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Diagnostic and prognostic performance and longitudinal changes in plasma neurofilament light chain concentrations in adults with Down syndrome: a cohort study

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Contributors statement:

MCI, DA, and JF conceived and designed the study.

MCI and JF coordinated the study.

MCI, IB, LV, LM, KvP, FS, DL, LK, GJ, SS, CM, SP, RH, IC, GN, JL, SZ, AS, ASR, EH, and JF acquired the data.

MCI, DA, JF analysed and interpreted the data.

DA and JF did the statistical analysis.

MCI, DA, and JF drafted the manuscript.

All authors revised the manuscript for important intellectual content and edited the manuscript.

MCI and JF, as corresponding authors, have accessed and verified the data and are responsible for the decision to submit the manuscript.

MCI and JF confirm that all authors have seen and approved the final text.

Data sharing

We would consider sharing de-identified, individual participant-level data that underlie the results reported in this Article. Data will be available with the publication of our main manuscript on receipt of a request detailing the study hypothesis and statistical analysis plan. All requests should be sent to the corresponding authors and they will contact with the responsible for each participating center. The steering committee of this study will discuss all requests and decide on the basis of the novelty and scientific rigor of the proposal whether data sharing is appropriate. All applicants are asked to sign a data Access agreement.

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Summary

Background—We sought to validate the clinical utility of plasma Neurofilament light (NfL), its prognostic value, and the longitudinal changes in a multicentre cohort of adults with Down syndrome.

Methods—We included adults with Down syndrome with longitudinal follow-up and at least two plasma samples from six centres. Participants were classified as asymptomatic, prodromal Alzheimer's disease, or Alzheimer's disease dementia, blind to biomarker data. We classified as "Progressors" those individuals that progressed along the Alzheimer's continuum during the follow up. Plasma NfL levels were measured using commercial kits for the Simoa SR-X™. We performed ANOVA to evaluate differences in baseline NfL levels, Cox regression to study their prognostic value, and linear mixed models to estimate longitudinal changes.

Findings—We analysed 572 samples from 226 participants with Down syndrome (165 asymptomatic (70%), 32 prodromal Alzheimer's (14%), and 29 Alzheimer's dementia (12%)). Mean follow-up was 3·6 (SD 1·6) years. Baseline plasma NfL levels showed an area under the ROC curve of 0·83 (95%CI 0·76–0·91) and 0·94 (95%CI 0·90–0·97) in differentiating asymptomatic participants from those in the prodromal and dementia groups, respectively. An increase in 1pg/ml in baseline NfL levels was associated to 1·04-fold risk of clinical progression (95%CI 1·02–1·07). Plasma NfL adjusted levels remained stable in non-progressors, but they showed an annual increase of 2·3 pg/ml (0·8–3·9) in the group of asymptomatic progressors, 3·3 pg/ml (1·6–4·9) in prodromal Alzheimer's disease progressors, and 6·5 pg/ml (3·2–9·8 pg/ml) in participants with Alzheimer's disease dementia.

Interpretation—Plasma NfL levels have excellent diagnostic and prognostic performance for the diagnosis of symptomatic Alzheimer in Down syndrome. The longitudinal trajectory of plasma NfL enables its use as a theragnostic marker in clinical trials.

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1. Background

Trisomy 21 is the most common genetic cause of intellectual disability, affecting 5.8 million people worldwide.[1] Due to the extra copy of the amyloid precursor protein gene caused by trisomy of chromosome 21, nearly all adults with Down syndrome have Alzheimer's disease neuropathology in their forties, and have an ultra-high lifetime risk of developing symptoms of Alzheimer's disease.[2–4]

The diagnosis of symptomatic Alzheimer's disease in Down syndrome is difficult, mainly because of the variability in cognitive performance, due in part to level of intellectual disability, and the lack of validated standardised assessment tools specifically designed for this population. However, core cerebrospinal fluid Alzheimer's disease biomarkers (e.g. Aβ42, total tau, 181-phospho-tau) have proven to be useful in the diagnosis of prodromal Alzheimer's disease and Alzheimer's disease dementia in the general population[5] and in Down syndrome.[6] A diagnostic blood-based biomarker would therefore have clear advantages, particularly for people with Down syndrome, where acquiring cerebrospinal fluid samples can be a challenge. Neurofilament light (NfL) is a scaffolding cytoskeleton protein of myelinated subcortical axons that can be reliably measured in plasma through single molecule array (SIMOA).[7] Although NfL is a non-specific biomarker of axonal damage,[8] in the context of Down syndrome, NfL levels may indeed be also specific for the diagnosis of symptomatic Alzheimer's disease, as in these early onset forms, alternative diagnosis affecting NfL levels are exceedingly rare.[6] NfL is a treatment-sensitive biomarker in some neurodegenerative diseases.[9,10] NfL levels can predict disease progression and brain neurodegeneration in preclinical sporadic and autosomal dominant Alzheimer's disease.[11–14] In Down syndrome, plasma NfL levels correlate in cross-sectional studies with cerebrospinal fluid levels of total and 181-phospho-tau, and also with cognitive performance.[6,15,16] High NfL levels also predict worse adaptive behaviour scores at one year.[17] However, the prognostic value along the Alzheimer's disease continuum or the longitudinal changes in plasma NfL levels have not been assessed in Down syndrome.

