Association of Neurofilament light chain, [^{18}F]PI-2620 tau-PET, TSPO-PET and clinical progression in patients with amyloid-β-negative CBS

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Abstract

Background and Objectives

Corticobasal syndrome (CBS) with underlying 4-repeat tauopathy is a progressive neurodegenerative disease characterized by declining cognitive and motor functions. Biomarkers for assessing pathological brain changes in CBS including tau-PET, TSPO-PET, structural MRI, neurofilament light chain (NfL) or glial fibrillary acidic protein (GFAP) have recently been evaluated for differential diagnosis and disease staging, yet their association with disease trajectories remains unclear. Therefore, we performed a head-to-head comparison of neuroimaging (tau-PET, TSPO-PET, structural MRI) and plasma biomarkers (NfL, GFAP) as prognostic tools for longitudinal clinical trajectories in amyloid-β-negative CBS.

Methods

We included clinically diagnosed amyloid-β-negative CBS patients with clinical follow-up data who underwent baseline structural MRI and plasma-NfL analysis for assessing neurodegeneration, [^{18}F]PI-2620-PET for assessing tau pathology, [^{18}F]GE-180-PET for assessing microglia activation and plasma-GFAP analysis for assessing astrocytosis. To quantify tau and microglia load we assessed summary scores of whole-brain, cortical and subcortical PET signal. For structural MRI analysis, we quantified subcortical and cortical grey matter volume. Plasma NfL and GFAP values were assessed using Simoa-based immunoassays. Symptom progression was determined using a battery of cognitive and motor tests (i.e. Progressive Supranuclear Palsy Rating Scale, PSPRS). Using linear mixed models, we tested whether the assessed biomarkers at baseline were associated with faster symptom progression over time (i.e. time x biomarker interaction).

Results

Overall, 21 amyloid-β-negative CBS patients with ~2-year clinical follow-up data were included. CBS patients with more widespread global tau-PET signal showed faster clinical progression (PSPRS: B/SE=0.001/0.0005, p=0.025), driven by cortical rather than subcortical tau-PET. In contrast, patients with higher global [^{18}F]GE-180-PET readouts showed slower clinical progression (PSPRS: B/SE=-0.056/0.023, p=0.019). No association was found between grey matter volume and clinical progression. Concerning fluid biomarkers, only higher plasma-NfL (PSPRS: B/SE=0.176/0.046, p<0.001) but not GFAP was associated with faster clinical deterioration. In a subsequent sensitivity analysis, we found that tau-PET, TSPO-PET and plasma-NfL showed significant interaction effects with time on clinical trajectories when tested in the same model.

Discussion

[^{18}F]PI-2620 tau-PET, [^{18}F]GE-180 TSPO-PET and plasma-NfL show prognostic potential for clinical progression in amyloid-β-negative CBS patients with probable 4-repeat tauopathy, which can be useful for clinical decision making as well as stratifying patients in clinical trials.

Introduction

Corticobasal syndrome (CBS) with underlying 4-repeat (4R) tau pathology, characterized by intracellular neuronal and glial 4R tau aggregates, is a progressive neurodegenerative disorder characterized by declining cognitive and motor functions[1-3]. 4R tauopathies are subclassified mainly as Corticobasal Degeneration (CBD) or Progressive Supranuclear Palsy (PSP)[1], which most commonly manifest as atypical Parkinson syndromes corticobasal syndrome (CBS)[2, 3] or PSP Richardson syndrome (PSP-RS), depending on the expression of cortical and subcortical symptoms[3, 4]. The clinical phenotypes of 4R tauopathies are potentially driven by heterogeneous 4R tau deposition patterns, with predominant brainstem and subcortical tau accumulation and only late-stage cortical tau in PSP-RS[5], vs. more widespread cortical tau aggregation in patients presenting as CBS[6, 7]. Clinically, amyloid-β-peptide (Aβ) negative CBS can be diagnosed using the Movement Disorders Society (MDS) criteria for PSP[3], or CBD criteria[1].

Various biomarkers for assessing the underlying pathological brain changes in Aβ-negative CBS patients have recently been evaluated for differential diagnosis and disease staging, yet the prognostic accuracy of these biomarkers for predicting future disease trajectories remains unclear. The next-generation tau-PET tracer [^{18}F]PI-2620 has shown a discrimination of CBS and PSP versus healthy controls as imaging biomarker to detect 4R tauopathies *in vivo*[4-7]. The [^{18}F]PI-2620 PET signal patterns were congruent with the histopathologically expected 4R tau accumulation, showing a shift towards cortical tau in CBS[4, 6]. Microglial activation, measured with [18F]GE-180 PET tracer targeting the 18 kDa translocator protein (TSPO), has been apparent in 4R tauopathies PSP and Aβ-negative CBS[8] as well as in AD[9]. As fluid biomarker, levels of neurofilament light chain (NfL) have been identified to be a surrogate for neuroaxonal injury in various neurological diseases[10-12], besides structural MRI as an imaging-based marker of neurodegeneration. Belonging to the family of class IV intermediate filaments, NfL is a component of the neuronal cytoskeleton in both central and peripheral neurons. In case of neurodegeneration or axonal damage, NfL can be released from neurons into the cerebrospinal fluid (CSF) and blood[13]. Elevation of glial fibrillary acid protein (GFAP), a structural component of fibrillary astrocytes, has been suggested as a marker for reactive astrogliosis in blood and CSF in various neurodegenerative diseases[14]. In cohorts with clinical diagnosis of 4R tauopathies, levels of NfL and GFAP in both blood and CSF have been shown to be elevated in comparison to idiopathic Parkinson's disease and healthy controls[15-17]. High NfL levels have been associated with shorter survival in a retrospective study with PSP-RS patients[18] and with predicting disease progression in PSP[19, 20]. However, the role of NfL for predicting disease progression in CBS is still unclear. The identification of prognostic biomarkers is crucial both for patient care itself and for the development of interventional trials. To address this, we performed a head-to-head comparison of neuroimaging (i.e. tau-PET, TSPO-

PET, structural MRI) and plasma (i.e. NfL, GFAP) biomarkers to evaluate their impact on future clinical trajectories in 21 amyloid-β-negative CBS patients with clinical diagnosis of a probable 4R tauopathy[3].

