Comparison of the Simoa and MSD R-PLEX assay to assess serum neurofilament light chain levels in hereditary transthyretin amyloidosis

Milou Berends[a,e], Johan Bijzet[b,e], Elisabeth Brouwer[c], Charlotte E. Teunissen[f], Sjors G.J.G. In 't Veld[f], Reinold O.B. Gans[a,e], Bouke P.C. Hazenberg[c,e], Paul van der Zwaag[d,e], Hans L.A. Nienhuis[a,e] and Bart-Jan Kroesen[d,e]

Depts of Internal Medicine[a], Laboratory Medicine[b], Rheumatology & Clinical Immunology[c], Genetics[d], Groningen Amyloidosis Center of Expertise[e], University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; Laboratory Medicine[f], Amsterdam Neuroscience, Amsterdam UMC location Vrije Universiteit Amsterdam, Amsterdam, the Netherlands.

INTRODUCTION

Neurofilament light chain (NfL), a biomarker of neuronal damage[1], can be detected in serum and has shown to be a sensitive biomarker for polyneuropathy in patients with hereditary transthyretin (ATTRv) amyloidosis.[2] A rise in serum NfL (sNfL) precedes the onset of polyneuropathy symptoms.[3] Blood biomarkers, such as NfL, have the potential to improve diagnosis. The Single molecule array (Simoa) assay is currently regarded as the gold stardard for NfL measurement.[4-5] It is a highly sensitive and specific immunoassay that allows measurement of sNfL in very low concentrations.[5] However, the Simoa assay is not widely available, which hampers implementation of NfL measurement into a clinical setting.

OBJECTIVE

We aimed to identify a high performance and well accessible assay for sNfL measurement in ATTRv amyloidosis patients. Here, we present a direct comparison of the Simoa assay with the Meso Scale Discovery (MSD) R-PLEX assay for measuring sNfL.

METHODS

sNfL levels were measured in samples collected between January 2000 and December 2021 from pathogenic transthyretin gene variant (*TTRv*) carriers and ATTRv amyloidosis patients. In each sample, sNfL levels were measured using both the Simoa assay and the MSD R-PLEX assay allowing direct comparison of the assays (Figure 1A). An additional data set with samples of healthy controls was added. Their sNfL levels were measured with the MSD R-PLEX only (Figure 1B). The internal standard for both assays was measured with the MSD R-PLEX assay allowing direct comparison of the quantitative difference between the two assays (Figure 1C).

RESULTS

A total of 332 samples were evaluated in 72 subjects. sNfL levels measured with the MSD R-PLEX assay (median 128.7pg/mL, interquartile range [IQR] 58.8–279.3) were consistently 4.9 times higher than those measured by the Simoa assay (26.4pg/mL [11.3–50.2]), p<.0001. All median sNfL levels per study group are displayed in Figure 1D-E. A strong correlation was found between levels measured with both assays (Pearson correlation coefficient 0.94, p<.0001) (Figure 1H). sNfL levels in healthy contols were 33.2 pg/mL [20.7-51.8] (Figure 1F) and correlated with age (p<.0001) (Figure 1I). Bland-Altman analysis showed, within the 95% limit of agreement, a mean constant bias of 130.4% for sNfL concentrations determined by the MSD R-PLEX assay compared to the Simoa assay. The quantitative difference as determined with the two assays related to a difference in the concentration of the internal standards provided with the tests (Figure 1G and 1J).