Leveraging a large multicentre cohort of adults with Down syndrome, this collaborative effort aimed to: (1) validate the diagnostic performance of plasma NfL levels to diagnose symptomatic Alzheimer's disease in Down syndrome in a multicentre cohort; (2) assess the prognostic performance of plasma NfL levels; and (3) determine the longitudinal trajectory of plasma NfL levels along the Alzheimer's disease continuum in Down syndrome. This information is essential to improve diagnostic accuracy for Alzheimer's disease in Down syndrome and to implement plasma NfL as an outcome measure in Alzheimer's disease clinical trials in Down syndrome.

2. Methods

2.1. Study participants

We included adults with Down syndrome above 18 years of age evaluated between August 2nd 2010 and July 16th 2019 in six different centres: Hospital de la Santa Creu i Sant Pau (Spain), University of Kentucky (USA), Institute Jerôme Lejeune (France), King's College London (United Kingdom), University of Cambridge (United Kingdom), and Ludwig-Maximilians-University of Munich (Germany). All participants were included if they provided at least two plasma samples for this study. Following the recommendations of the National Task Group on Intellectual Disabilities and Dementia Practices Consensus Recommendations for the Evaluation and Management of Dementia in Adults With Intellectual Disabilities,[18] clinical dementia status was determined individually for each participant in a Consensus Case Conference.[4,19,20] These discussions included at least two clinicians with longstanding expertise in evaluating dementia in the DS population and included the review of (1) the medical and psychiatric history as well as findings from the neurological exam, (2) informant interviews, and (3) the participant's performance in the neuropsychological evaluation taking into consideration the participants' baseline IQ, medical and psychiatric conditions, and any major life events.[19] Importantly, the clinicians were masked to biomarker data.

The participants with Down syndrome were classified into the following groups: asymptomatic, in those with no clinical or neuropsychological suspicion of Alzheimer's disease; prodromal Alzheimer's disease, in those for whom there was a suspicion of Alzheimer's disease, but symptoms did not fulfil criteria for dementia; or Down syndrome with Alzheimer's disease dementia in those with full blown dementia. Functional status to differentiate prodromal Alzheimer's disease and Alzheimer's disease dementia diagnoses was assessed on the basis of anamnesis, and with the support of validated questionnaires (see supplementary material for the questionnaires administered at each site) to differentiate decline due to cognitive impairment from pre-existing intellectual disability, placing a particular emphasis on establishing change from the individual's best level of functioning.[4,19,20] These procedures were performed at each clinical visit. Progression was defined as change in the clinical status of the participants in a follow up visit.

Those participants who had significant medical, pharmacological, or psychiatric conditions considered likely to be interfering in cognition and/or in functional levels were classified as "Uncertain" and excluded from the study. Functional status to differentiate prodromal and dementia groups was assessed based on clinical interview aimed to detect functional decline due to cognitive impairment taking into account the level of intellectual disability, placing a particular emphasis on establishing change from the individuals' best level of functioning. Clinical classification of participants was blinded to biomarker results, and specifically to NfL levels. For prognostic evaluation, asymptomatic and prodromal participants were subsequently classified as "Progressors" when there was clinical progression along the AD continuum or death due to AD. Those participants that remained in the same diagnostic category at the end of follow-up were classified as "Non-progressors".

2.2. Cognitive evaluation

Level of intellectual disability was categorized as mild, moderate, severe, or profound according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, based on care-givers' reports of the individuals' best-ever level of functioning. Due to the low number of participants with severe and profound intellectual disability, these two categories were merged for all analyses.

Cognitive assessment included a neurological and neuropsychological examination covering several cognitive domains. Details for cognitive tests at each participating centre are detailed in the Supplementary Material.

2.3. Standard approvals and participant consents

All participants or their legal guardians gave written consent or assent, and the local ethics committee of each centre approved all procedures included in this study.

2.4. Collection of samples and plasma analysis

After blood collection, all samples were transferred to each local laboratory where they were centrifuged, aliquoted and frozen at −80°C after extraction, following international recommendations. *APOE* genotype was determined at each centre. Plasma samples were shipped in dry ice to the laboratory in Hospital Sant Pau (Barcelona, Spain) where they were stored at −80°C until analysis.

Levels of plasma NfL were centrally measured in Hospital Sant Pau using the ultrasensitive equipment Simoa SR-X™ (Quanterix). All samples were measured in duplicate, and within one round of experiments between August and September 2019 using commercially available kits (NF-light™, Quanterix). Intra- and inter-assay coefficients of variation were 3·4% and 16·7%, respectively. Baseline and longitudinal samples obtained from each participant were measured side by side in the same run to avoid the effect of run-to-run variability. All analyses were performed by one technician, who was blind to clinical diagnosis. A subset of samples from this study had been previously analysed in a Simoa HD-1™ equipment (Montpellier, France). There was a high correlation between both assays (R^2=0·94, see Supplementary material **and supplementary figure 4**).

2.5. Statistical analysis

Levels of plasma NfL were log-transformed to attain a normal distribution. We used analysis of variance to compare baseline ages between groups and Chi-square test to compare the proportion of sex (male or female), intellectual disability and *APOE*-ε4 status across diagnostic categories. The association of plasma NfL levels with baseline age, sex, and intellectual disability was assessed by analysis of covariance in the group of asymptomatic non-progressors.

Down syndrome is a genetically determined form of Alzheimer's disease.[21] Thus, age is intrinsically and robustly linked to the development of symptomatic Alzheimer's disease.[4] For this reason, age was included as a covariate together with sex and intellectual disability in all the analysis throughout the manuscript. However, as this approach could potentially

obscure the relationships between these two intrinsically linked variables, we confirmed the analysis following an alternative approach. We calculated W-scores applying a linear model in the asymptomatic non-progressors including age, sex and the level of intellectual disability. Using this model, W-score-adjusted plasma NfL levels were calculated in the whole sample as the difference between measured levels and predicted levels. Results based on W-score-adjusted values and also the analyses based in raw NfL values are available as Supplementary Material.

