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ORIGINAL COMMUNICATION

Diagnostic and prognostic value of blood neurofilament light chain in ischemic stroke: an individual patient data meta-analysis

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Abstract

Background We aimed to conduct an individual patient data meta-analysis on blood neurofilament light chain (NfL) in ischemic stroke (IS) to enhance its clinical applicability.

Methods We performed a systematic literature search of studies on blood NfL measured in adult patients within 30 days after IS onset and derived age- and BMI-adjusted Z-scores based on a previously published reference population of healthy controls. We collected clinical, radiological and biochemical parameters of IS patients and tested associations of NfL at defined timepoints after IS onset (D1: < 24 h; D2: 24–48 h; D3: 48–72 h; D4–5: 72–120 h; D6–7: 120–168 h; D8–30: > 168 h) with baseline characteristics and 3-month follow-up outcomes (modified Rankin Scale, mRS; survival).

Results We included 4081 blood NfL values from 2872 participants (IS n = 1985, transient ischemic attack n = 88, healthy controls n = 799) of 18 published studies and 3 unpublished cohorts. In patients with IS, NfL Z-score progressively increased from D1 [median: 2.0 (IQR: 0.9–2.9)] to D6–7 [median: 3.5 (IQR: 3.0–3.8)], with discriminative ability being high for IS vs. controls (AUC: 0.79–0.97) and fair for IS vs. TIA (AUC: 0.64–0.80). Higher NfL Z-score at D1 was associated with greater risk of symptomatic intracranial hemorrhage (aOR = 1.33, p = 0.014) and, from D2 onwards, with larger infarct lesion volume (highest Spearman's rho: 0.795 at D6-7). NfL independently predicted a mRS > 2 (aOR = 1.31, p < 0.001) and mortality (aOR = 1.67, p < 0.001) at 3 months.

Conclusions Blood NfL level was progressively elevated after IS, could discriminate IS from healthy controls with high accuracy and had prognostic value for intra-hospital complications and 3-month clinical outcomes in IS.

Keywords Neurofilament light chain · Stroke · Transient ischemic attack · Functional outcome · Symptomatic intracranial hemorrhage · Modified Rankin scale

Introduction

Neurofilament light chain protein (NfL) represents the most promising biomarker candidate for detecting and monitoring neuronal injury after ischemic stroke (IS). Previous studies have provided evidence of increased blood NfL level after IS, with elevated biomarker concentrations predicting clinical outcomes when used in addition to standard clinical and radiological examination [1–5]. Despite encouraging preliminary results, numerous unresolved issues limit the routine use of blood NfL for clinical use in stroke medicine. First, blood NfL concentrations change rapidly over time in the days after IS and may remain elevated for weeks/months [2, 4]. Also, the prognostic value of blood NfL seems to be higher at later compared to earlier timepoints of quantification [2], but the optimal single quantification timepoint, as

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well as the extent of biomarker changes at individual level, remain uncertain. Second, despite the efforts to improve reproducibility and robustness of NfL measurements [6], absolute concentrations reported in published studies were quantified with different methods and/or assays and in distinct matrices (i.e., serum and plasma) and are therefore difficult to compare. In addition, well-known factors for influencing blood NfL concentrations, such as age, renal function and body-mass index (BMI), as well as systemic comorbidities [7–9] are not always accounted for. Third, most studies reported on single-center and small stroke cohorts, and these results have not been replicated across stroke centers from different countries.

To assess the clinical applicability of blood NfL for decision-making in stroke medicine, we aimed at conducting an individual patient data (IPD) meta-analysis of data obtained from international IS cohorts. In this way, we could better evaluate the temporal changes and the prognostic value of the biomarker for clinical outcomes by leveraging possible confounding factors and targeting pre-analytical variability of biomarker measurement at individual level.

Methods

Study protocol and search strategy

This IPD meta-analysis is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines for IPD systematic reviews (PRISMA-IPD) [10]. The study protocol was pre-registered in the PROSPERO database (registration No. CRD42024538833). A comprehensive search was conducted in MEDLINE (PubMed), Scopus (search string in the Appendix) and grey literature from database inception to June 24th, 2025. Study selection was conducted on Rayyan online platform (*rayyan.ai*) by five independent authors (LB, MR, SAR, MF, LD). Original articles in English language were selected according to pre-defined inclusion criteria: 1) participants aged 18 years or older with a diagnosis of IS; 2) blood NfL concentrations (serum and/or plasma) measured within 30 days from IS onset; 3) available data on at least one primary outcome measure at 3-month follow-up (i.e., all-cause mortality; modified Rankin Scale, mRS). After title and abstract screening, full text manuscripts were assessed for eligibility and disagreements were resolved by consensus (Supplemental Fig. 1). Neurologically healthy control subjects of selected studies were also included for comparison. Given that some studies enrolled patients with transient ischemic attack (TIA, defined without acute ischemic lesions visible