REFERENCES

1. Khalil et al. 2018. *Nat Rev Neurol*; 2. Berends et al. 2024. *Int J Mol Sci*; 3. Berends et al. 2024. *Amyloid*; 4. Kuhle et al. 2016. *Clin Chem Lab Med*; 5. Ulndreaj et al. 2023. *Diagnosis.*

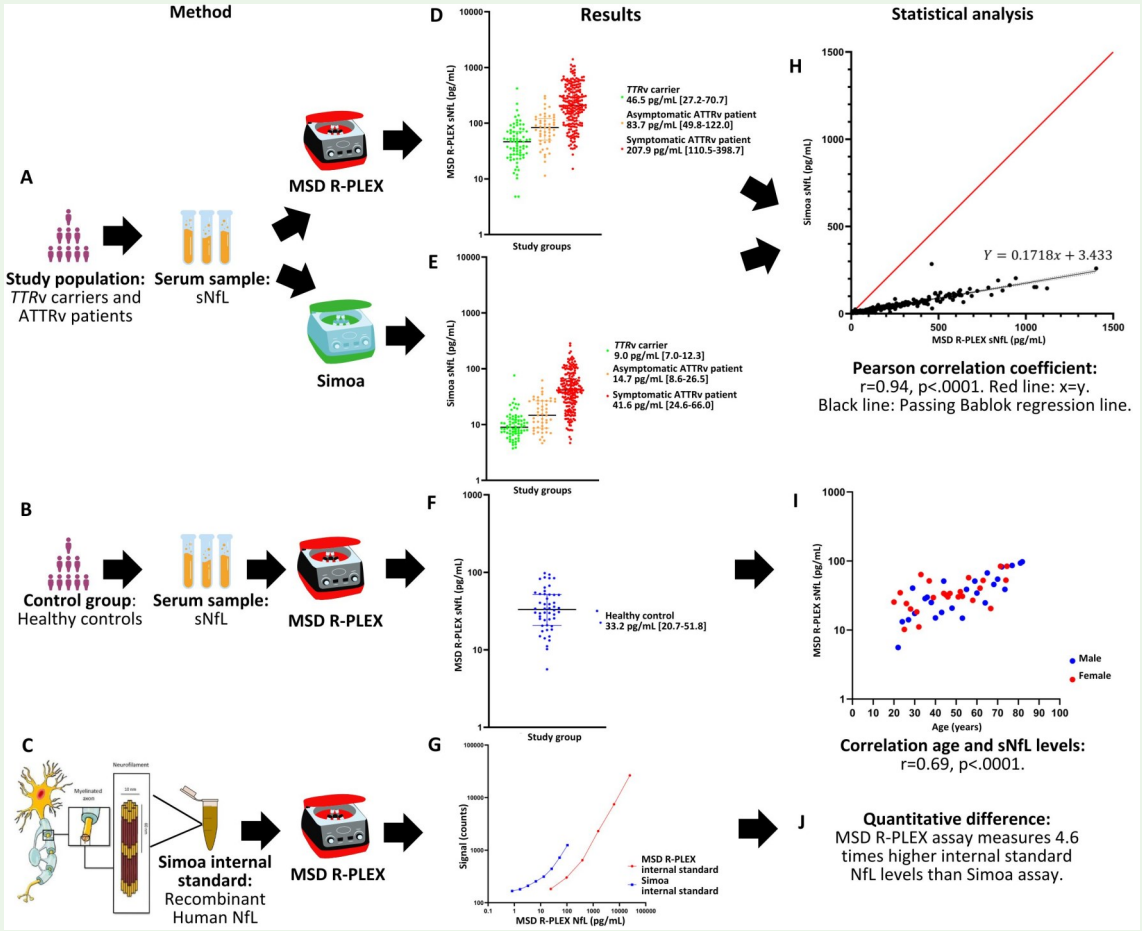

Figure 1. *Study design and results.*

Abbreviations: ATTRv: hereditary transthryetin amyloidosis; MSD: MesoScale Discovery; NfL: neurofilament light chain; Simoa: single molecule array; sNfL: serum neurofilament light chain; TTRv: transthyretin gene variant.

CONCLUSIONS

- **The MSD R-PLEX assay is a robust and sensitive alternative to the Simoa assay for measuring sNfL levels in ATTRv amyloidosis.**
- **The quantitative difference between the assays is related to the internal standard provided with the tests.**

UNIVERSITY MEDICAL CENTER GRONINGEN | *Gr*oningen *A*myloidosis *C*enter of *E*xpertise | university of groningen

Contact: scan QR-code or email m.berends@umcg.nl