We performed receiver operating characteristic (ROC) analysis for baseline plasma NfL levels to calculate areas under the curve with 95% confidence intervals,[22] and we selected cut-off values that maximized the Youden J index (sensitivity + specificity − 1). Clinical progression and its association with baseline plasma NfL levels were assessed by modelling Kaplan-Meier curves and multi-variable Cox regression analysis. Longitudinal changes in plasma NfL levels and their association with clinical progression status were assessed through linear mixed models. The initial model included baseline NfL levels, diagnostic category, age, sex, intellectual disability, and time from baseline sample (years) including its interaction with diagnostic category as fixed effects. We included a random intercept for centre and for assay run to account for inter-centre and inter-run variability, respectively. Random intercepts and slopes were defined at the participant level to account for repeated measures. Outliers were detected by visual inspection of their influence on the residuals. We used backward selection to choose the final model.

We used packages “car” (v.3.0–7), “pROC” (v.1.16.2), “survival” (v.3.1–12), “survminer” (v.0.4.6), “nlme” (v.3.1–147), “multcomp” (v.1.4–13), “ggplot2” (v.3.3.0) and “ggpubr” (v.0.3.0), as implemented in R statistical software (v 3.6.2) for plots and statistical analysis (references in supplemental material).

2.6. Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

We analysed 608 plasma samples from 236 participants from six different centres in Europe and the US. The clinical diagnoses were asymptomatic Down syndrome (n=165, 70%), prodromal Alzheimer’s disease (n=32, 14%) and Alzheimer’s disease dementia (n=29, 12%). Participants classified as “uncertain” (n=10, 4%) were excluded from the study (see Supplementary Material for more details on this group). Table 1 displays demographic, clinical, and biomarker variables across groups by baseline diagnosis and by clinical progression. Asymptomatic participants were significantly younger than those in the prodromal Alzheimer’s disease and Alzheimer’s disease dementia groups. Within the asymptomatic group, those who remained stable during follow-up were younger than those who showed clinical progression. Participants were followed up for 3·6 years (range 0·6 to 9·2 years), although follow-up time was shorter in the Alzheimer’s disease dementia

group compared to asymptomatic participants. There were no significant differences in the distribution of sex, intellectual disability, or *APOE*-ε4 status across groups.

To assess the influence of demographics and level of intellectual disability on NfL levels, we performed analysis in the asymptomatic non-progressors group. We found significant associations of age, sex, and intellectual disability with baseline plasma NfL levels. An increase of 1 year in baseline age was associated with a 3·8% increase in plasma NfL levels ($p<0·001$), males showed 14·8% lower levels of plasma NfL compared to females ($p=0·02$), and there was a linear association between plasma NfL and level of intellectual disability ($\beta=1·16$, $p=0·049$). To account for these potential confounders, we included age, sex and intellectual disability as covariates in the analysis. We repeated the analysis with W-score-adjusted levels that were calculated as the difference between actual measured levels and the predicted levels estimated from a linear model in the asymptomatic non-progressors group where age together with sex and intellectual disability were considered. The analysis of W-score-adjusted levels and that of raw values can be found in Supplementary Material.

Figure 1 displays the levels of baseline plasma NfL levels across diagnostic categories. After adjusting for age, sex and intellectual disability, levels of NfL were 79% higher ($p<0·001$) in dementia and 40% higher ($p<0·001$) in prodromal groups compared to those in the asymptomatic group (Figure 1A). The effect was similar when participants in the asymptomatic and prodromal Alzheimer's disease groups were subclassified as "Progressors" and "Non-progressors" (Figure 1B). Similar differences were found when raw NfL levels or adjusted NfL levels were compared (Supplementary Material).

We used ROC analysis to evaluate the diagnostic performance of baseline plasma NfL levels. As shown in Figure 2, baseline plasma NfL levels showed an AUC of 0·83 (95%CI 0·76–0·91) to differentiate asymptomatic participants from those in the prodromal group. This value increased to 0·94 (95%CI 0·90–0·97) in the discrimination between asymptomatic and dementia groups. Overall, plasma NfL levels showed an accuracy of 0·88 (95%CI 0·83–0·93) to distinguish asymptomatic from symptomatic participants (prodromal and dementia combined). Two cut-off values, 13pg/ml and 18·7pg/ml, yielded identical maximum Youden indices to discriminate between asymptomatic and prodromal groups showing sensitivities of 0·81 and 0·66, and specificities of 0·74 and 0·90, respectively. A cut-off value of 17pg/ml distinguished asymptomatic from dementia groups with a sensitivity of 0·90 and specificity of 0·86. Two cut-off values, 13pg/ml and 16·9pg/ml, were also found to yield the optimal balance between sensitivity and specificity to discriminate asymptomatic from symptomatic participants (prodromal and dementia combined).

We analysed the association of baseline plasma NfL levels with clinical progression along the Alzheimer's disease continuum. Fifty-four (27%) out of 197 participants without dementia (asymptomatic and prodromal groups) changed the clinical diagnosis during follow-up. As represented in Kaplan-Meier curves (Figures 3A and 3B), the whole sample had a median time to progression of 6·7 (IQR 4·2) years, shorter in the prodromal group (2 years) than in the asymptomatic group (7·9 years; $p<0·001$). We studied the association between baseline plasma NfL levels and the risk of progression through a multi-variable Cox regression analysis. Including age, sex, intellectual disability as covariates, and

diagnosis as categorical predictor, an increase in 1pg/ml in baseline NfL adjusted levels was associated to a 1·04-fold risk of clinical progression (95%CI 1·01–1·07; p=0.003). For graphical representation of the adjusted Cox curves (Figures 3C and 3D), participants were categorized into three tertiles according to their baseline plasma NfL levels and using the aDS group tertile cutoffs.