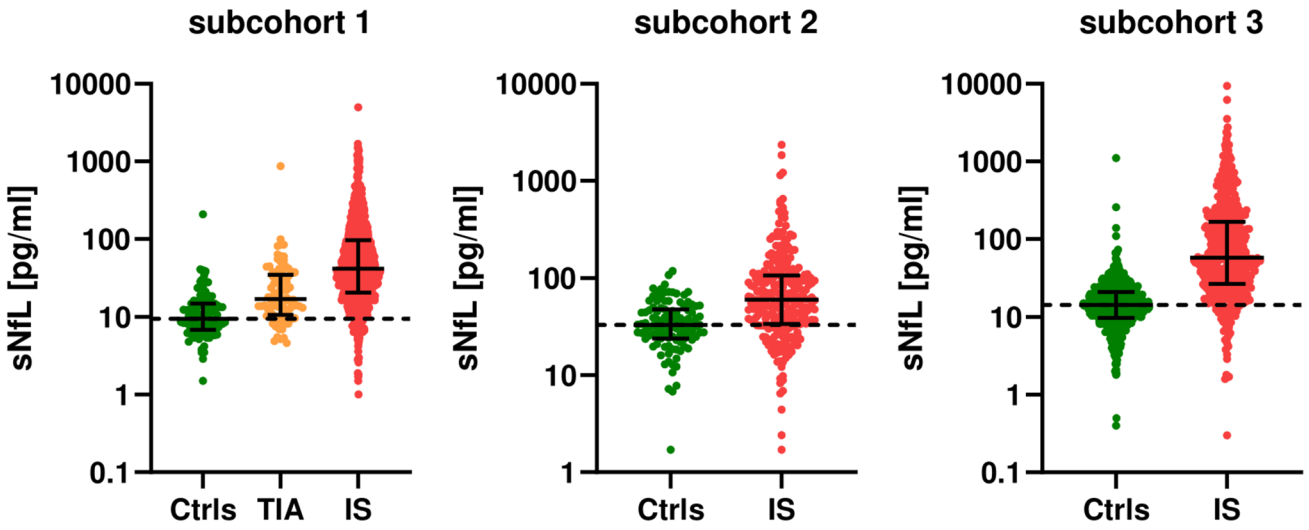

Fig. 1 sNfL concentrations in the study subcohorts. Subcohorts were distinguished according to the assay used for sNfL measurement, namely commercial Simoa assays (subcohort 1: controls n = 101, TIA n = 86, IS n = 1129) and two homebrew assays performed in two independent laboratory in Basel, Switzerland (subcohort 2: controls n = 108, TIA n = 2, IS n = 277) and Gothenburg, Sweden (subcohort 3: controls n = 590, IS n = 579). We included in subcohort 1 also patients whose samples were initially measured with commercial Ella assays (IS n = 588) whose sNfL values were converted to the corresponding Simoa values according to internally validated conversion formulas (see Methods for details). Data of 2 TIA patients of subcohort 2 (58.7 pg/ml and 31.7 pg/ml) were not illustrated. Dashed lines represent median sNfL concentrations in control groups [subcohort 1: 9.5 pg/ml (IQR: 6.9–14.9 pg/ml): subcohort 2: 33.0 pg/ml (IQR: 24.2–48.0 pg/ml); subcohort 3: 14.3 pg/ml (IQR: 9.8–21.1 pg/ml)]

at MRI) [11], we included them in the analysis when essential data were available [2, 11–13]. In addition to published cohorts, coauthors were allowed to share data of additional patients that were still unpublished at the time of data collection. Recruiting centers and number of cases per center included in the final analysis are reported in Supplemental Table 1 and visualized in Supplemental Fig. 2 [2–5, 12–25].

Data extraction and collection

We contacted the corresponding authors of the included studies and invited them to share IPD on a standardized data recording sheet or the dataset used for the study in its original form. IPD were then harmonized in accordance with pre-defined variable types (collected variables listed in Supplemental Methods).

Bias assessment

The risk of bias for the included observational studies was assessed using the Quality in Prognostic Studies (QUIPS) tool for Risk of Bias [26]. Two independent reviewers (MR, VT) conducted the bias evaluation, with discrepancies resolved by consensus, or by a third reviewer (LB). Assessment focused on six key domains: study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis and reporting. For each domain, studies were rated as having low, moderate, serious, or unclear risk of bias based on predefined criteria (Supplemental Table 2, Supplemental Fig. 3).

Quantification of blood NfL level

Blood NfL quantification was performed either with Single molecule arrays (Simoa) (Quanterix Inc., Billerica, Massachusetts, USA) or ELLA microfluidic system (BioTechne, Minneapolis, Minnesota, USA) assays (Supplemental Table 1) [2, 4, 19, 22, 27–30]. NfL concentrations measured on either platform are highly correlated with each other (correlation coefficients > 0.9) but absolute values are not interchangeable [27–30]. In this study, we considered Simoa NfL concentrations as reference given that all patients were measured with this method except for 581/1985 IS patients (29.3%) and 7/88 TIA patients (8.0%) (all with serum samples, see below). These subjects were recruited in two independent German cohorts (Halle and Würzburg) and measured at a single laboratory at the Martin-Luther-University Halle-Wittenberg (Halle, Germany). To evaluate comparability of Ella and Simoa assays in these two cohorts, we measured with both methods 28 serum samples from the Würzburg cohort (Supplemental Fig. 4). Spearman's rho coefficient between Simoa and Ella values was 0.974. Linear regression yielded the following coefficients: Simoa NfL (pg/ml) = Ella NfL [pg/ml] × 0.6717–2.3574. We adopted this formula to convert Ella values into Simoa values. Concerning the biological matrix, NfL level was measured in serum (sNfL) in 736/799 (92.1%) control subjects, 79/88 (89.8%) patients with TIA and 1723/1985 (86.8%) patients with IS (total serum samples n = 2538/2872, 88.4%). All plasma samples were measured with Simoa assays. To compare data on serum and plasma, we adopted conversion formulas previously validated and published for plasma/serum samples measured with Simoa assays: serum NfL [pg/ml] = −0.33 [pg/ml] + 1.11 × plasma NfL [pg/ml] [7, 31].

For samples measured with Simoa, three types of assays were used: commercially purchased kits (type of lot numbers of the adopted assays are reported in Supplemental Table 1), and two homebrew assays with antibodies purchased from Uman Diagnostics (Umeå, Sweden) and transferred onto the Simoa platform run in two independent laboratories (assay A: Basel, Switzerland; assay B: Gothenburg, Sweden) [2, 4, 19, 22]. Given that absolute concentrations measured with different assays in independent laboratories are not comparable, we distinguished three subcohorts according to the quantification method, namely subcohort 1 (commercial assays), subcohort 2 (homebrew assay A) and cohort 3 (homebrew assay B) (Supplemental Table 1). Core data of participants included in each subcohort is shown in Supplemental Table 3.

Derivation of sNfL Z-scores

Blood NfL concentrations can be influenced by several physiological and pathological factors [6, 7]. To mitigate the effect of the most relevant influencing factors, we derived age-adjusted and, if available, BMI-adjusted sNfL Z-scores for patients with NfL concentrations measured with commercial immunoassay and with available lot number data (subcohort 1) [32]. As a continuous measure indicating the variations (in terms of standard deviations) with respect to a reference healthy population, sNfL Z-scores we modelled on Generalized Additive Models for Location, Scale and Shape (GAMLSS) by using a large database of control subjects previously published (n = 4532) [7, 32]. The mean value of the reference population corresponds to a Z-score of 0 and each 1-unit increase of the Z-score corresponds to an increase of 1 standard deviation above the reference mean. sNfL Z-scores were calculated in n = 1091 participants with sNfL concentrations measured with commercial Simoa immunoassays after accounting for lot-to-lot variability (IS n = 969, controls n = 40, TIA n = 82).

