Assessing the temporal relationship of serum neurofilament light (NfL) and subclinical disease activity: Findings from APLIOS trial

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Disclosures

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Background and objective

- Several studies have shown serum NfL (sNfL) as a potential biomarker of future disease prognosis as well as treatment response at the group level in patients with MS[1-4]
- However, studies investigating sNfL as a marker of on-going disease activity at an individual patient level are needed
- In the 12-week, randomized, open-label, Phase 2 APLIOS study, frequent sNfL sampling and monthly MRI scans were performed
- For the first time, we explored the temporal association of sNfL concentration and subclinical disease activity using data from the APLIOS trial

Objective

To evaluate the potential of sNfL as a biomarker for predicting and monitoring subclinical disease activity in individual RMS patients

MRI, magnetic resonance imaging; MS, multiple sclerosis; RMS, relapsing MS; sNfL, serum neurofilament light chain

1. Manouchehrinia A, et al. Neurology 2020;94:2457–2467. 2. Kuhle J, et al. Mult Scler. 2019 doi:10.1177/1352458519885613. 3. Khalil M, et al. Nat Rev Neurol 2018;14:577–589. 4. Disanto G, et al. Ann Neurol 2017;81:857–870.

Methods: Study assessments and analysis

Assessments

sNfL levels were measured at 14 time points over 12 weeks using a novel, validated assay - Siemens sNfL assay on ADVIA Centaur®*

MRI scans were done every 4 weeks over 12 weeks

Analysis

1. sNfL longitudinally, as a function of ongoing disease activity

sNfL[1] was longitudinally estimated for the following three subgroups of patients:

- With on-study relapses (**R+**), irrespective of the occurrence of lesions
- With gadolinium-enhancing T1 lesions but without relapses (**GdT1+ R−**)
- Free of lesions and free of relapses (**GdT1− R−**)

2. Baseline sNfL and risk of on-study Gd+T1 lesions

Risk of on-study Gd+T1 lesion presence based on baseline 'high' vs 'low' sNfL[2]:

- All patients
- Subgroup of patients free of Gd+T1 at baseline

3. Temporal association of sNfL levels and MRI activity

sNfL levels in

- Patients with Gd+T1 lesion on Day 28 scan only
- Patients with no Gd+T1 lesions on any scans

4. Patient-level predictions of on-study Gd+T1 lesion activity

Prediction (sensitivity and specificity using area under the receiver operating characteristics curve) of the presence of on-study Gd+T1 lesions (at Week 4, 8, and 12) based on:

- Baseline Gd+T1 status
- Baseline sNfL ('high' vs 'low')
- Monthly sNfL values[3]

*The analytical sensitivity was confirmed to be 1.85 pg/mL, and the reportable range was 2 – 500 pg/mL. Linearity was established for 1 – 646 pg/mL NfL in serum. Linear regression result was R^2 = 0.996. Repeatability within runs was evaluated across 20 days using samples that spanned 80% of the assay measurement range. Within-run CV results were 1.5–3.4%, and total CV results were 2.5–5.1%. Analysis of analytical panel samples spiked with NfL antigen (n = 10), and individual serum samples (N = 122) from patients with MS demonstrated high correlation (R^2 = 0.998, in lower assay range of ≤ 100 pg/mL; and R^2 = 0.838, overall) between NfL results derived from the Siemens automated analyzer and from the Quanterix SIMOA platform.

[1]Age adjusted geometric mean; [2]Cox regression and Kaplan-Meier curves for 'high' (>9.1 pg/mL) vs 'low' NfL (≤9.1 pg/mL). [3]The last sNfL value prior to each MRI scan was used to predict the presence / absence of lesions on the next scan

CV, coefficient of variance; AUC, area under curve; Gd+T1, gadolinium-enhancing T1 lesion; MRI, magnetic resonance imaging; ROC, receiver operating characteristics; sNfL, serum neurofilament light chain