Methods

Participants and Clinical Evaluation

Patients were recruited and clinically tracked at the Department of Neurology at at Ludwig-Maximilians-Universität (LMU) Munich between February 2018 and March 2022. They were diagnosed by a movement disorders specialist as PSP-CBS phenotype with probable underlying 4R tauopathy according to the MDS-PSP criteria[3]. They also fulfilled the Armstrong criteria of probable or possible CBD-CBS[1]. Inclusion criteria were i) stable pharmacotherapy for at least one week before PET examination, ii) a negative family history for Parkinson's disease, frontotemporal dementia and Alzheimer's disease (AD), iii) no severe neurological or psychiatric disorders other than CBS and iv) negative Aβ status as determined via standardized diagnostic procedures at the LMU to rule out confounding AD pathology. Specifically, negative Aβ status was determined via CSF (i.e. Aβ42/40 ratio >5.5% or $A\beta_{1-42}$>375pg/ml) or negative [^{18}F]Flutemetamol-PET visual read[5, 6, 8].

At baseline, all patients underwent detailed clinical assessment, MR imaging, blood sampling and [^{18}F]PI-2620 PET. A subset also underwent baseline [^{18}F]GE-180 TSPO-PET. Since binding properties of this TSPO tracer have been found to depend on genetic polymorphism of the TSPO gene[21], all individuals underwent rs6971 SNP genotyping like described previously[22].

Clinical assessments at baseline and follow-up were performed by a movement disorders specialist at the LMU outpatient's clinic for movement disorders. Two experts performed baseline visits, while all follow-up visits were conducted by the same movement disorders specialist to reduce inter-rater variability. All experts were specifically trained for all study procedures prior to patient inclusion. Assessments included the PSP rating scale (PSPRS)[23], Unified Parkinson's Disease Rating Scale – Motor Part (UPDRS-III) including the modified Hoehn and Yahr (H&Y) score[24], and the PSP-clinical deficits scale (PSP-CDS)[25]. Functional independence was measured using the Schwab and England activities of daily living (SEADL) scale[26]. Global cognitive status was assessed with Montreal Cognitive Assessment (MoCA) scale[27]. For follow-up visits, different versions of the MoCA were used to avoid training effects. Disease duration was classified as the time between reported symptom onset and baseline clinical assessment. For all participants, clinical diagnosis was reviewed and confirmed at follow-up visits. By the time of the data cut-off for this study, eight patients had died. Autopsies were not available.

Standard Protocol Approvals, Registrations, and Patient Consent

All patients were recruited within the Activity of Cerebral Networks, Amyloid and Microglia in Aging and Alzheimer's Disease (ActiGliA) study, a prospective cohort study of the Munich Cluster for Systems Neurology (SyNergy) at Ludwig-Maximilians-University (LMU). The study and data analyses were approved by the local ethics committee LMU (ethics-applications: 17-569, 17-755 and 19-022) and the

German radiation protection authorities (BfS-application: Z 5 − 22464/2017-047-K-G). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. Patients did not receive compensation for study participation.

Neuroimaging, Acquisition and Processing

All PET procedures, including radiochemistry, acquisition and pre-processing, were conducted using established standardized protocols[5, 22, 28-30]. All Patients were scanned at the Department of Nuclear Medicine, LMU, using a Biograph 64 PET/CT scanner or a harmonized Biograph mCT (Siemens, Erlangen, Germany). For detection of microglial activation, [^{18}F]GE-180 TSPO-PET recordings (average dose: 179 ± 13 MBq) with an emission window of 60-80 min after injection were performed[22]. Dynamic [^{18}F]PI-2620 tau-PET (average dose: 188 ± 15 MBq) with emission recording 0-60 min after injection was obtained to assess tau aggregation. Static frames of the late phase (20-40 min)[31] were reconstructed for assessing tau binding.

All PET data analyses were perfomed using PMOD (V3.9; PMOD Technologies LLC; Zurich; Switzerland). For primary analysis, static emission recordings which were coregistered to the Montreal Neurology Institute (MNI) space using non-linear warping (16 iterations, frequency cutoff 25, transient input smoothing 8x8x8mm^3) to tracer-specific templates acquired in previous in-house studies, were used[5, 22, 28, 29]. For TSPO-PET, a late-phase template, consisting of cognitively unimpaired controls with intact motor function, was used (n_t=11, 60-80 min p.i.). Tau-PET images were coregistered to a late-phase template, consisting of a mixed population of healthy controls and patients with 4R-tauopathies without distinction of tau-PET-positivity (n_t=28, 20-40 min p.i.). Intensity normalization of all PET images was assessed by calculation of standardized uptake value ratios (SUVR). Herefore, the cerebellum was used as pseudo-reference tissue for microglia-PET[32]. The cerebellum was chosen as a unified pseudo-reference tissue since it was also used for tau-PET analysis. The dentate nucleus and superior and posterior layers of the cerebellum were excluded to account for potential tau-PET positivity in cerebellar areas and in adjacent extracerebral structures. Using the Brainnetome atlas[33], the brain was divided into 210 cortical and 36 subcortical volume-of-interests (VOIs) and SUVR were calculated. Per patient, the standardized regional deviation of SUVR (z-score) was computed versus the readouts of already established age- and sex- matched control cohorts for [^{18}F]GE-180 (n_c=13[34]) and [^{18}F]PI-2620 (n_c=14[35]).

Since patterns of abnormal tau- or TSPO-PET can be heterogeneous in Aβ-negative CBS patients, we computed summary measures of brain-wide PET abnormality. Specifically, we determined number of VOIs of the Brainnetome atlas in which PET SUVRs fell above a z-score of 1.5. This cut-off of 1.5 SD is based on experience on a sample of healthy controls without any evidence for neurological disease or

cognitive deficits, increasing the sensitivity to detect early PET abnormalities in patients with suspected 4R tauopathies. Summary measures of PET abnormality were determined for the whole brain, as well as for the cortex and subcortex separately, to determine whether subcortical and/or cortical tau- or TSPO-PET abnormalities were associated with clinical CBS trajectories.

For structural MRI, three-dimensional T1 MRI data (TR=2060ms, 0.8mm isotropic voxel size) recorded on a 3T SIEMENS Magnetom Prisma system (Siemens Healthineers, Erlangen, Germany) were non-linearly spatially normalized to MNI standard space using PNEURO tool (V3.9; PMOD Technologies LLC) and segmented into tissue specific probability maps. Regional grey matter density as a proxy of grey matter volume was extracted for each of the Brainnetome VOI.