Analysis of NfL at different timepoints of quantification

We analyzed NfL concentrations by considering the first NfL measurement available for each participant; for patients

Table 1 Summary data of the study population

	Controls [n = 799]	TIA [n = 88]	IS [n = 1985]	p*
Age [mean (± SD)]	57.5 (± 11.2)	68.8 (± 12.6)	68.1 (± 14.1)	$< 0.001^a$, $< 0.001^b$, 0.763^c
Female sex	302/799 (37.8)	35/88 (39.8)	889/1985 (44.8)	$< 0.001^a$, 0.805^b, 0.414^c
BMI	25.9 (23.7–28.5) [n = 684]	25.4 (23.8–29.4) [n = 85]	26.1 (23.9–28.8) [n = 1064]	0.179^a, 0.699^b, 0.864^c
Stroke characteristics				
NIHSS score on admission	–	–	8 (3–15) [n = 1936]	–
NIHSS score on admission ≥ 6 points	–	–	1151/1936 (59.5%)	–
AF (previously known or newly diagnosed)	3/82 (3.7)	4/18 (22.2)	473/1673 (28.3)	$< 0.001^a$, 0.022^b, 0.761^c
Etiology				$< 0.001^c$
LAA	–	9/83 (10.8)	322/1879 (17.1)	
CE	–	5/83 (6.0)	622/1879 (33.1)	
SVD	–	35/83 (42.2)	279/1879 (14.8)	
other determined etiology	–	1/83 (1.2)	106/1879 (5.6)	
undetermined etiology	–	33/83 (39.8)	550/1879 (29.4)	–
Revascularization therapy				
only IVT	–	–	214/1496 (14.3)	–
only EVT	–	–	434/1496 (29.0)	–
EVT + IVT	–	–	357/1496 (23.9)	–
if EVT, mTICI ≥ 2b	–	–	695/760 (91.4)	-
Outcome measures during hospitalization				
SICH	–	–	65/597 (10.9)	–
need for mechanical ventilation	–	–	49/569 (8.6)	–
need for hemicraniectomy	–	–	13/531 (2.4)	–
all-cause mortality	–	–	47/977 (4.8)	–
hospitalization time (days)	–	–	8 (4.5–13.5) [n = 367]	–
Outcome measures at 3 months				
all-cause mortality	–	–	264/1943 (13.6)	–
mRS		–	2 (1–4)	–
mRS of 3–6	–	–	786/1857 (42.3)	–
Sample available				
D1	–	26 (29.5)	944 (47.6)	–
D2	–	66 (75.0)	715 (36.0)	–
D3	–	13 (14.8)	452 (22.8)	–
D4-5	–	–	268 (13.5)	–
D6-7	–	8 (9.1)	359 (18.1)	–
D8-30	–	2 (2.3)	220 (11.1)	–
N. samples collected				0.354^c
1	799 (100.0)	74 (84.1)	1526 (76.8)	
2	–	5 (5.7)	148 (7.5)	
3	–	5 (5.7)	222 (11.2)	
4	–	4 (4.5)	89 (4.5)	

Categorical variables are reported as n. cases/total cases with available data (%), whereas continuous data are reported as median value (interquartile range) with the exception of age (reported as mean ± SD). *Reported p-values refer to Mann–Whitney *U* or chi-squared test for the comparison: a) IS vs. controls; b) TIA vs. controls; c) IS vs. TIA. *AF* atrial fibrillation, *CE* cardioembolism, *EVT* endovascular treatment, *IS* ischemic stroke, *IVT* intravenous thrombolysis, *LAA* large-artery arterosclerosis, *mRS* modified Rankin Scale, *mTICI* modified Thrombolysis In Cerebral Infarction score, *NIHSS* National Institute of Health Stroke Scale, *SICH* symptomatic intracranial haemorrhage, *SVD* small-vessel disease, *TIA* transient ischemic attack

