analysed using mixed-effects models to account for repeated measures across study visits.

Secondary analyses will investigate the influence of sNfL on therapy decisions by assessing changes in DMTs, including initiation, continuation or modification of treatment, in response to sNfL fluctuations. Logistic regression models will assess the probability of therapy changes based on sNfL levels, controlling for other disease activity indicators like relapses and MRI findings as well as comorbidities where appropriate. Differences in diagnostic and therapeutic decisions between group A (immediate reporting of sNfL concentrations to treating physicians) and group B (delayed reporting of sNfL concentrations to treating physicians) will be analysed using χ^2 tests for categorical variables and t-tests or Mann-Whitney U tests for continuous variables. Interaction effects in regression models will be explored to determine if the effect of sNfL on outcomes varies by randomisation group.

Subgroup analyses will be performed, stratified by MS subtype (RRMS vs SPMS) and disease stage (newly diagnosed vs established) to investigate potential differential effects of sNfL across these categories. Handling of missing data will involve multiple imputation techniques, assuming data are missing at random, and sensitivity analyses will assess the impact of missing data on study conclusions. To control for type I error due to multiple comparisons, appropriate corrections such as the Bonferroni correction or false discovery rate will be applied, particularly in secondary and exploratory analyses.

All statistical analyses will be conducted using R (V.4.4.0). Results will be presented with 95% CIs and p values where applicable. At the end of the study, sNfL and sGFAP levels will be correlated with the above parameters, adjusted for age, sex, BMI and comorbidities where applicable.

Patient and public involvement

Patients and members of the public were not directly involved in the design of this protocol. However, the NeuroFilMS study will collaborate with the associations of

patients with MS and professional networks (eg, the DGN and the ECTRIMS) to support patient engagement and dissemination. Patient organisations will be consulted in the later phases of the study to ensure that findings are communicated in accessible formats (eg, lay summaries, newsletters and patient days). Their feedback will also inform the design of dissemination strategies to maximise patient benefit and clinical translation

DISCUSSION

In the management of MS, the early initiation of high-efficacy DMTs is crucial to reduce the risk of long-term disability and disease progression. However, the unpredictable and heterogeneous nature of MS, characterised by varying disease trajectories and individual responses to treatment, complicates the optimisation of therapeutic strategies. Current monitoring tools (eg, EDSS) and imaging modalities (eg, MRI) guide treatment decisions but have important limitations. These include delayed detection of neurodegeneration, limited sensitivity in predicting long-term outcomes and high resource demands. Consequently, there is a pressing need for reliable and accessible serum biomarkers that can provide real-time insights into disease activity, predict progression and facilitate personalised treatment approaches.

To address this need, the NeuroFilMS study has been established to evaluate the application of sNfL as a biomarker in routine MS care. sNfL is a neuroaxonal damage marker that has shown promise in reflecting both acute inflammatory activity and chronic neurodegeneration in patients with MS.[25 83–85] By integrating sNfL measurements into clinical workflows, NeuroFilMS aims to enhance the precision of disease monitoring, enable timely adjustments to DMTs and ultimately improve patient outcomes.

sGFAP and sNfL have emerged as complementary serum biomarkers in MS, offering valuable insights into disease activity and progression. sGFAP is further associated with disability status as measured by EDSS[86] and with PIRA.[87] A large multicentre study (n=355) demonstrated that sGFAP has prognostic value for future PIRA events and provides additive predictive utility when used alongside sNfL.[64]

Moreover, recent evidence highlights the stratification potential of sGFAP and sNfL in the context of B-cell depleting therapy (BCDT). In a cohort of 362 patients with MS, increased sGFAP levels and the absence of an early sNfL decline after BCDT initiation were associated with a heightened risk of future PIRA.[65] Notably, a sustained rise in sGFAP over time suggests that BCDT may have limited impact on progressive, glial-driven disease processes. In contrast, declining sNfL levels in patients with RRMS without PIRA indicate modulation of acute inflammatory activity. However, persistently elevated sNfL levels in progressive MS underscore ongoing neuroaxonal degeneration that may remain unaddressed by BCDT. Additionally, sGFAP and sNfL levels measured before and after discontinuation of DMT can help stratify stable patients, aiding in risk assessment and therapeutic decision-making.[88] By assessing both sNfL and sGFAP, the NeuroFilMS study aims to further elucidate the relationship between these biomarkers and investigate their potential synergistic value in routine clinical care across Germany.

Another key strength of the NeuroFilMS study is its large sample size and multicentre approach including centre-specific randomisation, which enhance the generalisability of the findings and ensure a comprehensive representation of the MS population. The longitudinal design allows for the observation of sNfL dynamics over time, providing valuable data on its correlation with disease progression and treatment efficacy. Additionally, the embedded design within the German MS Registry ensures easy onboarding of centres and reduces the need for double data entry and repeated measurements. It further allows access to previously collected high-quality, real-world data, enabling robust analyses of sNfL's clinical utility, retrospective evaluation of sGFAP and the potential inclusion of additional biomarkers in future exploratory analyses. sNfL seems to be an increasingly accessible biomarker that can be easily assessed in routine blood samples as part of standard clinical care. It holds the potential to become a valid tool for individualised diagnostic and therapeutic decision-making over the long term, particularly since MRI examinations are less accessible, more expensive, often delayed and more burdensome for patients. Ultimately, the goal is to establish a meaningful and clinically feasible use of combined biomarkers—including sNfL, sGFAP and imaging tools such as MRI and optical coherence tomography (OCT)[9]—to support individualised patient care and more precise treatment decisions.

However, the study also faces potential limitations. Variability in sNfL assay measurements across different laboratories and platforms may introduce measurement inconsistencies, despite the implementation of standardised protocols and cross-validation procedures. Furthermore, the anticipated dropout rate of approximately 15% over the 2-year period could impact the study's power and introduce bias if attrition is not random. To mitigate these issues, NeuroFilMS incorporates strategies such as regular participant engagement, flexible scheduling of follow-up visits and advanced statistical methods to handle missing data. Additionally, the study acknowledges the challenge of integrating a new biomarker into clinical practice, which may require extensive training and adjustment by healthcare providers.

The NeuroFilMS study is designed to evaluate the integration of sNfL into routine clinical practice for the management of MS. By systematically assessing how sNfL can be incorporated into existing clinical workflows, the study aims to enhance disease monitoring and support evidence-informed therapeutic decisions. The implementation of sNfL as a biomarker offers several potential benefits, including the ability to enable timely adjustments to

treatment regimens, more accurate tracking of disease activity and the facilitation of personalised patient care. However, several challenges need to be considered, including the requirement for standardised assay procedures across laboratories to ensure consistent measurements, evaluation of the cost-effectiveness of widespread sNfL testing and need for appropriate training of healthcare providers to interpret and apply sNfL data in clinical practice.

In contrast, the use of GFAP in clinical practice remains at an earlier stage, with no widespread implementation as a routine biomarker, especially compared with sNfL. Within NeuroFilMS, sGFAP will be measured retrospectively, limiting its role in direct clinical decision-making but offering valuable insights into disease progression and its complementary relationship to sNfL. Logistical considerations, including the integration of biomarker testing into routine blood draws and the management of associated data, also need to be addressed to facilitate implementation.

Overall, the NeuroFilMS study's robust multicentre design, large representative cohort and collaboration with the German MS Registry are expected to generate robust real-world evidence and contribute to informing future clinical use of sNfL and, secondarily, sGFAP in MS care.

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