Assessment of a CBS clinical composite score

As there was no consensus on a single clinical scale that captures CBS symptom severity at the beginning of the study in 2018, we composed a CBS clinical composite across available clinical scales including PSPRS, MoCA and SEADL. We specifically selected these scales to summarize global clinical status, including cognitive symptom severity (i.e. MoCA), subcortical symptom severity (i.e. PSPRS) and impaired activities of daily living (i.e. SEADL). Specifically, MoCA, PSPRS and SEADL scores were fed into principal component analyses (i.e. prcomp command of the stats package in R statistical software). The first principal component that captured most of the variance across MoCA, PSPRS and SEADL scores was extracted as a summary measure of clinical disease severity (i.e. CBS clinical composite). The variance explained for the first principal component was 89.11%, suggesting that this component captures a large proportion of the variance across the clinical measures. For future clinical studies in CBS, the cortical basal ganglia functional scale, published in 2020, [36, 37] may be implemented as an additional single clinical scale for CBS symptoms.

Plasma biomarker assessment

NfL and GFAP levels were quantitatively determined in plasma samples using a commercial SIMOA kit (#103345, Quanterix, USA) following the manufacturer's instructions. All samples were analyzed in the same plate blinded to clinical information and were measured on the same day. The used aliquot underwent only one thaw/freeze cycle. NfL concentrations were measured using the Simoa HD-X analyzer (Quanterix, USA).

Statistical Analyses

First, we assessed whether Aβ-negative CBS patients declined during the follow-up period. To this end, we used linear mixed effects models using time from baseline as a predictor of the clinical composite

or PSPRS, controlling for age, sex, education, BMI, disease duration as well as random slope and intercept. We then determined whether baseline tau-PET, TSPO-PET, structural MRI, NfL or GFAP levels moderated clinical trajectories. Specifically, we tested the interaction effect of each baseline biomarker (i.e. tau-PET, TSPO-PET or structural MRI abnormality, NfL, GFAP) with time on the clinical composite or PSPRS. Again, models were controlled for age, sex, education, BMI, disease duration as well as random slope and intercept. For tau-PET or TSPO-PET abnormality, analyses were conducted using global PET abnormality scores as well as subcortical and cortical PET abnormality scores, to determine the impact of either cortical or subcortical PET abnormality on clinical trajectories. In addition, we performed simulated interventions to determine whether baseline PET or fluid biomarkers can help select patients at high risk of clinical progression to reduce sample sizes for potential intervention effects. To this end, we determined patient-specific annual change rates in the clinical composite and PSPRS using linear mixed effects models. Using these subject-specific change rates, we ran simulated interventions with hypothetical intervention effects of 20%/30%/40% using the R-package pwr (settings: two-sample t-test, two-tailed, type I error rate=0.05, power=0.8). Simulated interventions were performed for the whole CBS sample, as well as stratified by high and low biomarker groups (i.e. tau-PET, TSPO-PET, structural MRI, NfL, GFAP) determined by median split. All analyses were computed using R statistical software (r-project.org). Linear mixed models were run using the lmer package.

Data availability

Ethics approvals do not allow unrestricted and open-source sharing of patient-specific data with third-parties. Anonymized data that support the findings of this study are available on reasonable request from the corresponding author.

Results

21 Aβ-negative CBS patients with longitudinal clinical data were included in the current study (see Table 1 for demographic and clinical characteristics). At baseline, all patients underwent detailed clinical assessment, MR imaging, blood sampling and [^{18}F]PI-2620 PET. A subset of n=16 also underwent baseline [^{18}F]GE-180 TSPO-PET. All included patients were medium or high affinity binder for [^{18}F]GE-180 TSPO-PET. Cross-sectional analysis of [^{18}F]PI-2620 PET and [^{18}F]GE-180-PET[8] of parts of the cohort has been reported before[4-6]. Median clinical follow-up times were 1.95 years ranging between 0.75 and 2.72 years with an average of 3.1±0.7 visits. As expected, we found a significant effect of clinical follow-up time on the CBS clinical composite scores (B/SE=-12.126/2.279, p<0.001) and PSPRS (B/SE=7.847/1.240, p<0.001). Baseline z-score maps of [^{18}F]PI-2620 tau-PET and [^{18}F]GE-180 TSPO-PET are shown in Figure 1, showing widespread elevations of subcortical and cortical [^{18}F]PI-2620 tau-PET signals as well as limited cortical and moderate subcortical [^{18}F]GE-180 TSPO-PET signal increases. The PET summary scores for global, cortical and subcortical assessments are summarized in Table 1.

More widespread cortical tau-PET is associated with faster clinical deterioration

In a first step, we tested whether baseline [^{18}F]PI-2620 tau-PET predicted speed of clinical progression in Aβ-negative CBS. We hypothesized that higher [^{18}F]PI-26260 tau-PET readouts are associated with faster clinical progression. To test this, we assessed the interaction effect between [^{18}F]PI-2620 tau-PET and follow-up time on trajectories in the CBS clinical composite score and PSPRS using linear mixed models adjusting for age, sex, education, BMI, disease duration, as well as random slope and intercept. For global [^{18}F]PI-2620 tau-PET abnormality (i.e. number of Brainnetome ROIs with a z-score>1.5) we found a significant time by [^{18}F]PI-2620 tau-PET interaction on the CBS clinical composite score (B/SE=-0.059/0.025, p=0.021, Fig.2A) and PSPRS (B/SE=0.001/0.0005, p=0.025, Fig.2B), supporting the hypothesis that faster clinical deterioration in Aβ-negative CBS patients is associated with more widespread global [^{18}F]PI-2620 tau-PET signal. When stratifying this analysis by [^{18}F]PI-2620 tau-PET abnormality in the cortex vs. subcortex, cortical [^{18}F]PI-2620 tau-PET was associated with faster decline on the CBS clinical composite score (B/SE=-0.063/0.027, p=0.024) and PSPRS (B/SE=0.001/0.0005, p=0.026), while subcortical [^{18}F]PI-2620 tau-PET abnormality was not (CBS clinical composite: B/SE=-0.286/0.184, p=0.127; PSPRS: B/SE=0.005/0.004, p=0.191). This suggests that specifically the elevation of [^{18}F]PI-2620 tau-PET in the cortex is associated with faster clinical progression in Aβ-negative CBS. Linear mixed model statistics are summarized in Table 2.