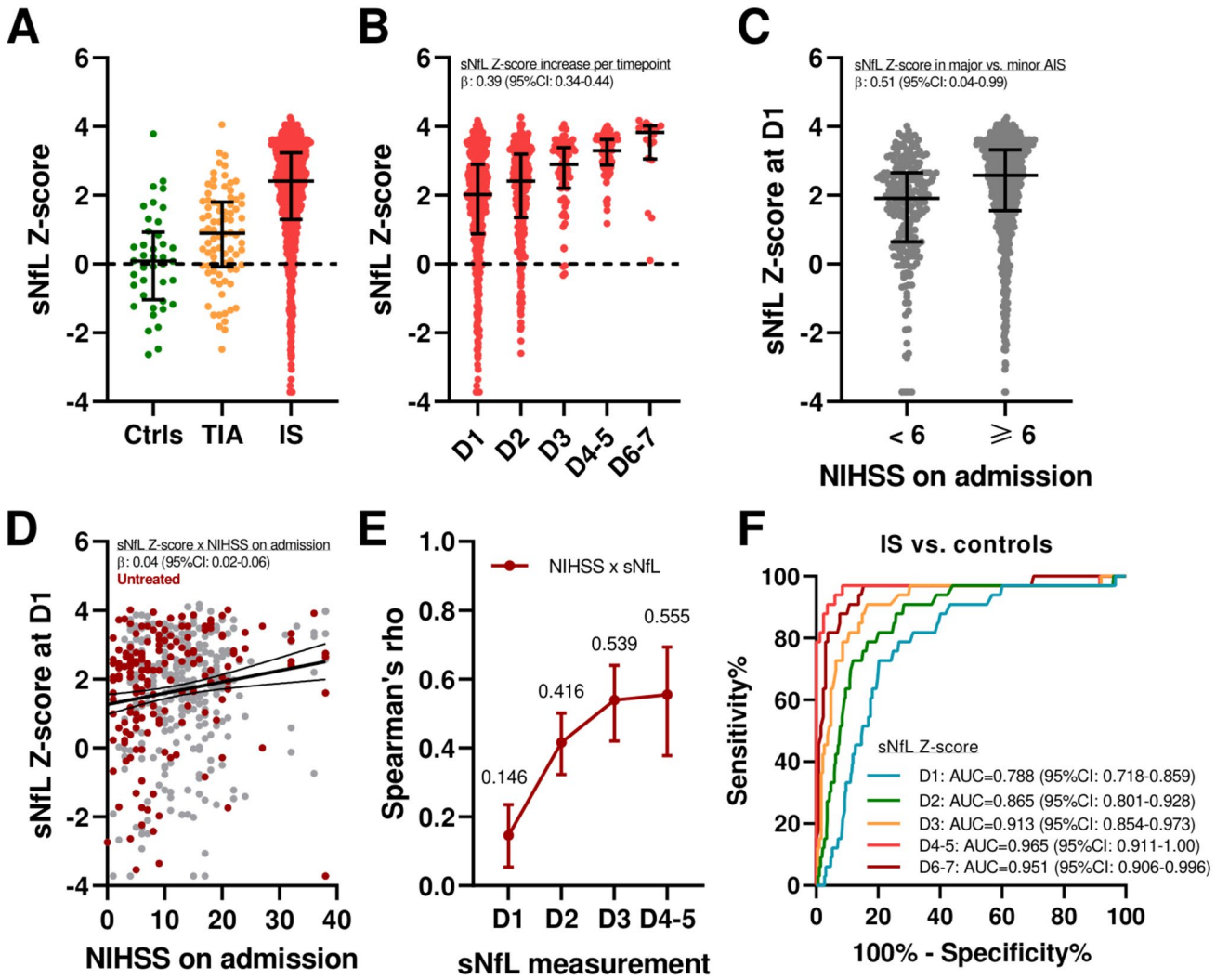

Fig. 2 sNfL Z-scores in IS. **A** sNfL Z-scores in diagnostic groups. **B** Temporal changes of sNfL in IS patients (subcohort 1). **C** sNfL Z-score in IS patients with major vs. minor IS (i.e., NIHSS score ≥ 6 vs. < 6). **D** Association between sNfL Z-score at D1 (< 24 h from IS onset) and NIHSS score on hospital admission in IS (red dots represent patients in which sNfL was measured before neuroimaging and treatment administration). **E** Spearman's correlation between sNfL Z-score and NIHSS at different timepoints (i.e., assessed on the same day). Points indicate Spearman's rho coefficient and error bars indicate 95% confidence interval. **F** ROC analysis of sNfL Z-score for the discrimination between IS patients and control subjects**.** *AUC* area under the curve, *EVT* endovascular therapy, *IS* acute ischemic stroke, *IVT* intravenous thrombolysis, *NIHSS* National Institute of Health Stroke Scale, *ROC* receiver operating characteristic, *sNfL* serum neurofilament light chain protein, *TIA* transient ischemic attack

with repeated NfL measurements, we used the closest value to clinical onset. Then, we distinguished NfL measurements according to the timepoint of biomarker quantification from IS symptom onset, namely D1 (day 1: < 24 h), D2 (day 2: 24–48 h), D3 (day 3: 48–72 h), D4–5 (days 4 and 5: 72–120 h), D6–7 (days 6 and 7: 120–168 h) and D8–30 (days 8 to 30: > 168 h). We repeated statistical analysis on NfL measurements at each timepoint of quantification. Regarding samples at D1, 572 samples were collected on hospital admission before neuroimaging and acute treatment, of which 162 samples were included in subcohort 1 being measured with commercial Simoa assays (hence, sNfL Z-scores were available). For the other D1 samples, we did not have available data on the exact time of blood collection with respect to hospital admission and/or acute treatment.

Statistical analysis

Statistical analyses were conducted on R studio v.4.2.2 (R foundation, Vienna, Austria) and visualization of results was carried out with GraphPad Prism v.8 (GraphPad Software, La Jolla, California, USA). Unadjusted comparison of continuous variables between groups was performed with Mann–Whitney *U* test. Categorical variables were compared

Table 2 Associations between sNfL Z-score and clinical outcomes at 3 months

mRS 3–6 vs. mRS 0–2 or unchanged	aOR (95%CI)	p-value	n. cases
first available*	1.31 (1.17–1.48)	< 0.001	821
D1	1.18 (1.02–1.36)	0.025	341
D2	1.28 (1.07–1.53)	0.008	468
D3	1.63 (1.05–2.52)	0.030	185
D4-5	3.37 (0.78–14.48)	0.102	83
D6-7	1.86 (1.03–3.34)	0.039	130
3-month mRS	aOR for ordinal shift (95%CI)	p-value	n. cases
first available*	1.37 (1.25–1.51)	< 0.001	821
D1	1.36 (1.23–1.51)	< 0.001	341
D2	1.32 (1.15–1.52)	< 0.001	468
D3	1.64 (1.24–2.17)	< 0.001	185
D4-5	4.51 (1.95–10.42)	< 0.001	83
D6-7	1.92 (1.28–2.88)	0.002	130
all-cause mortality	aOR (95%CI)	p-value	n. cases
first available*	1.67 (1.37–2.05)	< 0.001	826
D1	1.71 (1.29–2.23)	< 0.001	346
D2	1.71 (1.23–2.36)	0.001	469
D3	4.40 (1.70–11.44)	0.002	186
D4-5	1.40 (0.48–4.03)	0.535	83
D6-7	6.17 (1.26–30.11)	0.024	131

