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Age-Related Normative Data and Percentile Ranges for Serum Neurofilament Light Chain Levels

Dados Normativos Ajustados a Idade e Intervalos Percentuais para os Níveis Séricos de Neurofilamentos de Cadeia Leve

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Abstract

Introduction: Blood-based neurofilament light chain (NfL), a neuronal cytoskeleton protein, has proven to be a dynamic and robust biomarker for neurodegeneration. The influence of age on NfL levels has been extensively documented in cerebrospinal fluid studies, with blood quantification showing similar trends. To aid the clinical interpretation of NfL values, it is crucial to establish normal reference intervals through population-specific studies that account for NfL's dynamic range.

Methods: In this study, we determined reliable age-stratified reference values for serum NfL (sNfL) using the single-molecule array (SiMoA) immunoassay in individuals with no evidence of cognitive impairment according to age- and education-adjusted normative data for the Mini-Mental State Examination (MMSE), recruited from the central region of Portugal, across a broad age range.

Results: Our sample comprised 335 individuals (median age of 53 years, 57% female) aged 20 to 99 years. We found a strong correlation between age and sNfL levels (rho=0.72, p<0.001). Age-stratified reference limits (upper 95th percentile in each decade) were established as follows: 20-30 years= 9.77 pg/mL; 31-40 years= 13.3 pg/mL; 41-50 years= 16.5 pg/mL; 51-60 years= 21.2 pg/mL; 61-70 years= 41.3 pg/mL; 71-80 years= 48.1 pg/mL; 80+ years= 62.5 pg/mL.

Conclusion: These findings provide a unique national normative baseline for the Portuguese population.

Resumo

Introdução: Os neurofilamentos de cadeia leve (NfL), uma proteína do citoesqueleto neuronal, têm-se revelado um biomarcador dinâmico e robusto de neurodegeneração no sangue periférico. A influência da idade nos níveis de NfL foi já amplamente documentada no líquido cefalorraquidiano, tendo a quantificação sanguínea apresentado tendências semelhantes. Para auxiliar a interpretação clínica dos valores do NfL é crucial estabelecer intervalos de referência normais através de estudos de populações que englobem toda a gama dinâmica do NfL.

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Métodos: Neste estudo, determinámos pontos de corte estratificados para a idade do NfL sérico (sNfL) utilizando a metodologia de Single Molecule Array (SiMoA) em indivíduos sem evidência de défice cognitivo, de acordo com os dados normativos ajustados à idade e escolaridade do Mini-Mental State Examination (MMSE) para a população portuguesa, recrutados na região centro do país e abrangendo uma vasta faixa etária.

Resultados: A nossa amostra consistiu em 335 indivíduos (idade mediana de 53 anos, 57% do sexo feminino) com idades compreendidas entre os 20 e os 99 anos. Encontrámos uma forte correlação entre a idade e os níveis de sNfL (rho=0,72, p<0,001). Os limites de referência estratificados por idade (percentil 95 em cada grupo etário) foram estabelecidos da seguinte forma: 20-30 anos= 9,77 pg/mL; 31-40 anos = 13,3 pg/mL; 41-50 anos= 16,5 pg/mL; 51-60 anos= 21,2 pg/mL; 61-70 anos= 41,3 pg/mL; 71-80 anos= 48,1 pg/mL; 80+ anos= 62,5 pg/mL.

Conclusão: Os nossos resultados fornecem uma base normativa única a nível nacional para a população portuguesa.

Introduction

Blood-based neurofilament light chain (NfL) has emerged as a leading biomarker candidate for neurodegeneration across various diseases.[1] It is a neuroaxonal biomarker with clinically proven value, enabled by the development of ultrasensitive immunoassays.[1,2] The highest NfL concentrations are observed in HIV-associated cognitive deficits, frontotemporal degeneration, and vascular dementia.[2] NfL quantification has proven relevant in differentiating between types of dementia[3] and in monitoring treatment response in multiple sclerosis, being considered the gold standard for analytical sensitivity.[4]

Studies have consistently shown that NfL levels increase with age in patients and healthy individuals,[2] highlighting the importance of reference values to distinguish between pathological changes and normal aging. This study aims to assess the relationship between serum NfL (sNfL) and normal aging in a cognitively unimpaired cohort from the central region of Portugal. We also provide age-stratified reference values for sNfL, reported in percentiles.