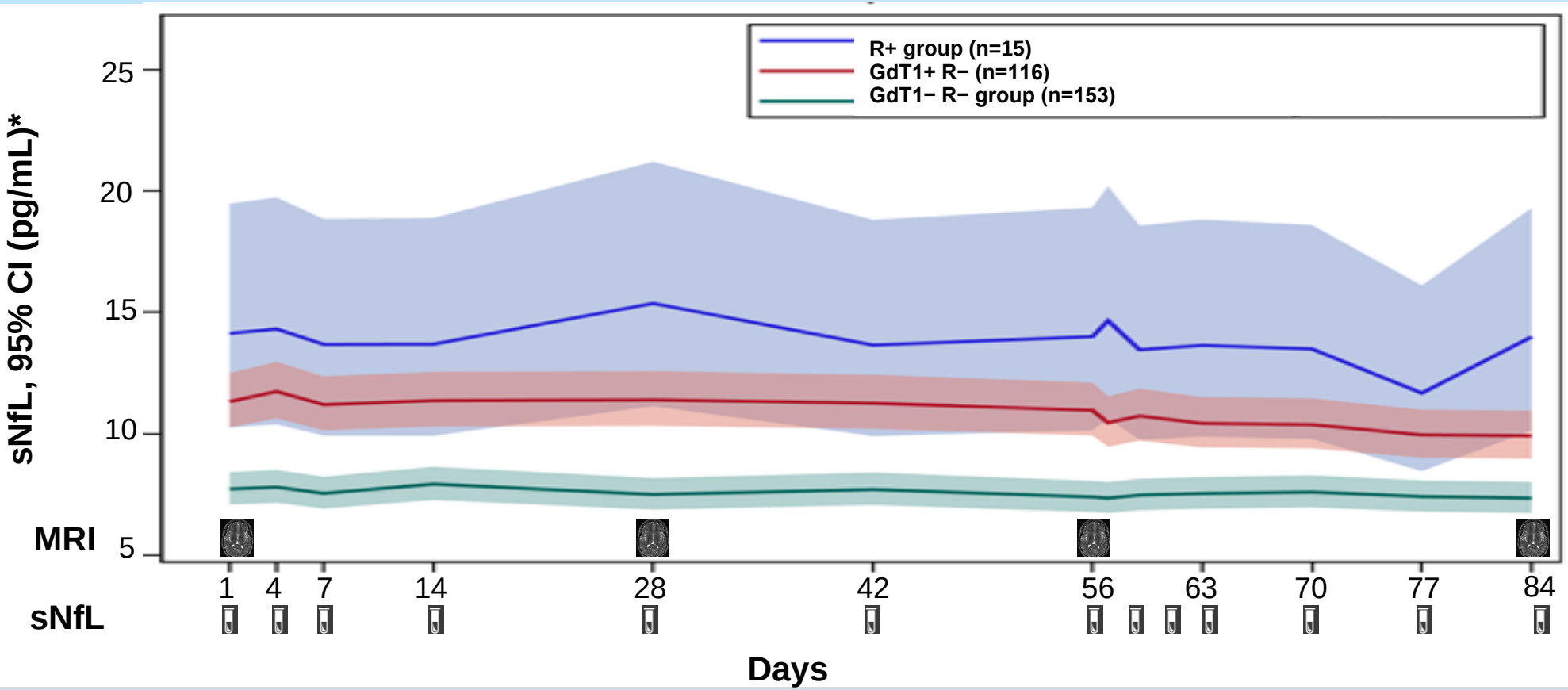
Patient characteristics at baseline

Characteristics	All patients N=284	R+ group n=15	GdT1+ R− group n=116	GdT1− R− group n=153
Age (years)	37.3±8.92	31.3±8.71	35.6±8.64	39.2±8.67
Sex, female, n (%)	199 (70.1)	12 (80.0)	75 (64.7)	112 (73.2)
Race, White, n (%)	275 (96.8)	15 (100.0)	108 (93.1)	152 (99.3)
Weight (kg)	73.7±18.38	68.5±29.63	75.5±19.70	72.9±15.77
BMI (kg/m^2)	25.5±6.13	25.1±10.18	25.9±6.79	25.3±5.03
MS duration since first symptom (years)	9.3±7.75	7.3±6.87	9.0±7.84	9.7±7.77
No. of relapses in the year before the study	1.3±0.72	1.9±0.99	1.3±0.74	1.3±0.65
No. of relapses in the M12 to M24 before the study	1.0±1.58	1.6±1.5	0.9±1.12	1.0±1.86
EDSS score	3.0±1.30	2.9±1.37	2.7±1.27	3.1±1.29
No. of Gd+T1 lesions	1.5±4.97	5.0±7.02	3.1±6.87	0.0±0.00
sNfL (pg/mL), median	9.1	13.6	10.5	7.7