Plasma NfL but not GFAP predicts clinical progression

Next, we assessed whether plasma NfL and GFAP are associated with faster clinical worsening. For NfL, we found a significant interaction with time on worsening in the clinical composite (B/SE=-0.230/0.111, p=0.044, Fig.3A) and PSPRS (B/SE=0.176/0.046, p<0.001, Fig.3B), indicating that stronger neurodegeneration is associated with faster clinical worsening. Yet, no time by biomarker abnormality interaction was found for GFAP, neither for the clinical composite (B/SE=-0.020/0.027, p=0.455, Fig.3C) nor for PSPRS (B/SE=-0.007/0.0127, p=0.574, Fig.3D). This suggests that GFAP as a marker of abnormal astrocyte function is not associated with subsequent clinical worsening in Aβ-negative CBS. A summary of linear mixed model statistics can be found in Table 2.

More widespread TSPO-PET readout is associated with slower clinical progression

For TSPO-PET we found that more widespread global signal was associated with slower worsening both on the clinical composite (B/SE=0.109/0.050, p=0.037, Fig.3C) and PSPRS (B/SE=-0.056/0.023, p=0.019, Fig.3D). In contrast to tau-PET this effect was stronger for subcortical TSPO-PET (clinical composite: B/SE=0.660/0.243, p=0.011; PSPRS: B/SE=-0.369/0.106, p=0.002) rather than cortical TSPO-PET abnormality (clinical composite: B/SE=0.118/0.060, p=0.057; PSPRS: B/SE=-0.059/0.028, p=0.040). These findings suggest that more widespread microglial activation is associated with attenuated clinical progression in Aβ-negative CBS. For summary statistics, see Table 2.

MRI-based volumetry is not predictive of clinical progression

When assessing the number of VOIs falling below an atrophy z-score cut-off of -1.5 on structural MRI, we did not find any association with faster decline in the clinical CBS composite score or PSPRS, neither for global (clinical composite: B/SE=0.040/0.031, p=0.209; Fig.3E; PSPRS: -0.039/0.020, p=0.072, Fig.3F), nor for cortical (clinical composite: B/SE=0.044/0.033, p=0.190; PSPRS: B/SE=-0.041/0.021, p=0.076) or subcortical VOIs (clinical composite: B/SE=0.171/0.351, p=0.628; PSPRS: -0.403/0.231, p=0.103). Detailed statistics are summarized in Table 2. As we did not find that pronounced brain atrophy was associated with faster clinical disease progression, we do not expect systematic confounding effects of grey matter atrophy on the respective PET study results.

Sample size estimation for clinical trials

Lastly, we assessed whether [^{18}F]PI-2620 tau-PET, [^{18}F]GE-180 TSPO-PET or NfL, all of which have been shown to predict clinical trajectories in Aβ-negative CBS individually, can help identifying patients at risk of clinical progression. This would help in the future with reducing (as compared to solely relying on the clinical diagnosis) sample sizes required for detecting intervention effects in clinical trials. To this end, we performed simulated interventions (i.e. 10/20/30% attenuation of annualized change rates on the clinical composite or PSPRS) using either the whole CBS sample or patients above or below

median biomarker abnormalities (i.e. global [^{18}F]PI-2620 tau-PET, global [^{18}F]GE-180 TSPO-PET, NfL). Based on these samples, we determined the minimum sample size required for detecting intervention effects at a significance level of 0.05 and a power of 80%. We found that selecting Aβ-negative CBS patients with [^{18}F]PI-2620 tau-PET signals above median reduced the number of patients required to detect simulated intervention effects by 50%. Similarly, selecting patients with [^{18}F]GE-180 TSPO-PET signals below-median reduced required sample sizes by 24%. Strongest sample size reductions capacities were found for plasma NfL: Selecting patients with above-median plasma NfL levels reduced sample sizes by 60%. Detailed sample size estimates for different intervention strengths and biomarker selection criteria are summarized in Table 3.

Discussion

Our major aim was to test the impact of neuroimaging and plasma biomarkers on disease progression in patients with Aβ-negative CBS of the clinical category "with probable underlying 4R tauopathy". Our head-to-head-comparison included biomarkers concerning neurodegeneration (NfL and structural MRI), pathology (tau-PET), neuroinflammation (TSPO-PET) and astrogliosis (GFAP). To our knowledge this study is the first to investigate the impact of potential biomarkers on clinical trajectories in Aβ-negative CBS patients. For the clinical scores PSPRS and the CBS clinical composite score (consisting of PSPRS, SEADL and MoCA), we observed a highly significant effect of follow-up time in CBS patients over a ~2-year follow-up period, suggesting worsening of symptoms over time. In our mixed linear model, the PSPRS rate of change in CBS patients was 7.8±1.2 points per year. In one study assessing longitudinal changes in PSPRS in nine histopathologically proven cases of 4R-tauopathy with CBS phenotype, average decline of PSPRS was ~ 10 points per year[38], hence slightly above our observed clinical decline. This may be partially due to our study's inclusion criteria requiring our patients to undergo three brain scans at baseline, possibly rendering them as less clinically affected.

Assessing the interaction effect between biomarkers and time on clinical trajectories, we showed that more widespread [^{18}F]PI-2620 tau PET signal as well as higher levels of NfL in plasma at baseline are associated with disease progression in our Aβ-negative CBS cohort. The impact of [^{18}F]PI-2620 tau load on clinical progression was specifically driven by cortical rather than subcortical elevation of [^{18}F]PI-2620 tau-PET signal. In contrast, we found that higher levels of [^{18}F]GE-180-PET tracer uptake were associated with a slower disease course. In our sample, GFAP in plasma and structural MRI were not associated with clinical progression. Lastly, we assessed whether the biomarkers with prognostic value (i.e. [^{18}F]PI-2620 tau-PET, [^{18}F]GE-180 TSPO-PET and plasma-NfL) could help identifying patients at risk of clinical progression to reduce sample sizes required for detecting intervention effects in clinical trials. Strongest sample size reduction capacities were found for plasma NfL, followed by [^{18}F]PI-2620 tau-PET.

Concerning neuroimaging biomarkers, our findings align with studies of AD, a 3-repeat/4-repeat tauopathy, and PSP patients with Richardson syndrome phenotype, in which *in vivo* PET markers of tau burden were associated with the disease course at baseline[36-38]. In our Aβ-negative CBS cohort, the impact of [^{18}F]PI-2620 tau-PET on disease progression is driven by cortical rather than subcortical tracer signal. For [^{18}F]PI-2620 tau-PET, the reason for the observed limited value of subcortical tau tracer enhancement concerning clinical progression may be a ceiling effect with high subcortical binding in predilection sites, like the basal ganglia, with high tracer uptake independently from disease duration and disease severity[5].