Results were obtained with GLMMs (CLMM for ordinal shift) after accounting for the recruiting center and for age, eGFR, NIHSS on admission, IVT and EVT as covariables. *Models were adjusted also for time-to-sampling. *aOR* adjusted odds ratio, *CLMM* cumulative link mixed models, *eGFR* estimated glomerular filtration rate, *EVT* endovascular treatment, *GLMM* generalized linear mixed-effect models, *IVT* intravenous thrombolysis, *mRS* modified Rankin Scale, *NIHSS* National Institute of Health Stroke Scale, *sNfL* serum neurofilament light chain protein

with the chi-squared test. Correlations were computed with Spearman's rho coefficient. We used one-sample Wilcoxon signed-rank test to assess difference between sNfL Z-score values with the reference value in the standard population (sNfL Z-score = 0). Meta-analysis was performed using generalized linear mixed-effects models (GLMMs) to test associations between NfL values (either raw concentrations or Z-scores) and other variables [if not other specified: age, estimated glomerular filtration rate (eGFR), National Institute of Health Stroke Scale (NIHSS) score on hospital admission, intravenous thrombolysis (IVT), endovascular treatment (EVT)] by accounting the cohort of origin as random effect covariate. To evaluate the ordinal shift on the mRS, we built cumulative link mixed models (CLMM) adjusted for the same covariables. To assess changes of sNfL over time, we built linear mixed-effect models (LME) with sNfL as dependent variable, timepoint of quantification, age and renal function as covariables and both the recruiting center and individual patients as random effect. In IS patients of our cohort, age and BMI were significantly correlated with sNfL concentrations when measured early after IS (i.e., D1), whereas correlations were weaker or not significant for late sNfL measurements (Supplemental Table 4). Given the greater robustness of adjusted Z-scores compared to raw biomarker concentrations [7], we performed first statistical analysis by using available sNfL Z-scores of subcohort 1 and then replicated the main findings on raw sNfL concentrations in subcohorts 2 and 3. We assessed the equality of variance of sNfL in each timepoint of quantification with the Brown-Forsythe test by measuring the spread of values with respect to the median, and computed confidence intervals for each comparison with the Games-Howell's multiple comparison test. Discriminative accuracy of NfL was assessed with receiver operating characteristic (ROC) analysis, and best cutoff values were computed by maximizing the Youden index. Results of statistical analysis were considered significant at two-sided p-values < 0.05.

Results

Systematic search results and risk of bias

Seventeen observational studies were included in the IPD meta-analysis. The risk of bias was moderate for 11 studies,