R+: Includes patients who had a confirmed relapse during treatment epoch (irrespective of the occurrence of lesions); **GdT1+ R− :** Includes patients who had Gd+T1 lesions from baseline until 30 days after the last injection date but no confirmed relapse; GdT1− R−: Includes the rest of the patients

BMI, body mass index; EDSS, expanded disability status scale; Gd+T1, gadolinium-enhancing T1 lesion; MS, multiple sclerosis; sNfL, serum neurofilament light chain

1. sNfL longitudinally as a function of ongoing disease activity



sNfL level was:

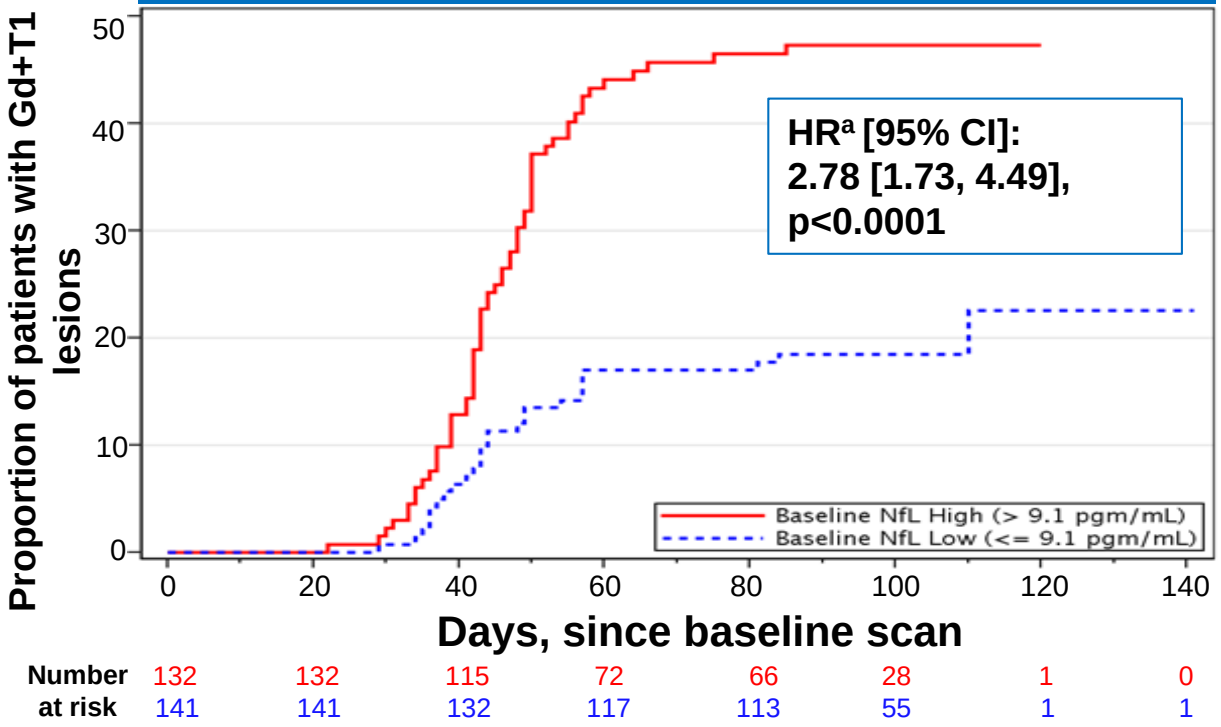
- **– highest in patients who experienced an on-study relapse (irrespective of lesion activity)**
- **– intermediate in patients with lesion activity (but without clinical relapses)**
- **– lowest in patients free of lesions and relapses**

*Adjusted geometric means and 95% CIs are from a repeated measures model, which includes time and patient group as factors, age as continuous covariate, and age-by-patient group and time-by-patient group interactions. Gd+T1 lesions from scans collected within 14 days after termination of steroid therapy are excluded from the analysis.