Regarding TSPO-PET, we found that less widespread microglial activity levels were associated with faster disease progression, particularly in the subcortex. Speculatively, microglial activation occurs early during the disease course. In our previous cross-sectional TSPO-PET study in Aβ-negative CBS, we found higher TSPO-PET signal in early stages of disease[22]. Microglial activation might, thus, be protective in terms of disease progression, with low TSPO-PET readouts in some patients reflecting a functional burnout of microglia indicative of a faster disease course. In keeping with this, a potential protective effect of microglia activation during early disease course has been shown in AD patients[39, 40]. On the contrary, in a study of 17 PSP-RS patients with a rate of change in PSPRS similar to that in the present study (~ 6-7 points per year), a PCA-based analysis of the TSPO-PET tracer data as obtained by the first-generation tracer [^{11}C]PK11195 revealed that subcortical neuroinflammation was associated with faster clinical progression[41]. The difference between our and the result of the above study may be due to differences in distribution of pathological brain changes in PSP-RS vs. CBS, with a shift towards cortical brain regions in case of CBS[42], and due to differences in disease duration and hence disease dynamics (~ 4.7 years vs. 2.7 years in our cohort). The latter might impact study results as microglial activation associated with progressive neuronal injury reflecting disease progression may occur in later disease stages[40]. Moreover, [^{11}C]PK11195 has a relatively low brain uptake[43] and may only be partially comparable to the tracer used in our study.

When assessing regional brain atrophy on structural MRI, no association was found between grey matter volume and clinical progression. This suggests that the degree of brain atrophy does not forecast future clinical trajectories, similar to previous findings regarding 4R-tauopathy PSP with Richardson Syndrome phenotype, showing that tau-PET outperforms MRI regarding the prognostic value[41].

Concerning fluid biomarkers, we found that stronger neurodegeneration measured by NfL in plasma is associated with faster clinical worsening. NfL has been associated with survival in a retrospective study with PSP-RS patients[18] and with a prognostic value concerning disease progression in PSP-RS[19, 20], but not yet in CBS patients. The range and variation rate of plasma/CSF NfL concentrations in neurodegenerative diseases are still not understood in detail, and further studies with longitudinal NfL measurements in CBS patients as well as other phenotypes of 4R tauopathies are warranted. In our Aβ-negative CBS cohort, GFAP in plasma as a potential marker of astrogliosis was not associated with disease progression. CSF/plasma GFAP has been identified as a possible diagnostic and disease course monitoring biomarker in various neurodegenerative diseases[14-17], while, so far, a prognostic value has only been shown for clinical outcome in stroke patients[44].

Overall, our findings on the relevance of imaging and blood biomarkers on clinical progression in Aβ-negative CBS patients suggest an important role for tau burden, microglial activation, and neuronal

damage on prediction clinical progression in CBS. In future investigations, the question of whether and to what degree clinical progression is paralleled by progression of the above biomarkers needs to be addressed. In particular, longitudinal imaging and blood analysis would allow to investigate potential changes in the interplay between these biomarkers over time, and to study the influence of potential biomarker interplay changes over time on the clinical phenotype course.

We acknowledge several limitations to the present study. Our cohort was diagnosed using clinical criteria without neuropathological verification. To reduce the risk of clinical misdiagnosis, all patients have been seen in a specialized outpatient clinic for movement disorders, diagnosis was reconfirmed at each follow-up visit and all patients fulfilled diagnostic criteria for a probable 4R tauopathy according to the MDS-PSP criteria[3]. A further clinical investigation of the cohort will be conducted to follow up on natural disease course and mortality, and to, eventually, evaluate postmortem histopathology in the context of antemortem biomarker profile and in-depth clinical characterization. This will allow us to validate sensitivity and specificity of the PET, MRI and blood biomarkers studied against the diagnostic gold standard, and to further assess clinical scores for patient stratification for potential therapeutic studies. A further limitation of our study is the relatively small number of patients. This might mask effects such as age-dependency of tracer binding and of fluid biomarker levels. Moreover, despite strong correlations between plasma and CSF-derived NfL ($r \sim 0.7$[45]), plasma NfL may be influenced by confounding factors such as renal function or neuropathies in comparison to CSF-derived NfL. However, we decided to use clinically easily accessible plasma measures in our study to maximize the sample size for our main analysis as not all participants underwent CSF sampling. The replication of our findings with larger clinical cohorts will, thus, be important to determine generalizability of our results and to evaluate a potential synergistic effect of tau-PET, TSPO-PET and plasma-NfL as baseline biomarkers on sample size reductions for clinical trials designs. Moreover, future studies should also include other subtypes of primary tauopathies to compare the biomarkers' potential prognostic role in different phenotypes of suspected 4R-tauopathies, as well as amyloid-β-positive CBS patients to evaluate a potential generalizability of our results for CBS in the course of AD.

In conclusion, tau-PET, TSPO-PET and plasma NfL show impact on future clinical progression in amyloid-β-negative CBS. Thus, this can be useful for future clinical decision making as well as for stratifying patients for clinical trials. Moreover, our power calculation data demonstrate that clinical trials in Aβ-negative CBS patients are feasible and that these baseline biomarkers have potential to reduce sample sizes when designing interventional trials in CBS.

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Tables

Table 1: Demographic and clinical sample characteristics at baseline

Variable	Amyloid-β-negative CBS (n=21)
Sex (male/female)	9/12
Age, years	68.7±12.0
Education, years	12.8±2.8
Disease duration, years	2.69±1.35
Follow-up, years (median [range])	1.95 [0.75;2.72]
PSPRS scores	22.2±13.7
SEADL scores	69.0±19.2
MoCA scores	23.7±4.1
CBS clinical composite scores	15.3±22.9
Global PI-2620 tau-PET summary score	107.28±69.88 (median=105)
Cortical PI-2620 tau-PET summary score	90.94±63.9 (median=88)
Subcortical PI-2620 tau-PET summary score	16.34±10.03 (median=16)
Global TSPO-PET summary score	49.12±36.98 (median=32)
Cortical TSPO-PET summary score	40.14±31.06 (median=21)
Subcortical TSPO-PET summary score	8.98±7.51 (median=7)

Data are presented as mean ± standard deviation, unless indicated otherwise. The CBS clinical composite score is defined by a principal component analysis using MoCA, PSPRS and SEADL scores. CBS = corticobasal syndrome, PSPRS = progressive supranuclear palsy rating scale, SEADL = Schwab and England Activities of Daily Living, MoCA = Montreal Cognitive Assessment.

