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

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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

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MRI, magnetic resonance imaging; MS, multiple sclerosis; RMS, relapsing MS; sNfL, serum neurofilament light chain
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\(^3\)

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\(^*\)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 sNfL in serum. Linear regression result was \(R^2 = 0.996\). Repeatability within runs was evaluted 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 sNfL 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 sNfL 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’ sNfL (≤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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All patients N=284</th>
<th>R+ group n=15</th>
<th>GdT1+ R− group n=116</th>
<th>GdT1− R− group n=153</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>37.3±8.92</td>
<td>31.3±8.71</td>
<td>35.6±8.64</td>
<td>39.2±8.67</td>
</tr>
<tr>
<td><strong>Sex, female, n (%)</strong></td>
<td>199 (70.1)</td>
<td>12 (80.0)</td>
<td>75 (64.7)</td>
<td>112 (73.2)</td>
</tr>
<tr>
<td><strong>Race, White, n (%)</strong></td>
<td>275 (96.8)</td>
<td>15 (100.0)</td>
<td>108 (93.1)</td>
<td>152 (99.3)</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>73.7±18.38</td>
<td>68.5±29.63</td>
<td>75.5±19.70</td>
<td>72.9±15.77</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.5±6.13</td>
<td>25.1±10.18</td>
<td>25.9±6.79</td>
<td>25.3±5.03</td>
</tr>
<tr>
<td><strong>MS duration since first symptom (years)</strong></td>
<td>9.3±7.75</td>
<td>7.3±6.87</td>
<td>9.0±7.84</td>
<td>9.7±7.77</td>
</tr>
<tr>
<td><strong>No. of relapses in the year before the study</strong></td>
<td>1.3±0.72</td>
<td>1.9±0.99</td>
<td>1.3±0.74</td>
<td>1.3±0.65</td>
</tr>
<tr>
<td><strong>No. of relapses in the M12 to M24 before the study</strong></td>
<td>1.0±1.58</td>
<td>1.6±1.5</td>
<td>0.9±1.12</td>
<td>1.0±1.86</td>
</tr>
<tr>
<td><strong>EDSS score</strong></td>
<td>3.0±1.30</td>
<td>2.9±1.37</td>
<td>2.7±1.27</td>
<td>3.1±1.29</td>
</tr>
<tr>
<td><strong>No. of Gd+T1 lesions</strong></td>
<td>1.5±4.97</td>
<td>5.0±7.02</td>
<td>3.1±6.87</td>
<td>0.0±0.00</td>
</tr>
<tr>
<td><strong>sNfL (pg/mL), median</strong></td>
<td>9.1</td>
<td>13.6</td>
<td>10.5</td>
<td>7.7</td>
</tr>
</tbody>
</table>

*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

- 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

sNfL level was:

*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

Cox regression model includes:
- baseline NfL category as factor, age, baseline number of Gd-enhancing T1 lesions, and baseline volume of T2 lesions as continuous covariates;
- 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

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

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

<table>
<thead>
<tr>
<th>Predictor:</th>
<th>baseline Gd+T1 count</th>
<th>baseline sNfL (&gt;9.1pg/mL vs ≤9.1 pg/mL)</th>
<th>sNfL longitudinally: last sNfL before MRI scan (&gt;9.1pg/mL vs ≤9.1 pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 4</td>
<td>AUC = 0.787</td>
<td>AUC = 0.759</td>
<td>AUC = 0.745</td>
</tr>
<tr>
<td>Week 8</td>
<td>AUC = 0.695</td>
<td>AUC = 0.737</td>
<td>AUC = 0.689</td>
</tr>
<tr>
<td>Week 12</td>
<td>AUC = 0.820</td>
<td>AUC = 0.8</td>
<td>AUC = 0.749</td>
</tr>
<tr>
<td>Average</td>
<td>AUC = 0.77</td>
<td>AUC = 0.765</td>
<td>AUC = 0.727</td>
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</tbody>
</table>

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