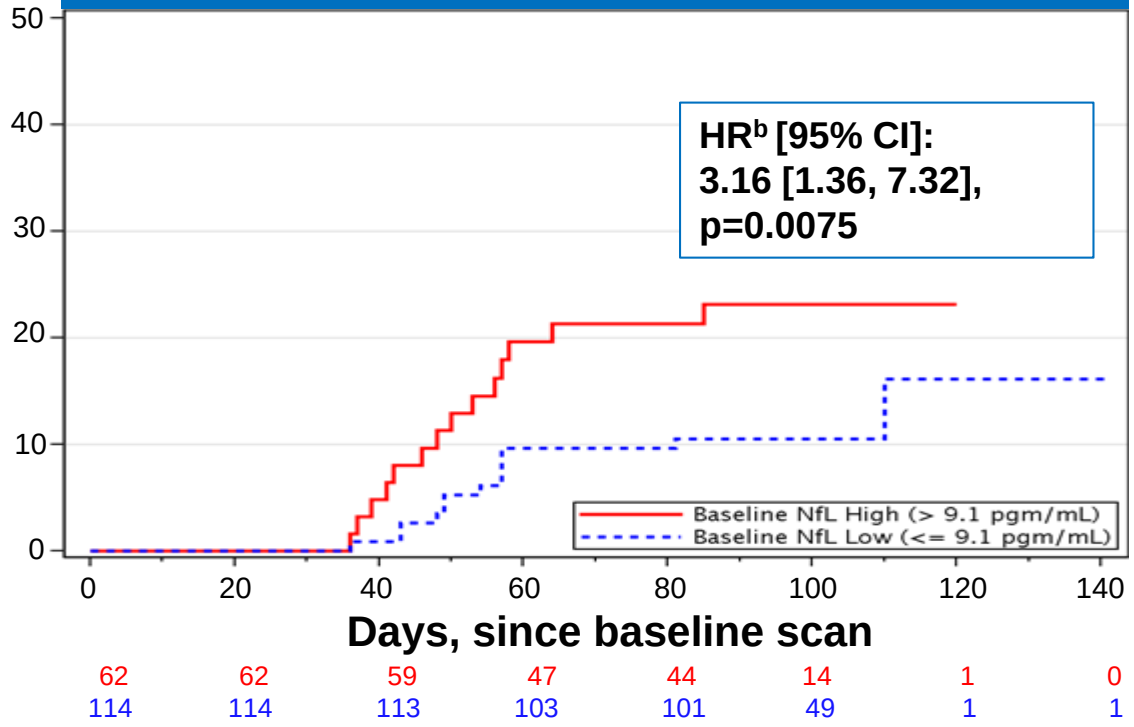
CI, confidence interval; Gd+T1, gadolinium-enhancing T1 lesion; MRI, magnetic resonance imaging; sNfL, serum neurofilament light chain

2. Baseline sNfL predicts risk of on-study Gd+T1 lesions

All patients[a]



Patients free of Gd+T1 at baseline[b]