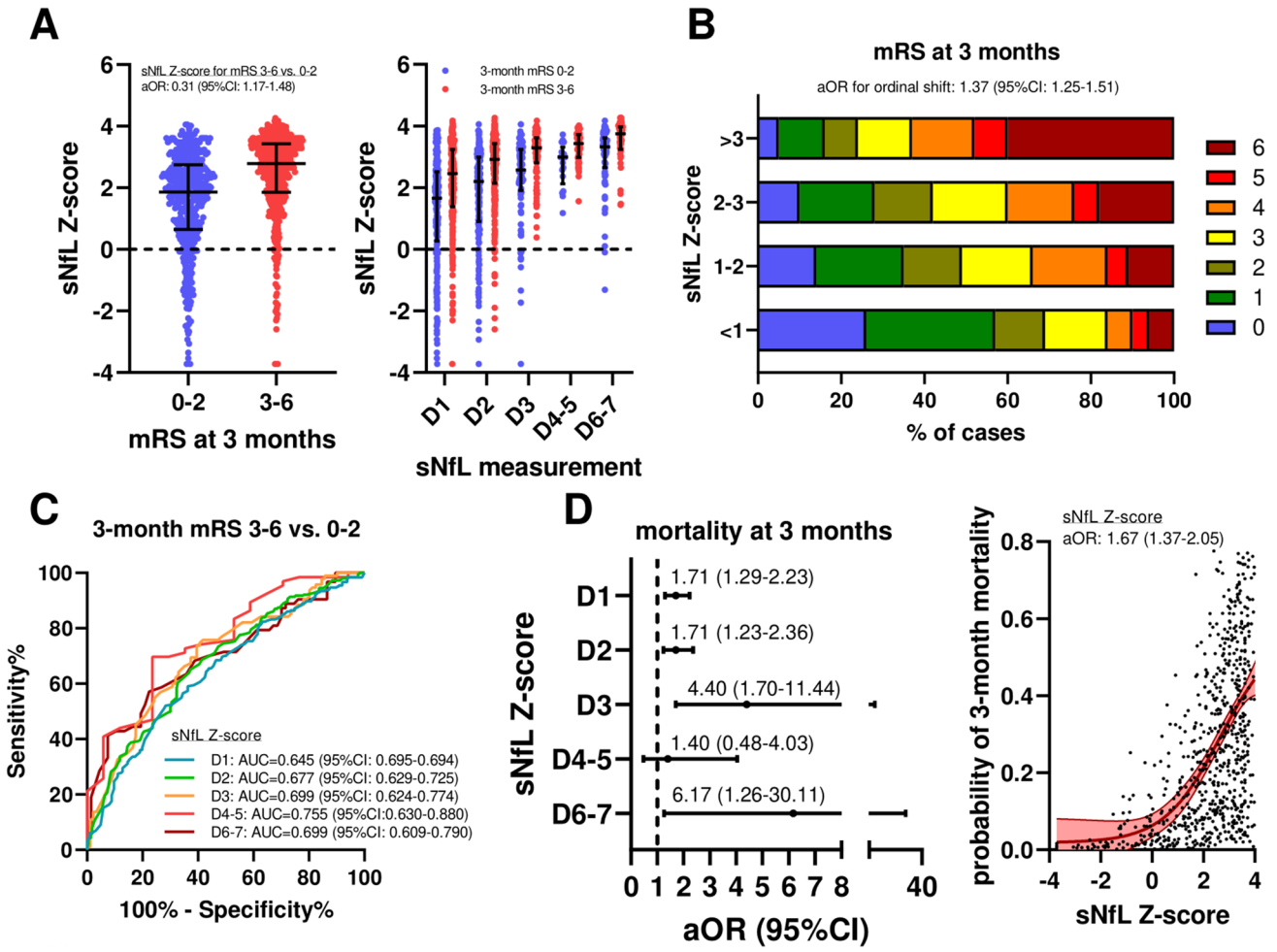

Fig. 3 Associations between sNfL and clinical outcomes at 3 months. **A** sNfL Z-score in IS patients with poor (mRS of 3–6) vs. good (0–2 or unchanged to pre-event mRS) outcome at 3 months. Left graph illustrates first available sNfL Z-score, whereas right graph represents the temporal change of sNfL in the two IS subgroups according to 3-month mRS. **B** Distribution of mRS scores at 3 months according to first available sNfL Z-score. **C** ROC analysis of sNfL for discriminating patients with good vs. poor outcome at 3 months. **D** Associations between sNfL at different timepoints and all-cause mortality. On the right graph, the association between first available sNfL Z-score and all-cause mortality at 3 months is illustrated. Adjusted odds ratio (aOR) refer to multivariable regression models accounting for age, eGFR, NIHSS on admission, intravenous thrombolysis and endovascular treatment as covariables (models with first available sNfL were adjusted also for time-to-sampling). In panel B, aOR was calculated with cumulative link mixed models for ordinal shift adjusted for the same covariables. *IS* ischemic stroke, *mRS* modified Rankin Scale, *ROC* receiver operating characteristic, *sNfL* serum neurofilament light chain protein

low for 3 studies and serious for 3 studies (Supplemental Table 2, Supplemental Fig. 3).

sNfL after IS

Overall, 4081 sNfL measurements from 2872 participants who had at least one available NfL value were included (complete demographic and clinical data in the Supplemental Results and Table 1). Among these, 1985 patients had IS, 88 patients had TIA and 799 were neurologically healthy control subjects. At hospital admission, patients with IS had median NIHSS scores of 8 points (IQR: 3–15) (Table 1). sNfL concentrations of first available samples in IS patients were significantly higher than in control subjects in all subcohorts ($p < 0.001$ for all) (Supplemental Table 3, Fig. 1). In subcohort 1, one-sample analysis showed that sNfL Z-scores were significantly elevated in patients with IS ($p < 0.001$) and in those with TIA ($p < 0.001$) compared to the reference (Z-score = 0). In contrast, sNfL Z-scores in our control group did not differ significantly from the reference population ($p = 0.398$) (Supplemental Table 3, Fig. 2A). sNfL Z-scores were significantly elevated above 0 at all sampled timepoints ($p < 0.001$ for all) (Supplemental Table 3, Fig. 2A), and variability analysis revealed that spreading of sNfL values was lower at later than earlier timepoints (Supplemental Fig. 5). Linear mixed-effect (LME) models built on 969 participants

with IS with repeated measurements (1227 individual sNfL Z-scores, n. of cases with single vs. repeated measurement at each timepoint in Supplemental Table 5) revealed a significant association between timepoint of measurement and age- and BMI-adjusted sNfL Z-scores. Models were also adjusted for renal function as a covariate and included both patient ID and recruiting center as random effects ($p < 0.001$). sNfL Z-scores were progressively more elevated from D1 to D6-7, increasing by 0.39 (95%CI: 0.34–0.44) units per sampled timepoint (Fig. 2B). Results were consistent in all subcohorts by analyzing sNfL absolute concentrations, with an average increase of approximately 70 pg/ml per timepoint (Supplemental Table 6, Supplemental Fig. 6). Results also remained robust across treatment subgroups [no acute therapy: $\beta = 0.38$ (95%CI: 0.29–0.48), $p < 0.001$; only IVT: $\beta = 0.35$ (95%CI: 0.25–0.44), $p < 0.001$; only EVT: $\beta = 0.40$ (95%CI: 0.31–0.49), $p = 0.001$; IVT + EVT: $\beta = 0.39$ (95%CI: 0.30–0.48), $p < 0.001$].

Association of sNfL with IS clinical severity

Data on NIHSS score on hospital admission was available for 1936 of 1985 IS patients (97.5%). We found significant associations between higher NIHSS scores and higher sNfL Z-score, especially at timepoints later than D1 (Fig. 2B–C, Supplemental Table 7, Supplemental Results).

Association between sNfL and infarct lesion size

We investigated the relationship between sNfL and infarct lesion size, assessed either at non-contrast CT performed 24–72 h after symptom onset (n = 261) or by diffusion-weighted MRI (n = 452) (detailed results presented in Supplemental Results). Briefly, sNfL levels were associated with infarct lesion size at D2, D3, D4–5, D6–7 and D8-30 but not at D1. The strength of correlations increased progressively with time after IS onset (e.g., Spearman's rho: 0.106 at D1, 0.671 at D2 and 0.795 at D6–7 in subcohort 3 with MRI data) (Supplemental Results, Supplemental Tables 8–9).

Discriminative accuracy of sNfL for IS

At ROC analysis, sNfL Z-score discriminated IS patients from control subjects with good to excellent accuracy, with area under the curve (AUC) values increasing from 0.788 at D1 to 0.965 and 0.951 at D4-5 and D6-7, respectively (Fig. 2F, Supplemental Table 10). Results were consistent by analyzing sNfL concentrations in all subcohorts (Supplemental Table 11). Moreover, we found a moderate to fair accuracy of sNfL for discriminating IS from TIA, with better results at D2 (AUC: 0.796) and D3 (AUC: 0.795) than at D1 (AUC: 0.643) (Supplemental Table 11).

Predictive value of sNfL for hemorrhagic transformation

In our cohort, 597 patients (30.1%) had available data on symptomatic intracranial hemorrhage (SICH) occurrence, defined according to ECASS II criteria [33]. Patients with available SICH data were female in 287/597 (48.1%) cases, had mean age of 71.5 years (SD: ± 13.4 years) and median NIHSS score admission of 10 points (IQR: 5–16 points). In comparison, patients without SICH data were younger [mean age: 66.6 years (SD: ± 14.1 years), $p < 0.001$] and had lower admission NIHSS scores [median: 7 points (IQR: 2–14 points), $p < 0.001$] but did not significantly differ in female sex distribution (43.4%, $p = 0.060$). Moreover, there was a slight imbalance in the indication of IVT (with SICH data: 43.4%, without SICH data: 34.7%, $p < 0.001$) but not of EVT (with SICH data: 52.9%, without SICH data: 52.8%, $p = 1.00$). In IS patients with data on SICH occurrence, the first sNfL measurement was obtained at D1 in 591 cases (before SICH occurrence, 99.0%), at D2 in three cases (0.5%), at D3 in two cases (0.35%) and at D5 in one case (0.15%).