Table 2: Linear mixed model statistics for time by biomarker interactions on clinical trajectories

Dependent variable	Biomarker	N (subjects/ observations)	B/SE	T	p
CBS clinical composite score	Global PI-2620 tau-PET	21/64	-0.059/0.025	-2.395	**0.021**
	Cortical PI-2620 tau-PET		-0.063/0.027	-2.352	**0.024**
	Subcortical PI-2620 tau-PET		-0.286/0.184	-1.577	0.127
	Global TSPO-PET	16/51	0.109/0.050	2.185	**0.036**
	Cortical TSPO-PET		0.118/0.060	1.971	0.057
	Subcortical TSPO-PET		0.660/0.243	2.716	**0.011**
	Global grey matter volume	17/53	0.040/0.031	1.282	0.209
	Cortical grey matter volume		0.044/0.033	1.336	0.190
	Subcortical grey matter volume		0.171/0.351	0.488	0.628
	NfL	20/62	-0.230/0.111	-2.073	**0.044**
	GFAP	19/59	-0.020/0.027	-0.755	0.455
PSPRS	Global PI-2620 tau-PET	21/64	0.001/0.0005	2.329	**0.025**
	Cortical PI-2620 tau-PET		0.001/0.0005	2.318	**0.026**
	Subcortical PI-2620 tau-PET		0.005/0.004	1.331	0.191
	Global TSPO-PET	16/51	-0.056/0.023	-2.462	**0.019**
	Cortical TSPO-PET		-0.059/0.028	-2.138	**0.040**
	Subcortical TSPO-PET		-0.369/0.106	-3.470	**0.002**
	Global grey matter volume	17/53	-0.039/0.020	-1.950	0.072
	Cortical grey matter volume		-0.041/0.021	-1.925	0.076
	Subcortical grey matter volume		-0.403/0.231	-1.747	0.103
	NfL	20/62	0.176/0.046	3.842	**0.0004**
	GFAP	19/59	-0.007/0.013	-0.567	0.574

Linear mixed model statistics were adjusted for age, sex, education, body mass index, disease duration, as well as random slope and intercept. The CBS clinical composite score is defined by a principal component analysis

using MoCA, PSPRS and SEADL scores. CBS = corticobasal syndrome, PSPRS = progressive supranuclear palsy rating scale, SEADL = Schwab and England Activities of Daily Living, MoCA = Montreal Cognitive Assessment.

Table 3: Sample size estimation for detecting simulated intervention effects using biomarker-based patient stratification strategies

		Primary endpoint: CBS clinical composite score				Primary endpoint: PSPRS			
			Intervention effect				Intervention effect		
		Median annualized change rate	10%	20%	30%	Median annualized change rate	10%	20%	30%
PI-2620 tau-PET	Pooled (N=21)		481	110	45		708	196	96
	>median	-14.77	234	54	22	9.44	295	82	41
	<median	-8.28	833	189	77	6.68	1279	353	173
TSPO-PET	Pooled (N=16)		796	181	74		879	243	119
	>median	-6.65	971	221	90	4.02	1264	348	171
	<median	-16.52	604	138	56	9.95	548	152	75
NfL	Pooled (N=21)		481	110	45		708	196	96
	>median	-14.06	189	44	18	10.0	255	71	35
	<median	-7.38	1049	283	97	3.41	1141	315	154

Numbers are N required per study arm (i.e. placebo vs. verum). CBS clinical composite score is defined by a principal component analysis using MoCA, PSPRS and SEADL scores. CBS = corticobasal syndrome, PSPRS = progressive supranuclear palsy rating scale.

Figures

Figure 1: Group-average PET abnormality in amyloid-β-negative CBS

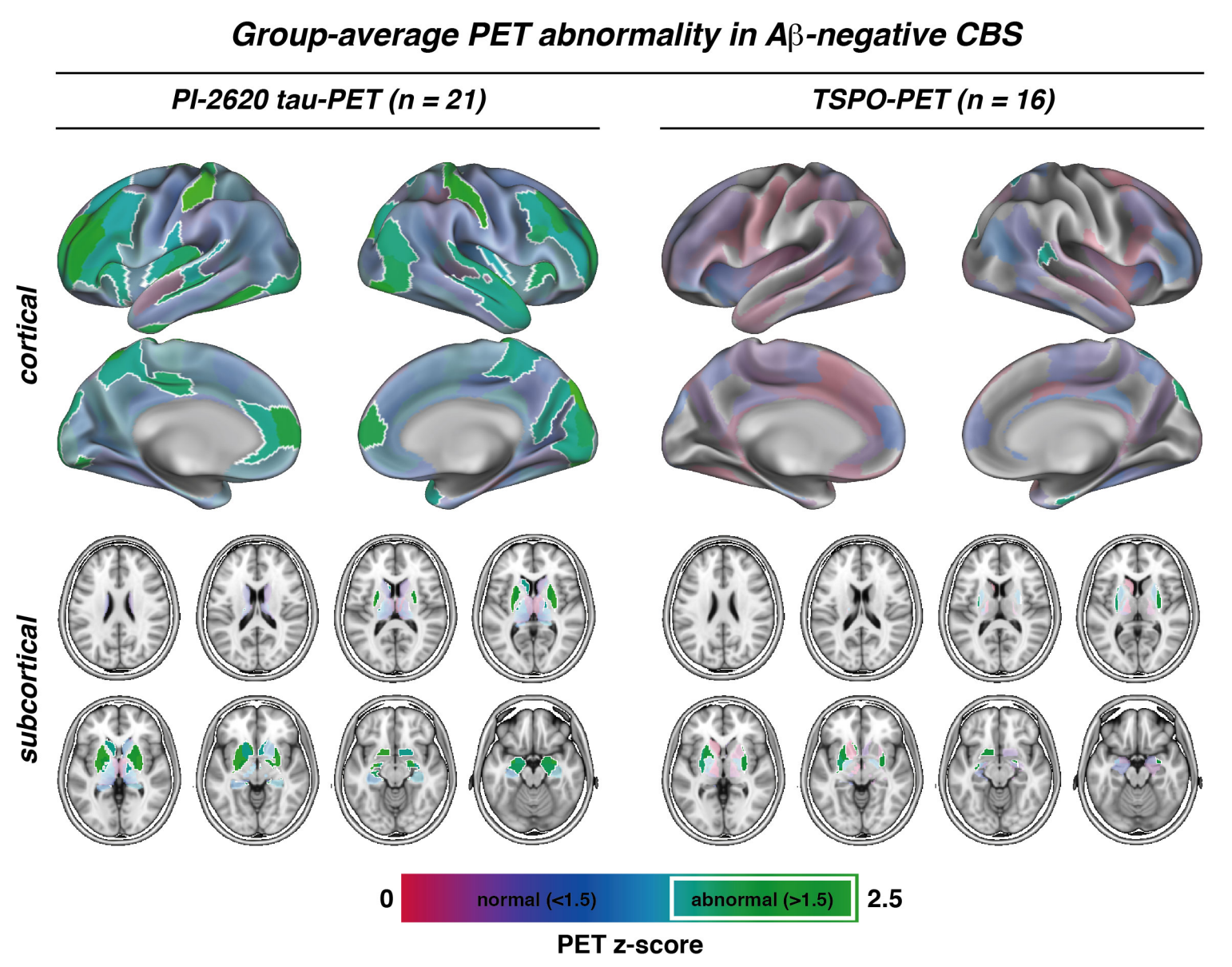
Surface and subcortical renderings of elevated group-level for PI-2620 tau-PET z-scores (left panels) and TSPO-PET z-scores (right-panels) that were referenced against PET images obtained in healthy controls. Z-scores greater than 1.5 are considered pathological and are highlighted by white margins.