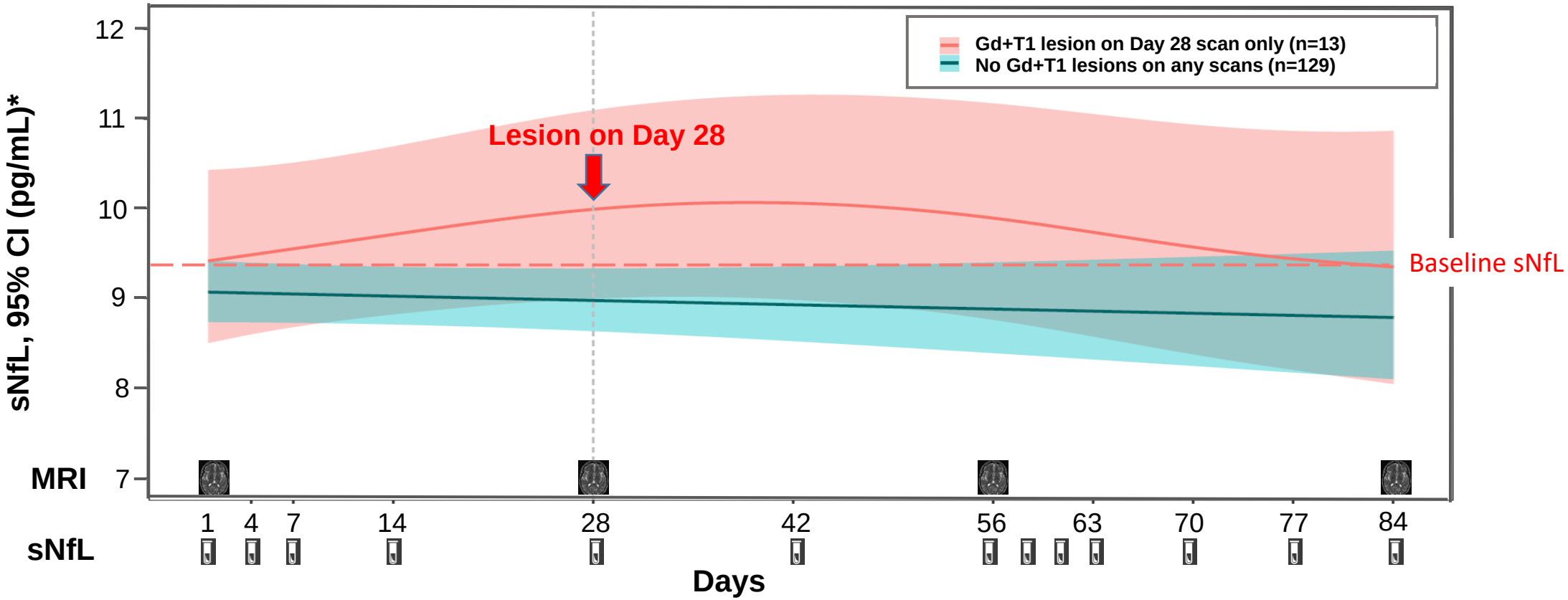


High baseline sNfL confers increased risk of on-study Gd+T1 lesion

Even in patients free of Gd+T1 lesions at baseline, sNfL at baseline predicted on-study lesions

Cox regression model includes: [a]baseline NfL category as factor, age, baseline number of Gd-enhancing T1 lesions, and baseline volume of T2 lesions as continuous covariates; [b]baseline NfL category as factor, age, and baseline volume of T2 lesions as continuous covariates.
CI, confidence interval; Gd+T1, gadolinium-enhancing T1 lesion; MRI, magnetic resonance imaging; sNfL, serum neurofilament light chain