sNfL Z-scores at D1 were significantly higher in patients who later experienced SICH (65 cases, 10.9%) compared to those who did not ($p < 0.001$). At multivariable analysis on 182 patients (of which 81 received IVT) adjusted for covariates (age, eGFR, NIHSS on admission, IVT, EVT), elevated sNfL Z-score at D1 was significantly associated with SICH [adjusted odds ratio, aOR: 1.33 (95%CI: 1.06–1.68), $p = 0.014$] (Supplemental Fig. 7). The discriminative performance of sNfL for predicting SICH was modest [AUC of 0.644 (95%CI: 0.556–0.732)], with best cutoff at D1 of sNfL Z-score > 2.29, which held a sensitivity of 62.0% (95%CI: 48.2–74.1%) and a specificity of 64.2% (95%CI: 58.2–69.8%) (Supplemental Fig. 7). Data on subcohorts 2 and 3 were not analyzed due to very few (n = 10, subcohort 2) or no (subcohort 3) data on SICH.

Prognostic value of sNfL for other intra-hospital outcomes

No significant association was found between sNfL levels and the indication for decompressive hemicraniectomy. Instead, elevated sNfL Z-scores were associated with higher risk of intra-hospital mortality (data for n = 977 patients, sNfL at D2, D3, D6-7), greater likelihood of requiring mechanical ventilation (data for n = 569 patients, sNfL at D1 and D2), and longer hospital stay (data for

n = 367 patients, Supplemental Results, Supplemental Table 12).

Association between sNfL and recanalization rate after EVT

Among patients who underwent EVT and had available data on mTICI (760/791, 96.1%), successful reperfusion (mTICI of 2b, 2c or 3) was achieved in 695 patients (91.4%). Recanalization success was not associated with the longitudinal changes of sNfL level, but elevated sNfL Z-score after treatment (D2, D3, D6-7) were associated with futile recanalization – defined as poor functional outcome or death at 3-month follow-up despite successful recanalization (Supplemental Fig. 8, Supplemental Results).

Prognostic value of sNfL for clinical outcomes at 3 months

Data on 3-month mRS scores and all-cause mortality were available for 1857 (93.6%) and 1943 (97.9%) participants with IS, respectively (Table 1). Patients with unfavorable outcome (mRS 3–6 at 3 months) had significantly higher sNfL Z-scores in first-available samples and at all timepoints compared to those with good outcome (mRS 0–2 or unchanged to pre-stroke status) (Fig. 3A–B, Supplemental Table 13). At multivariable analysis, higher sNfL Z-score was associated with poorer clinical outcome both when analyzed as a binary outcome and as an ordinal scale (Fig. 3B, Table 2), and results were consistent for D2 sNfL also after excluding patients who developed SICH (Supplemental Results). Discriminative accuracy of sNfL Z-score for 3-month mRS 3–6 was moderate, with highest AUC at D4-5 [AUC: 0.755 (95%CI: 0.628–0.882)] (Fig. 3C, Supplemental Table 14) (optimal sNfL Z-score cutoffs at each timepoint in Supplemental Table 15). Similar results were found in survival analysis, namely increased sNfL Z-score in IS non-survivors compared to survivors, significant associations between sNfL at different timepoints and all-cause mortality in multivariable models (Fig. 3D, Table 2) and moderate discriminative accuracy of sNfL for mortality (Supplemental Tables 14–15) (details in Supplemental Results).

Discussion

In this IPD meta-analysis, we systematically investigated the temporal pattern, the discriminative accuracy and the prognostic value of sNfL for IS to enhance its clinical applicability. Our main findings indicate that sNfL levels: (1) are elevated at all timepoints following IS, with a progressive increase over time; (2) correlate with early clinical and radiological metrics of disease severity (NIHSS, infarct lesion size on CT/MRI); (3) demonstrate a high discriminating accuracy for IS vs. healthy controls; (4) are associated with SICH occurrence, intra-hospital all-cause mortality and length of hospital stay; and (5) are associated with poorer 3-month clinical outcomes, including mortality and functional disability (mRS > 2). Collectively, these findings support the potential role of sNfL as a clinically informative biomarker in stroke management.

On a pathophysiological level, the rise of sNfL levels after IS likely indicates a combination of primary neurofilament disruption occurring shortly after the ischemic event and secondary Wallerian degeneration-like processes that follow the initial injury [2, 34, 35]. In the acute phase, brain ischemia leads to neurofilament degradation into fragments, which underlies the increased marker concentration detected in peripheral blood through blood–brain barrier disruption [35]. However, sNfL measured within 24 h does not pair with infarct size as assessed on CT/MRI, while correlations become progressively stronger after a few days up to one week after onset [2, 36]. The value of early sNfL quantification appears thus complementary to that of CT/MRI and may rather inform on the pre-existing neurodegenerative burden, on the individual vulnerability to ischemic injury and possible complications thereof. As an alternative hypothesis, they could also reflect neuroaxonal injury in brain regions apparently normal at neuroimaging [37]. Instead, the progressive increase of sNfL at later phases could rather reflect secondary degeneration of white matter tracts due to disconnection of remote cortical regions [2]. Such temporal pattern justifies the time-dependent accuracy of sNfL to discriminate patients with IS against neurologically healthy subjects, rising from good (AUC: 0.79–0.87) at stroke onset to excellent (AUC > 0.95) at the end of the first week after stroke.