Methods

Our convenience sample consisted of 335 individuals recruited from the Neurology Department of ULS Coimbra (individuals with non-neurological conditions and relatives of patients who attended the Dementia Clinic), volunteers from nursing homes, and healthy community participants. Inclusion criteria encompassed individuals aged 20 years and older, with no history of neurological pathology, namely stroke or traumatic brain injury, a collateral history of autonomous functional status, and a normal neurological and cognitive examination. Cognitive screening was based on the Portuguese version of the Mini-Mental State Examination (MMSE), and participants were considered cognitively preserved when their performance was within the normative range adjusted for age and education.[5]

Blood samples were collected into serum separation tubes, centrifuged at 1800 g, 10 min at 4ºC, aliquoted into polypropylene tubes, and stored at -80ºC until analysis. Serum levels of NfL were determined in duplicate using the NF-Light Advantage kit by Single Molecule Array (SiMoA) in the SR-X platform (Quanterix, USA), following the manufacturer's instructions.

Statistical analysis was performed using statistical software R (version 4.1.3). A normal distribution was assessed using the Shapiro-Wilk test. Spearman correlation was applied. The subjects were stratified into seven categories by decade: 20 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, 71 to 80, and over 80 years. Serum NfL concentrations in each category were evaluated in percentiles at 25th, median, 65th, 80th, 90th, and 95th.[6] Visualization consisted of a color scale obtained by spline interpolating the NfL levels at each percentile range over the age. The Brown-Forsythe (BF) test was applied to assess the variances of sNfL in each age category.

Results

Our cohort consists of 335 neurological controls, cognitively unimpaired patients, or healthy volunteers, with a median age of 53 years, ranging from 20 to 99 years, and 57% were female. We observed a strong correlation between age and sNfL (rho=0.72, $p<0.001$). To examine the relationship between age and sNfL levels, we calculated sNfL concentrations across different decades (**Table 1**). Age-stratified reference limits (95th percentile) were established as follows: 20-30 years (n=44)= 9.77 pg/mL; 31-40 years (n=51)= 13.3 pg/mL; 41-50 years (n=49)= 16.5 pg/mL; 51-60 years (n=67)= 21.2 pg/mL; 61-70 years (n=41)= 41.3 pg/mL; 71-80 years (n=42)= 48.1 pg/mL; 80+ years (n=41)= 62.5 pg/mL. Notably, sNfL levels increased nonlinearly with age, particularly after the sixth decade, agreeing with previous reports.[5] To visualize the progression of sNfL levels over the lifespan (**Fig. 1**), we analyzed the percentiles stratified by age, represented by colored areas. The analysis showed significant dispersion and heterogeneity across age groups ($p<0.001$, BF test), with older age groups having a wider range of sNfL values. However, certain age groups, such as those between 41-60 ($p=0.84$) and 71-99 ($p=0.06$), were relatively homogenous.

Table 1. Percentiles of serum NfL in each age group.

Age range	n	25th	50th	65th	80th	90th	95th
20-30	44	4.14	5.73	6.42	7.90	9.17	9.77
31-40	51	5.03	6.61	7.86	8.79	10.6	13.3
41-50	49	7.38	8.91	10.5	13.3	14.5	16.5
51-60	67	6.29	8.19	9.72	13.3	16.3	21.2
61-70	41	9.62	11.3	12.4	14.4	28.5	41.3
71-80	42	14.7	21.8	26.2	34.2	42.3	48.1
>80	41	17.8	26.7	32.0	40.3	59.6	62.5

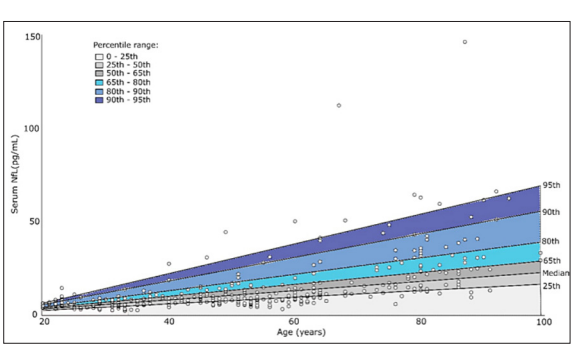

Figure 1. Percentile ranges of serum NfL. Percentile range areas of serum NfL during normal aging. The spline-interpolated percentile range curves are based on the measures in each age category.

Discussion

The blood NFL levels of our cohort align more closely with those reported by Khalil *et al* (2020) than with other studies,[7-9] where NfL levels were significantly lower. This discrepancy may be due to differences in cohort selection, as our cohort's characteristics are more representative of community-based studies. Additionally, it has been noted that sNfL tends to be higher than plasma levels,[9] which could further explain the observed increase in our data. Consistent with the literature, we found a strong correlation between age and sNfL levels,[2,6] reinforcing the age-related effect on this biomarker.

As shown in **Table 1**, our findings demonstrate the expected age-related increase in NfL levels, with slightly lower values in older groups than Khalil *et al* (2020). In contrast, Harp *et al* (2020) reported lower and less dispersed values, while Vermunt *et al* (2022) also found lower levels, particularly in younger individuals. Simrén *et al* (2022) showed slightly higher mean values in participants over 70, though their study lacked our percentile-based detail.