3. Temporal association of sNfL levels and MRI activity*

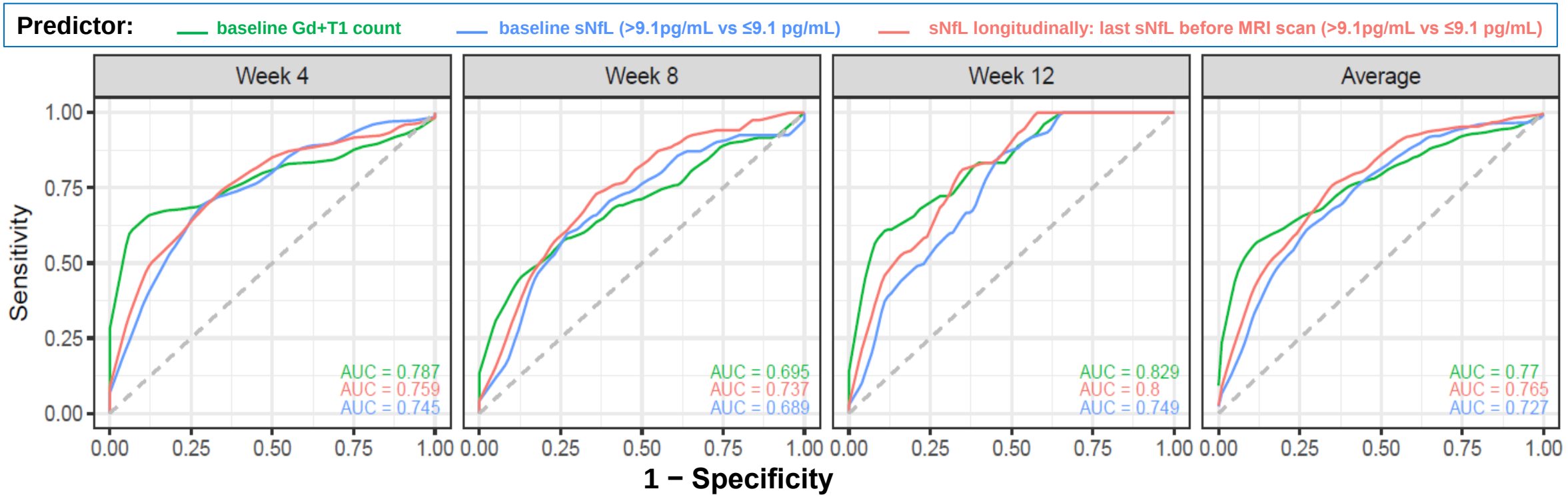


sNfL levels increased approximately one month before the lesion was visible on MRI (at day 28), and gradually decreased back to baseline within approximately 2 months

*Only patients with no missing scans are included in the analysis. For each group, an additive mixed model that adjusts for age and log of baseline NfL, and includes a non−parametric smoother of time, random intercept and slope.
CI, confidence interval; Gd+T1, gadolinium-enhancing T1 lesion; MRI, magnetic resonance imaging; RMS, relapsing multiple sclerosis; sNfL, serum neurofilament light chain

4. Patient-level predictions of on-study Gd+T1 lesion activity

ROC AUC curves for the prediction of on-study Gd+T1 lesions*



Over 12-weeks, prediction of on-study Gd+T1 lesions based on sNfL was comparable to that based on the Gd+T1 count on a baseline MRI scan

*Prediction of whether a patient would have an on-study Gd+T1 lesion using logistic regressions. All models adjusted for age. Model "baseline Gd+T1" used the number of Gd+T1 on the baseline scan as predictor; model "baseline sNfL" used the baseline sNfL measure (>9.1pg/mL vs ≤9.1 pg/mL) as predictor; "sNfL longitudinally" used the last sNfL value before each MRI scan (>9.1pg/mL vs ≤9.1 pg/mL) as the predictor of whether there would be lesions on the next scan. AUCs from Week 4, Week 8, and Week 12 scans are calculated from 10−fold cross−validation. 'Average' corresponds to the average AUC across scans.

AUC, area under the curve; Gd+T1, gadolinium-enhancing T1 lesion; MRI, magnetic resonance imaging; ROC AUC, area under the receiver operating characteristics curve; sNfL, serum neurofilament light chain

Conclusions

1. **Longitudinal sNfL levels were reflective of ongoing disease activity on a group level:**
   - sNfL levels were highest in patients with clinical relapses, intermediate in patients with subclinical activity and lowest in patients free of lesions and relapses
2. **Baseline sNfL had a predictive value for on-study lesion formation in all patients, but notably also in those who appeared free of Gd+T1 lesions at baseline**
3. **sNfL levels were increased for approximately 3 months around the time when lesions were visible on MRI**
4. **On an individual patient level, the predictive value of sNfL and Gd+T1 lesions are comparable:**
   - For baseline sNfL vs baseline Gd+T1 lesion
   - For serial assessments of sNfL vs baseline Gd+T1 lesion

- **Our analysis suggests that sNfL has utility in monitoring subclinical disease activity**
- **Quarterly monitoring of sNfL may be adequate for the surveillance of subclinical disease activity; sNfL may have advantages in terms of cost effectiveness and patient burden**
- **sNfL could complement and may provide an alternative in cases where standard MRI monitoring is infeasible**

Gd+T1, gadolinium-enhancing T1 lesion; MRI, magnetic resonance imaging; RMS, relapsing multiple sclerosis; sNfL, serum neurofilament light chain

Thank you