Due to its relatively slow temporal kinetics over the days and weeks following stroke onset, sNfL is supposed to have limited utility for guiding acute treatment decisions [38]. In our study, the rise of sNfL levels early after onset was associated with a higher risk of SICH. If validated in further studies and head-to-head comparisons with other candidate biomarkers, for example glial fibrillary acidic protein (GFAP) and tau protein [39–41], these results would improve acute stroke management for tailoring and monitoring reperfusion therapies at individual level. Moreover, we also found associations of high sNfL level measured within 24 or 48 h from IS onset with more frequent need of mechanical ventilation and longer hospital stay, similarly to what was previously observed for severely ill patients affected by other conditions such as severe acute respiratory syndrome coronavirus type-2 (SARS-CoV-2)-associated disease and in the perioperative care [6, 8, 42, 43]. Thus, sNfL concentrations measured early after IS onset may still be informative to identify patients with expected worse disease course and who may more likely benefit from initiation (or withdrawal)

of intensive care regimens. In this study, we lacked detailed information on medical management, systemic comorbidities as well as non-neurological comorbidities that could impact sNfL levels (e.g., renal disease, delirium etc.) [44, 45]. Hence, our preliminary results should be rather interpreted as hypothesis-generating and encourage further studies on this topic. On another level, our IPD meta-analysis demonstrates that a single measurement of sNfL within one week after onset can aid the stratification of patients at risk of poorer clinical outcome at follow-up. For this scope, additional biomarkers reflecting multiple aspects of stroke pathophysiology are emerging together with NfL, such as glial fibrillary acidic protein (GFAP), brain-derived tau and synaptic proteins (Barba et al. under review) [3, 5, 14, 16, 18]. Despite the encouraging preliminary results, currently used platforms for biomarker quantification are not appropriate for clinical use in real-world contexts. Here, point-of-care biosensor devices based on novel magnetic, electrochemical and optical detection mechanisms raise hopes for on-site measurement and thus rapid decision-making [46], which turns critical especially for pre-hospital triage of patients [47]. Furthermore, the net clinical benefit of integrating fluid biomarkers in predictive algorithms for stroke still needs to be proven in ad hoc validation studies [41].

As a minor finding of our study, we found increased sNfL Z-score in patients with TIA compared to controls, in contrast to previous reports [13, 48]. The discrepancy could be due to the smaller sample size of other cohorts as well as inconsistencies on blood sampling timepoint, given that TIA patients were not systematically included in study protocols on IS. Moreover, most of TIA patients included in this study had blood sampling at first at D2, which should be taken into account with respect to early initiation of secondary prophylaxis. Preclinical studies showed the differential structural stability of neuroaxons in transient vs. permanent brain ischemia, which supports the time-dependent effectiveness of reperfusion therapies [34, 35]. If validated in larger cohorts and with targeted studies, the finding of increased sNfL concentration after TIA would suggest a subclinical neuronal damage that could be not detected at neuroimaging [37]. Alternatively, elevated sNfL levels observed in TIA patients compared to controls could reflect the overall cardiovascular comorbidity burden of TIA patients, given that our TIA and control populations were not matched for the risk factor profile [49, 50].

As a limitation of our work, we acknowledge the heterogeneity of biomarker measurement techniques, which could have biased the analysis. Although applied to only a subset of participants, the use of age- and BMI-adjusted sNfL Z-scores helps to mitigate this limitation and improves the generalizability of our results relative to analyses using unadjusted absolute values. However, given the magnitude of sNfL increase in IS compared to normal values, adjustment could be not always necessary for clinical use in IS. On the other hand, other pathological factors known to influence sNfL values, including cardiovascular diseases such as heart failure and other neurological disorders including neurodegenerative processes, were not considered in multivariable models of our analysis. Hence, adjustment for renal function and stroke-related factors does not fully address this possible confounding source. Second, the diagnostic value of sNfL could not be comprehensively assessed in comparison to differential diagnoses of IS, such as hemorrhagic stroke, epileptic seizures, meningoencephalitis and others. Given that most of the studies included neurologically healthy controls [2, 4, 13, 21, 22], which derived mostly from a single population-based cohort [4], the discriminating accuracy of sNfL for IS vs. other neurological conditions should be further assessed. Third, we only performed exploratory analyses with neuroimaging data on final infarct size. Other radiological measures (for example, infarct lesion density, core/penumbra volume mismatch and white matter integrity) [2, 51] could be more informative for prognostic purposes and should be further analyzed, given also the little influence of successful reperfusion therapy on biomarker levels. Fourth, most patients included had only one sample collected, thus hampering analysis on temporal pattern of sNfL at individual level. Fifth, cohort composition was predominantly of European origin, thus limiting generalizability of results for other populations. Finally, although clinically meaningful, the analysis on SICH occurrence should be interpreted as exploratory given the small complete case sample used for multivariable modelling. Key strengths of the study lie in its multinational nature, robust sample size, and the solid statistical approach.

In conclusion, blood NfL level is increased since early IS stages, progressively rises in the first week after IS onset, reflects clinical and radiological disease severity, and is associated with short- and middle-term clinical outcomes. Our meta-analysis results derived from international IS cohorts strongly support the potential use of blood NfL as a neuronal biomarker in stroke medicine. Still, prospective validation studies for integrating NfL into standard clinical and radiological evaluation will ease identification of high-risk IS patients and decision-making at individual level.

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Data availability Deidentified participant data can be requested to the corresponding authors according to data sharing agreement.

Declarations

Conflicts of interest ST reports a patent on the use of BD-tau (WO 2025/056634) and consulting fees from Quanterix. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZpath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZ Therapies, Cognito Therapeutics, CogRx, Denali, Eisai, Enigma, LabCorp, Merck Sharp & Dohme, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Quanterix, Red Abbey Labs, reMYND, Roche, Samumed, ScandiBio Therapeutics AB, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures sponsored by Alzecure, BioArctic, Biogen, Cellectricon, Fujirebio, LabCorp, Lilly, Novo Nordisk, Oy Medix Biochemica AB, Roche, and WebMD, is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, and is a shareholder of CERimmune Therapeutics (outside submitted work). All other authors have no conflicts of interest related to this work to report.

Ethical approval Studies included in this IPD meta-analysis received approval by the local Ethics Committees of the recruiting centers. All patients or their legal representatives gave written informed consent to participate in the studies included in this IPD meta-analysis. Individual contributors ensured that data sharing was in agreement with local regulations. For the unpublished cohorts, study protocols were approved by the local Ethics Committees of Martin-Luther-University Halle-Wittenberg (reference n.: 2021–101) and University of Würzburg (reference n.: 05/20-am, DRKS00022064). The Graz cohort was registered on ClinicalTrials.gov (registration nr.: NCT05273216) and the study protocol was approved by the Ethics committee of the Medical University of Graz (ethic vote numbers: 24260 ex 11/12 and 30254 ex 17/18).

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