We performed linear mixed-model analysis to compare longitudinal changes in plasma NfL levels between diagnostic categories and to evaluate the association of these changes with clinical progression.

As displayed in Figure 4, we found that changes in longitudinal levels of plasma NfL differed between clinical categories and progression status (p<0·001). Plasma NfL levels showed an annual increase of 3% per year (0·4–5·8%) in the group of asymptomatic non-progressors, not significantly different from that of prodromal Alzheimer's disease non-progressors. However, we found an increase of 11·5% per year (4·9–18·5%, p=0·02) in the group of asymptomatic progressors and of 16% per year (8·4–2·4%, p=0·001) in prodromal Alzheimer's disease progressors. In participants with Alzheimer's disease dementia, NfL levels increased in average 24·3% per year (15·3–34·1, p<0·001). We found similar trajectories when the analysis was performed using W-score-adjusted values (Supplementary Material).

Discussion

This longitudinal study of a large multicentre cohort of people with Down syndrome confirms that plasma NfL levels are a useful biomarker for the diagnosis of symptomatic Alzheimer's disease in Down syndrome and have good prognostic performance. Moreover, the characterization of longitudinal trajectories of NfL in plasma showed that the rate of change in plasma NfL levels sharply increased along the Alzheimer's disease continuum. These longitudinal changes, which did not seem to plateau along the Alzheimer's disease continuum, posit plasma NfL levels as a particularly suitable biomarker for dementia diagnosis and as a surrogate marker of efficacy in clinical trials for Alzheimer disease in Down syndrome.

The positive association of plasma NfL levels with age is consistently found in sporadic Alzheimer's disease,[7,23–25] autosomal dominant Alzheimer's disease,[11,14] and Down syndrome.[6,15,26] In this respect, in a large multimodal biomarker study of the natural history of Alzheimer's disease in adults with Down syndrome, we found that plasma NfL levels differ from non-trisomic controls at age 30, 20 years before symptomatic Alzheimer's disease,[4] We also found differences in relation to the level of intellectual disability such that more severe or profound disability is associated with higher levels of NfL. We believe that this may relate to the difficulties derived from the clinical assessment of individuals with severe and profound intellectual disability, which might delay their Alzheimer's disease diagnosis. In sporadic Alzheimer's disease, no association of NfL levels with educational level has been described.[27]

Baseline plasma NfL levels differed between diagnostic groups replicating previous single-centre cross-sectional studies.[4,6,26] The excellent diagnostic performance for plasma NfL in our large multicentre cohort across 5 countries and languages, using a commercially available assay, reinforces the clinical relevance of this biomarker, as it can be easily and rapidly used by multiple centres effectively. We also showed that plasma NfL levels accurately identified prodromal and dementia Alzheimer's disease patients with Down syndrome, confirming the excellent diagnostic performance of cross-sectional NfL levels to detect symptomatic Alzheimer's disease in Down syndrome.[6]

To study the prognostic performance of baseline plasma NfL levels, we classified participants as "progressors" or "non-progressors" according to changes in their clinical diagnosis during the follow up. Higher baseline NfL levels were associated with clinical progression. Previous studies in small single centre cohorts of participants with Down syndrome report that higher plasma NfL levels predicted the likelihood of dementia and were associated with decreased adaptative behaviour scores in the follow-up.[17,26] Similar results are observed in one study in sporadic Alzheimer's disease, where high plasma NfL levels are associated with longitudinal cognitive decline and Alzheimer's-related brain atrophy.[7] However, in the same study and others, baseline NfL levels did not predict whether patients with mild cognitive impairment would progress to AD dementia or remain stable.[7,28] Our findings highlight the role of baseline levels of NfL, not only as a diagnostic biomarker, but also as a prognostic marker for AD-related cognitive impairment in DS.

The understanding of the role of biomarker changes to predict clinical progression is important to monitor the effect of disease-modifying drugs in clinical trials. The longitudinal changes of plasma NfL levels were different across the clinical groups and progression status. Plasma NfL levels showed an annual increase of 3% in the asymptomatic non-progressors group, but the estimated annual increase ranged from 11·5% in those asymptomatic progressors to 24·3% in participants with Alzheimer's disease dementia. In sporadic Alzheimer's disease, greater rates of plasma NfL increases are described among people with mild cognitive impairment compared with healthy controls and among Alzheimer's dementia patients compared with controls and mild cognitive impairment.[27] In our study, the annual rate of change in NfL levels was highest in the dementia group, suggesting that it does not reach a plateau at this stage. A recent study in autosomal dominant Alzheimer's disease using serial NfL measurements found that the NfL annual rate of change distinguished mutation carriers and non-carriers almost a decade earlier than NfL levels measured at a single timepoint.[12] Similarly, in our study, although cross-sectional data did not identify asymptomatic progressors, longitudinal changes did. The increase in longitudinal plasma levels in Down syndrome is in contrast with flattening of the curve of estimated annual increases that has been described in longitudinal studies measuring CSF total tau and 181-phospho-tau levels in autosomal dominant and sporadic Alzheimer's disease.[24,29–31] However, this finding is in agreement with the acceleration in the atrophy rates found in MRI along the AD continuum.[32–34] Future studies should further investigate the relationship between atrophy rates and NfL changes. Thus, the increase in the annual change along the Alzheimer's continuum in Down syndrome, without evidence for a plateau, facilitates the modelling and power analysis for the use of NfL levels as a surrogate marker of efficacy in clinical trials. The advantages in identifying surrogate biomarkers

in blood are evident. Plasma NfL levels are an easily accessible and inexpensive tool compared to others currently used, such as lumbar punctures, PET scans, or centre-specific neuropsychological assessments.