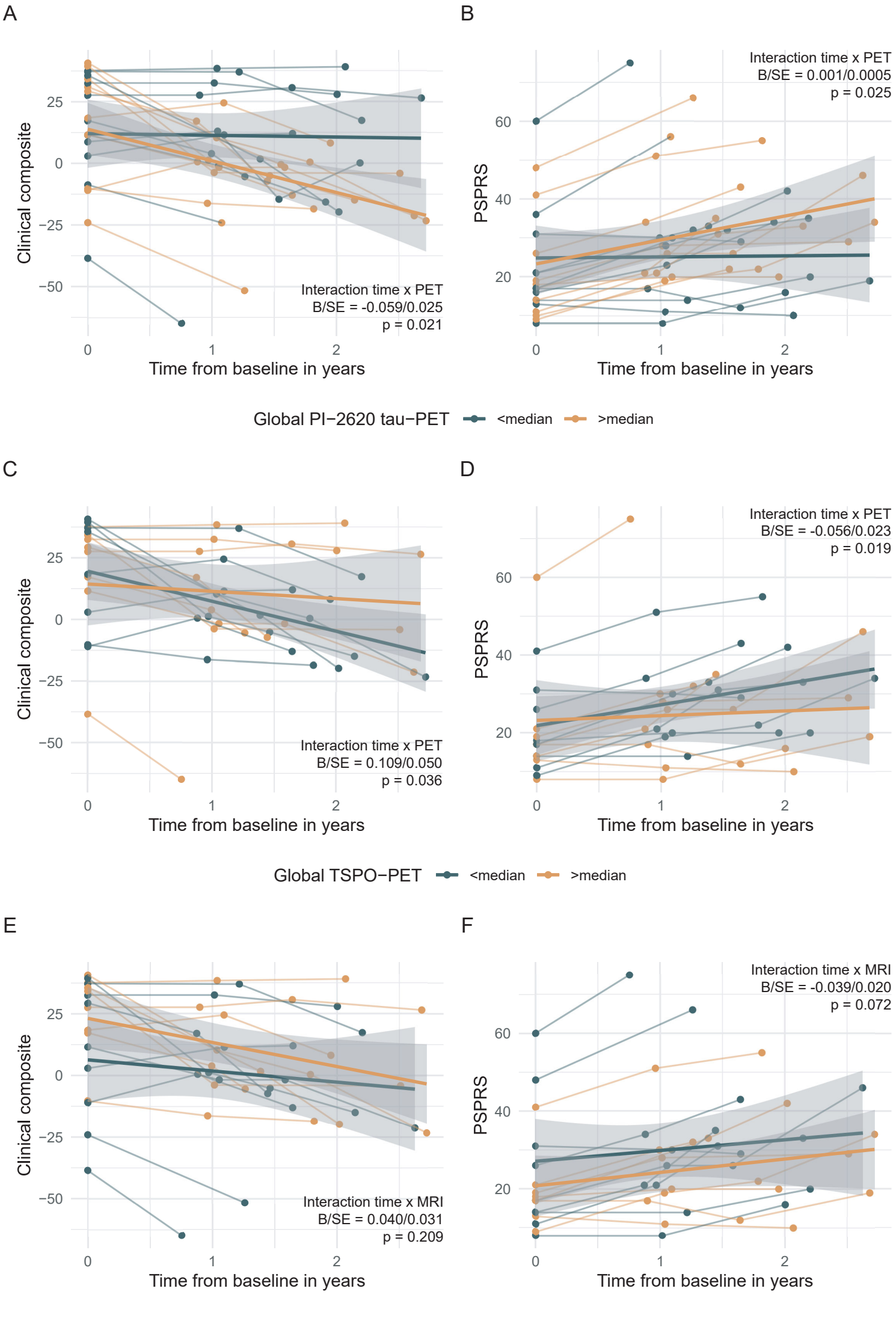
Figure 2: Neuroimaging-based prediction of clinical trajectories in amyloid-β-negative CBS