Consistent with our findings, the annual increase in sNfL levels is most pronounced in individuals over 60 years of age.[6] Moreover, older individuals exhibit greater variability in sNfL levels compared to younger groups,[6] reflecting significant inter-individual differences that must be considered when interpreting these concentrations. This variability is particularly crucial when comparing sNfL levels in elderly patients with age-matched healthy controls.[2] While normal aging is associated with neuronal loss and/or decreased cerebrospinal fluid turnover,[7] the higher prevalence of comorbidities in the elderly may also contribute to the observed rise in sNfL, suggesting that aging and comorbidities play a role in its increase.[10]

Strengths of this study include the report of age-stratified reference values of sNfL in Portuguese individuals across a broad age range, which is particularly valuable for diagnosing the presence of neuropathological processes and monitoring treatment efficacy. Our results highlight the importance of age-stratified interpretation in the clinical context, given the large dynamic range of the assay.

This study has limitations. The study was conducted in a single center, comprising individuals from the central region of Portugal only, which may limit its generalizability to other populations. Although the convenience sampling strategy could introduce selection bias, the recruitment was carried out in a regional reference center serving both urban and rural areas, which helped capture partici-

pants from diverse socioeconomic and educational backgrounds. Cognitive screening was based on the MMSE, which, although less sensitive to subtle impairments, is widely validated and particularly suitable for populations with low literacy levels in the elderly, as observed in Central Portugal.[5] In addition, neuroimaging data were not available to explore correlations between sNfL levels and structural brain measures; however, when accessible in the clinical setting, no evidence of significant structural abnormalities was reported. Moreover, clinical and physiological data such as body mass index (BMI), renal function, and other comorbidities (e.g., diabetes, smoking history) were not systematically recorded, as the study was designed to establish normative reference ranges in cognitively healthy adults rather than to model clinical predictors of sNfL. Although BMI and renal function have been associated with sNfL concentrations,[9,11] their influence appears to be modest compared to the effect of age. Polymeris *et al* (2022) reported an inverse association between BMI and sNfL, possibly reflecting a dilution effect due to larger blood volume. In contrast, Fitzgerald *et al* (2022) observed that the association was most pronounced in underweight individuals (≤ 1% of the variability). Renal function, typically estimated by estimated glomerular filtration rate (eGFR), also influences sNfL levels, but mainly in individuals with chronic kidney disease.[12] As our cohort consisted of cognitively unimpaired and healthy adults, these factors are unlikely to have substantially affected our findings.

Conclusion

In conclusion, we established age-stratified reference values for sNfL across a broad age range, providing a unique national normative baseline for the Portuguese population. ■

Contributorship Statement / Declaração de Contribuição

ASS: Performed biomarker quantification, the statistical analysis, and wrote the manuscript.

ML and SF: Contributed to data collection.

MJL: Contributed to sample collection and preparation, and biomarker analysis.

MTP, CB, and JD: Contributed to clinical assessment, sample, and data collection.

IS: Made substantial contributions to the study concept and design, coordinated clinical assessments, and critically reviewed the manuscript.

IB: Conceptualized the study, oversaw biomarker analysis, and wrote and revised the manuscript.

All authors revised the manuscript for important intellectual concepts and gave final approval for the publication of the version.

ASS: Realizou a quantificação dos biomarcadores, a análise estatística e redigiu o manuscrito.

ML e SF: Contribuíram para a recolha de dados.

MJL: Contribuiu para a recolha e preparação das amostras, bem como para a análise dos biomarcadores.

MTP, CB e JD: Contribuíram para a avaliação clínica e para a recolha de amostras e dados.

IS: Deu um contributo substancial para o conceito e desenho do estudo, coordenou as avaliações clínicas e fez a revisão crítica do manuscrito.

IB: Concebeu o estudo, supervisionou a análise dos biomarcadores e redigiu e reviu o manuscrito.

Todos os autores reviram o manuscrito quanto a aspetos intelectuais relevantes e deram a aprovação final para a publicação da versão.

Responsabilidades Éticas

Conflitos de Interesse: Os autores declaram a inexistência de conflitos de interesse na realização do presente trabalho.

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Confidencialidade dos Dados: Os autores declaram ter seguido os protocolos da sua instituição acerca da publicação dos dados de doentes.

Proteção de Pessoas e Animais: Os autores declaram que os procedimentos seguidos estavam de acordo com os regulamentos estabelecidos pela Comissão de Ética responsável e de acordo com a Declaração de Helsínquia revista em 2024 e da Associação Médica Mundial.

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Ethical Disclosures

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Confidentiality of Data: The authors declare that they have followed the protocols of their work center on the publication of patient data.

Protection of Human and Animal Subjects: The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and those of the Code of Ethics of the World Medical Association (Declaration of Helsinki as revised in 2024).

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