The major strength of our study is that we determined plasma NfL levels in a large well-characterized multicentre population of participants with Down syndrome, to our knowledge the largest to date, with a centralized analysis. However, our study has also some limitations. The clinical diagnosis of cognitive decline in Down syndrome, especially in the prodromal stages, is particularly challenging. This adds to the difficulty in assessing intellectual disability homogeneously across centres. Formal evaluations of intellectual disability show floor effects and might be impacted by the AD cognitive decline, making them unreliable in symptomatic patients. The heterogeneity in the cognitive evaluation protocols between different sites does not allow for thorough examination of associations between plasma NfL levels and cognitive measures. Instead, we have used the clinical diagnosis, which has been performed by expert consensus at each site and blind to biomarker results. The advantage of such a strategy is that it supports the external validity and generalizability of our results as the diagnosis of prodromal Alzheimer's disease or Alzheimer's dementia in Down syndrome, which is still based on clinical consensus, and not on specific sets of cognitive testing scores.[18] Another limitation is the relatively short follow-up time, but we would like to note that even in this short follow-up time, we were able to detect relevant differences. Furthermore, the clinical follow-up of the participants in this study is still active at each centre, and the next few years will certainly provide additional and more accurate prognostic results. Finally, as our study lacked additional biochemical and structural AD biomarkers, we could not analyse the relationship between markers of different pathophysiological processes, which should be considered in future studies.

In summary, our study confirms the clinical utility of plasma NfL for the diagnosis and prognosis of symptomatic Alzheimer in Down syndrome. The increases in the annual rates of change along the AD continuum enable the use of plasma NfL as a theragnostic marker in clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research in context

Evidence before this study

We searched and reviewed the literature using PubMed, meeting abstracts and presentations. We searched PubMed on June 30, 2020, for research studies that examined neurofilament light chain (NfL) in people with Down syndrome using the search terms “neurofilament light” OR “plasma biomarker” AND “Down syndrome” AND “Alzheimer” OR “dementia”. Eight studies had previously assessed plasma NfL levels in Down syndrome, however, none of them studied the prognostic performance and the longitudinal trajectories of plasma NfL in this population.

Added value of this study

This is the first study to assess the prognostic performance of plasma NfL levels to detect prodromal and Alzheimer’s disease dementia in people with Down syndrome and to describe the longitudinal trajectory of plasma NfL along the Alzheimer’s disease continuum in Down syndrome. In addition, this study replicates and confirms the good diagnostic performance of plasma NfL in a multicentre sample.

Implications of all the available evidence

Our multicentre study has immediate implications in clinical practice as it shows the clinical utility of plasma NfL for the diagnosis and prognosis of symptomatic Alzheimer’s disease in Down syndrome. In addition, the longitudinal trajectory of plasma NfL, with increasing rates of change along the Alzheimer’s disease continuum, enables its use as a theragnostic marker in clinical trials.