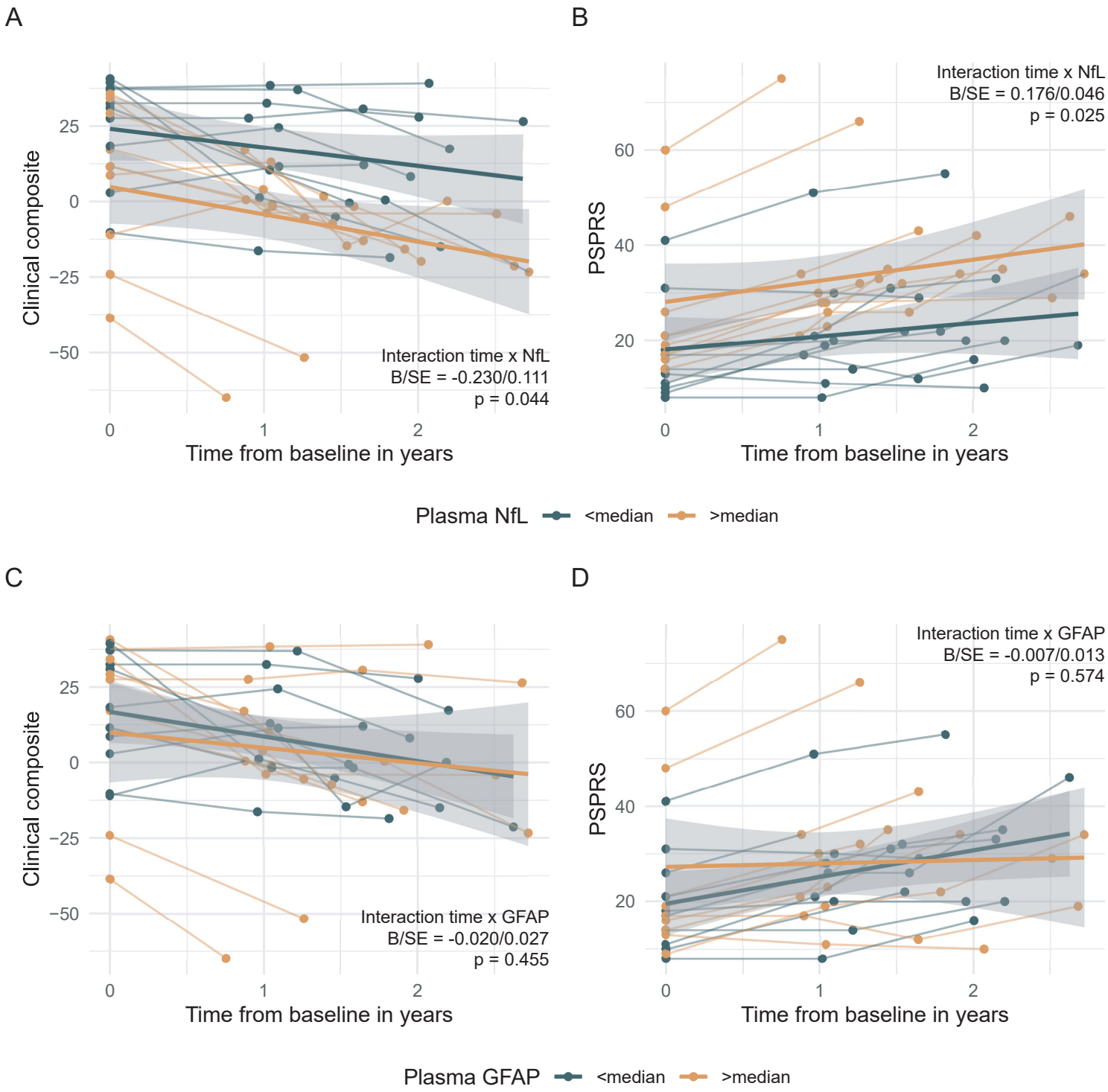
Line-plots, illustrating clinical trajectories on the CBS clinical composite score (panels A, C, E) and PSPRS (panels B, D, F) stratified by abnormality in global PI-2620 tau-PET (panels A, B), global TSPO-PET (panels C, D) or global MRI-based grey matter atrophy (panels E, F). For visualization, regression fits were split into above and below median groups to illustrated disease trajectories relative to imaging signal abnormality, however, interactions were computed using continuous measures. Statistics are based on linear mixed models controlling for age, sex, education, body mass index, disease duration, as well as random slope and intercept. CBS clinical composite score is defined by a principal component analysis using MoCA, PSPRS and SEADL scores. A decrease in score value of the CBS clinical composite score indicates a clinical deterioration, while in PSPRS an increase in score value indicates clinical worsening. Linear model fits (i.e. least squares line) are indicated together with 95% confidence intervals. CBS = corticobasal syndrome, PSPRS = progressive supranuclear palsy rating scale.

Figure 3: Fluid biomarker-based prediction of clinical trajectories in amyloid-β-negative CBS

Line-plots, illustrating clinical trajectories on the clinical composite (panels A, C) and PSPRS (B, D) stratified by abnormality in plasma NfL (A, B) and plasma GFAP (C, D). For visualization, regression fits were split into above and below median groups to illustrated disease trajectories relative to NfL and GFAP levels, however, interactions were computed using continuous measures. Statistics are based on linear mixed models controlling for age, sex, education, body mass index, disease duration as well as random slope and intercept. CBS clinical composite score is defined by a principal component analysis using MoCA, PSPRS and SEADL scores. A decrease in score value of the CBS clinical composite score indicates a clinical deterioration, while in PSPRS an increase in score value indicates clinical worsening. CBS = corticobasal syndrome, PSPRS = progressive supranuclear palsy rating scale, NfL = neurofilament light chain, GFAP = glial fibrillary acidic protein.


Group-average PET abnormality in Aβ-negative CBS
PI-2620 tau-PET (n = 21)
TSPO-PET (n = 16)
cortical
subcortical
0
normal (<1.5)
abnormal (>1.5)
2.5
PET z-score


A
B
Clinical composite
PSPRS
Time from baseline in years
Interaction time x PET
B/SE = -0.059/0.025
p = 0.021
Interaction time x PET
B/SE = 0.001/0.0005
p = 0.025
Global PI−2620 tau−PET
<median
>median
C
D
Interaction time x PET
B/SE = 0.109/0.050
p = 0.036
Interaction time x PET
B/SE = -0.056/0.023
p = 0.019
Global TSPO−PET
<median
>median
E
F
Interaction time x MRI
B/SE = 0.040/0.031
p = 0.209
Interaction time x MRI
B/SE = -0.039/0.020
p = 0.072


Fluid biomarker-based prediction of clinical trajectories in Aβ-negative CBS
A
B
Clinical composite
PSPRS
Time from baseline in years
Interaction time x NfL
B/SE = -0.230/0.111
p = 0.044
Interaction time x NfL
B/SE = 0.176/0.046
p = 0.025
Plasma NfL
<median
>median
C
D
Interaction time x GFAP
B/SE = -0.020/0.027
p = 0.455
Interaction time x GFAP
B/SE = -0.007/0.013
p = 0.574
Plasma GFAP
<median
>median