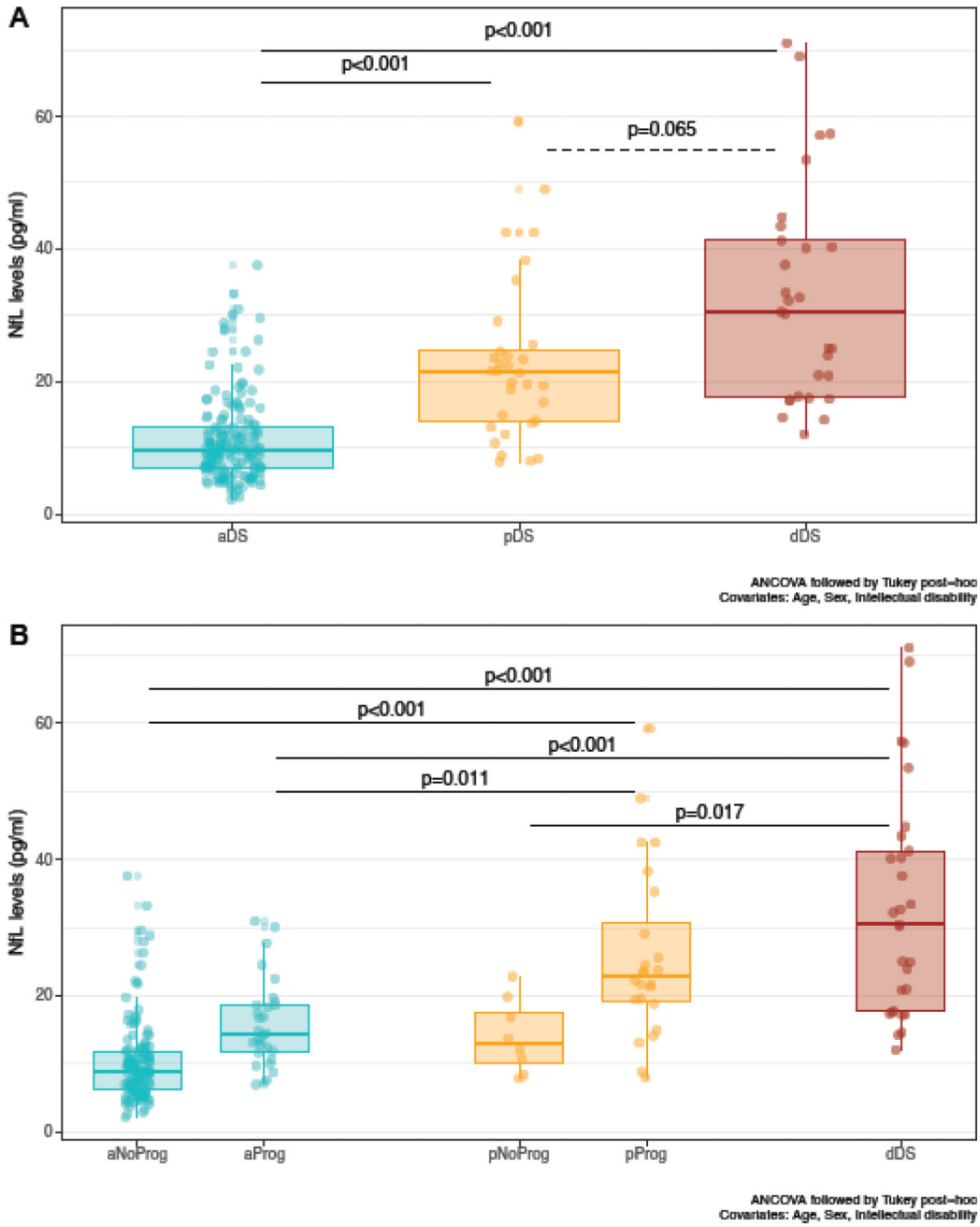

Figure 1. Baseline plasma NfL levels across baseline diagnostic categories (A) and across diagnostic categories considering clinical progression during follow-up (B).

Age, sex and intellectual disability were included as covariates in the analysis. Only statistically significant associations are shown.

aDS: asymptomatic Down syndrome; pDS: prodromal Alzheimer's disease in Down syndrome; dDS: Alzheimer's disease dementia in Down syndrome. aNoProg: asymptomatic Down syndrome, non-progressor; aProg: asymptomatic Down syndrome, progressor; pNoProg; prodromal Alzheimer's disease in Down syndrome, non-progressor; pProg: prodromal Alzheimer's disease in Down syndrome, progressor.

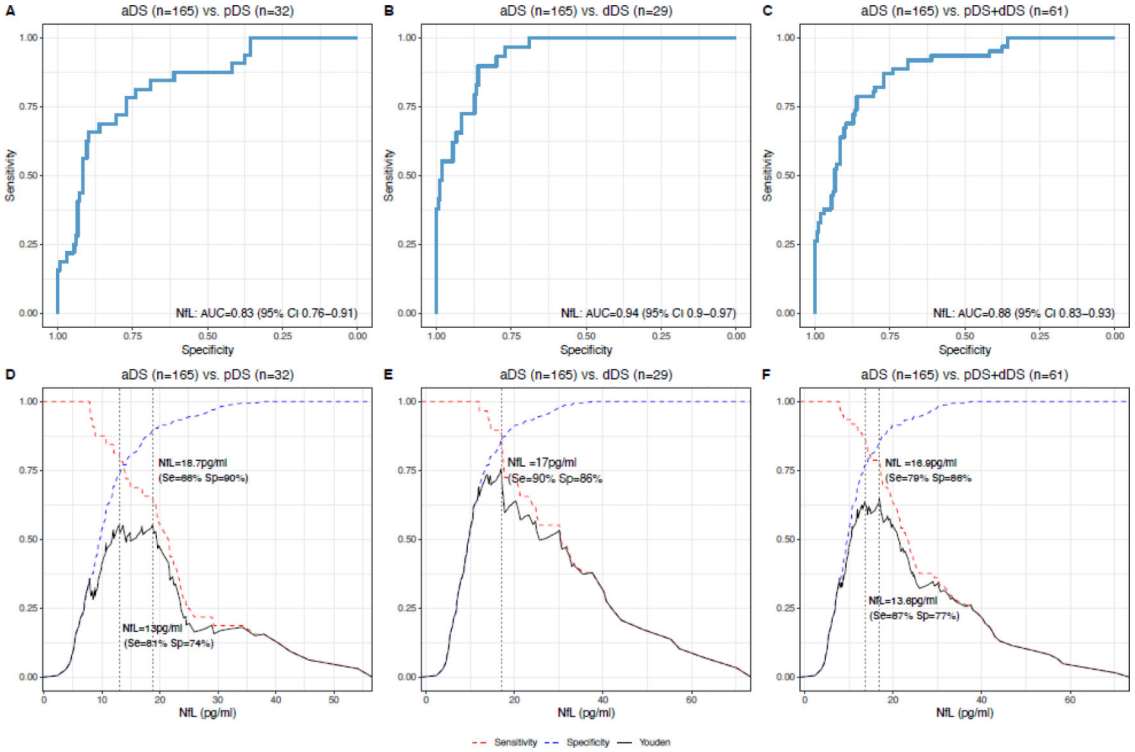

Figure 2. Receiver operating characteristic (ROC) analysis of baseline plasma NfL levels for the discrimination between asymptomatic and symptomatic participants

aDS: asymptomatic Down syndrome; pDS: prodromal Alzheimer's disease in Down syndrome; dDS: Alzheimer's disease dementia in Down syndrome; AUC: area under the curve; CI: confidence interval; Se: sensitivity; Sp: specificity, NfL: baseline plasma NfL levels.

Vertical dashed lines in panels D, E and F indicate cut-off values that yielded an optimal balance between sensitivity and specificity (maximum Youden J index). When two cut-off values yielded the same Youden J indices both values are indicated (panels D and F).

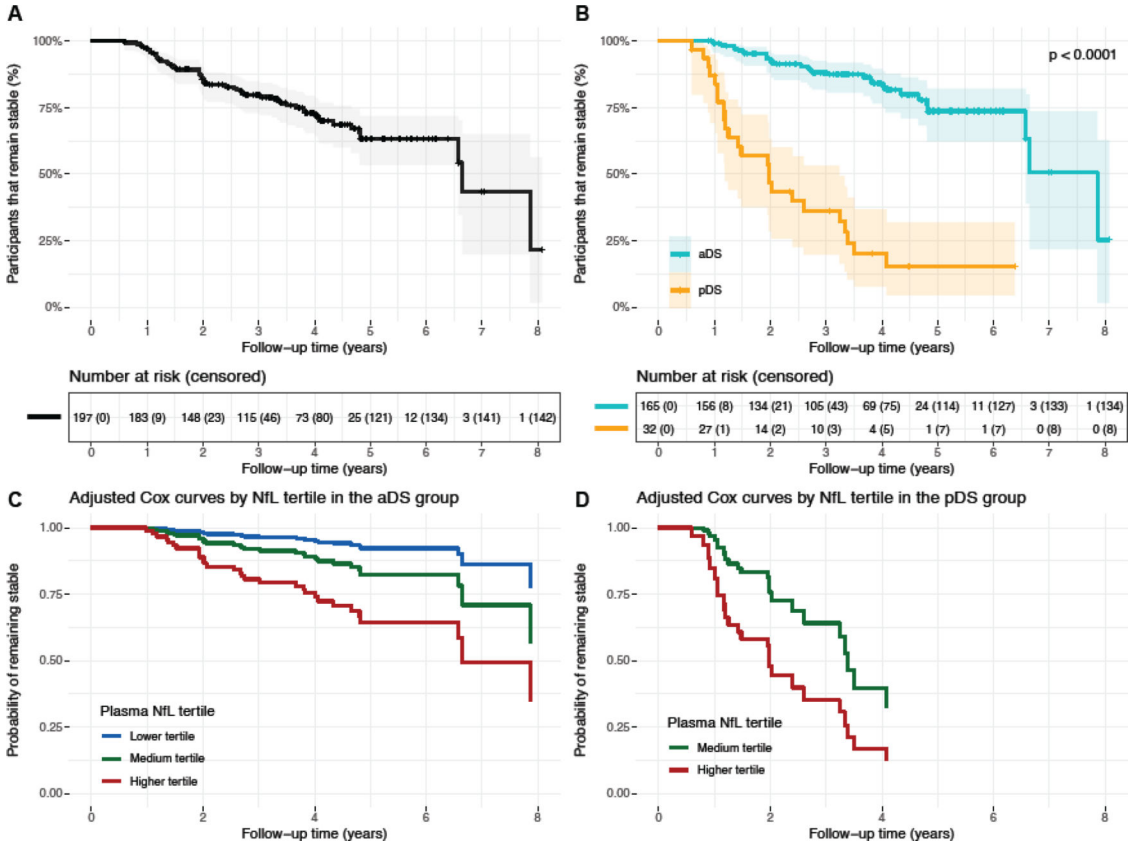

Figure 3. Clinical progression of participants without dementia and association with baseline NfL levels

Panels A and B show Kaplan-Meier curves in all participants and by clinical diagnosis, respectively. Panels C and D represent the predicted progression through adjusted Cox curves for participants in the aDS group (C) and in the pDS group (D) according to baseline NfL levels.

aDS: asymptomatic Down syndrome; pDS: prodromal Alzheimer's disease in Down syndrome.

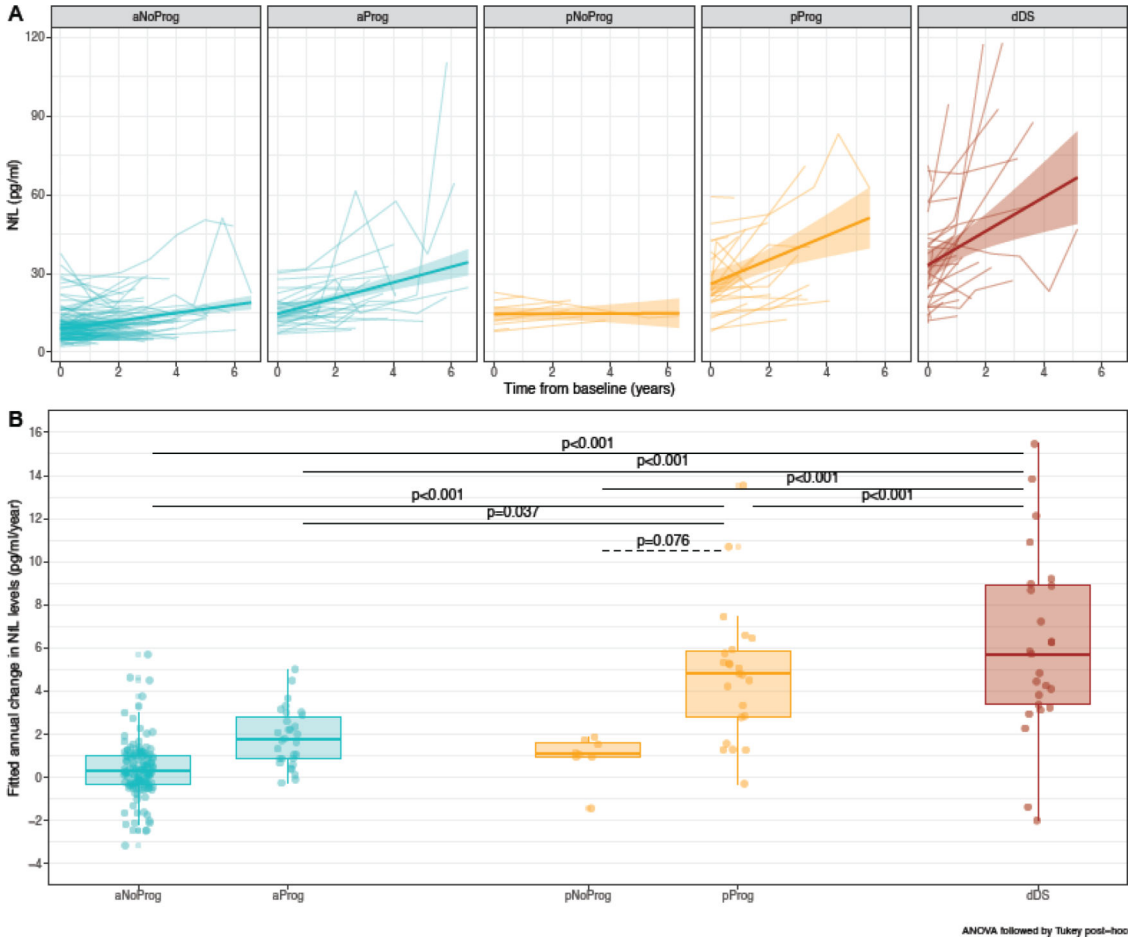

Figure 4. Trajectories (A) and estimation of the annual increase (B) in plasma NfL levels across diagnostic categories.

aNoProg: asymptomatic Down syndrome, non-progressor; aProg: asymptomatic Down syndrome, progressor; pNoProg; prodromal Alzheimer's disease in Down syndrome, non-progressor; pProg: prodromal Alzheimer's disease in Down syndrome, progressor; dDS: Alzheimer's disease dementia in Down syndrome.

Table 1.
Demographics, clinical, and biomarker variables by diagnostic categories

Unless otherwise specified, values are expressed as mean (standard deviation).

	By baseline diagnosis				By clinical progression				
	aDS	**pDS**	**dDS**	**P value**	**aNoProg**	**aProg**	**pNoProg**	**pProg**	**P value**
Number of participants-baseline samples (%)	165 (70%)	32 (14%)	29 (12%)	N.A.	135 (57%)	30 (13%)	8 (3%)	24 (10%)	N.A.
Number of longitudinal samples (%)	263 (43%)	43 (7%)	40 (7%)	N.A.	206 (34%)	57 (9%)	9 (2%)	34 (6%)	N.A.
Age, years	38·9 (9·7)	50·6 (5·5)	53·3 (5)	<0·001[a]	36·7 (8·7)	48·9 (7·5)	46·4 (3·4)	52 (5·4)	<0·001
Sex, Female/Male (% Female)	75/90 (45%)	14/18 (44%)	13/16 (45%)	0·98[b]	62/73 (46%)	13/17 (43%)	2/6 (25%)	12/12 (50%)	0·81[b]
Number of participants with intellectual disability (%)									
Mild	49 (30%)	12 (38%)	5 (17%)	0·31[b]	41 (30%)	8 (27%)	3 (38%)	9 (38%)	0·69[b]
Moderate	89 (54%)	18 (56%)	18 (62%)		71 (53%)	18 (60%)	4 (50%)	14 (58%)	
Severe/profound	26 (16%)	2 (6%)	6 (21%)		22 (16%)	4 (13%)	1 (2%)	1 (4%)	
Follow-up time, years	3·8 (1·7)	3·2 (16)	2·8 (1·4)	<0·007[a]	3·6 (1·5)	4·4 (2·1)	3·3 (19)	3·2 (1·5)	0·031[a]
Plasma NfL levels, pg/ml	11·2 (6·5)	22·9 (12·4)	33 (16·5)	<0 001[a]	10·2 (6)	15·8 (6·4)	14 (5·4)	25·8 (12·7)	<0·001[a]
Plasma NfL adjusted levels, pg/ml*	0·8 (4·7)	8·1 (11·2)	16·3 (166)	<0·001[a]†	0·8 (4·5)	1·3 (5·4)	1·6 (66)	10·3 (11·7)	<0·001[a]
APOE-ε4+/APOE-ε4-(% APOE-ε4+)	27/103 (21%)	6/20 (23%)	6/19 (24%)	0·92[b]	21/82 (20%)	6/21 (22%)	2/6 (25%)	4/14 (22%)	0·99[b]

aDS: asymptomatic Down syndrome; pDS: prodromal Alzheimer's disease in Down syndrome; dDS: Alzheimer's disease dementia in Down syndrome; aNoProg: asymptomatic Down syndrome, non-progressor; aProg: asymptomatic Down syndrome, progressor; pNoProg; prodromal Alzheimer's disease in Down syndrome, non-progressor; pProg: prodromal Alzheimer's disease in Down syndrome, progressor; N.A.: not applicable·

[a] Analysis of Variance

[b] Chi-squared test

* Plasma NfL adjusted levels were calculated as the difference between measured levels and the predicted levels estimated from a linear model in the aDS non-progressors group where age together with sex and intellectual disability were considered·