nature portfolio

Peer Review File

Plasma phosphorylated tau 217 and longitudinal trajectories of Aβ, tau, and cognition in cognitively unimpaired older adults

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The present study investigated the associations between plasma p-tau217, measured using the C2N mass spectrometry method, and longitudinal changes in amyloid-PET, tau-PET, and cognitive decline in cognitively unimpaired individuals from the Harvard Aging Brain Study. Of particular relevance, the authors also examined whether plasma p-tau217 levels are associated with subsequent amyloid and tau accumulation among participants who were Aβ-PET negative at baseline (< 24 Centiloids), based on the hypothesis that plasma p-tau217 may serve as an earlier indicator of Alzheimer's disease pathology than amyloid-PET. The study addresses an underexplored area, as little is currently known about predictors of amyloid-β positivity in cognitively normal individuals. However, given the uncertain clinical relevance of amyloid positivity alone, the potential impact of these findings for Alzheimer's disease therapeutics and patient care remains unclear. Overall, the manuscript is well written and easy to follow, although some minor typos are present. The authors conclude that plasma p-tau217 changes early—earlier than amyloid-PET—and that baseline plasma p-tau217 levels could predict amyloid and tau accumulation among initially amyloid-negative unimpaired individuals. However, this conclusion is not fully supported by their analyses, which, in my opinion, overlook other important predictors of amyloid-PET conversion that previous studies have found to be more strongly associated with progression to amyloid positivity.
1) Across all analyses, the authors do not consider what is likely the strongest predictor of future Aβ-PET positivity: baseline Centiloid values. Previous work by investigators at the Mayo Clinic, addressing this same research question, demonstrated that baseline Centiloid levels among amyloid-negative unimpaired individuals were actually a better predictor of future amyloid positivity than plasma %p-tau217 measured by mass spectrometry (doi: 10.1002/alz.14629). This raises an important and practical question: if readily available baseline information from the amyloid-PET scan can better predict progression risk, why should an additional, expensive mass-spectrometry plasma assay be performed? The authors should directly address this issue and clarify the potential incremental value, if any, of plasma p-tau217 over baseline PET metrics.
2) Another important issue is the high number of ad-hoc points used in the study. In the Results section, the authors state, “These values were very close to previous reported AUC (0.94) and %pTau217 cut point (4.2%) to identify Aβ PET >25 CL using the same %pTau217 assay in a different cohort,21 demonstrating consistent performance of the plasma %pTau217 in detecting elevated cortical Aβ burden.” However, this interpretation appears selective and potentially misleading. Different cut-points for the C2N assay have been reported across studies, reflecting variability in assay performance depending on the cohort and context. For example, in a recent publication in JAMA (doi: 10.1001/jama.2024.13855), a cut-point of 3.26% was proposed. Based on the distributions shown in Figure 1a of the present manuscript, applying a 3.26% threshold would likely lead to a substantial increase in false positive cases, significantly altering the author’s results. Citing only studies that align with the authors' preferred cut-point may give a false sense of consistency. Another example of cut-point variability is the AHEAD study (doi: 10.1002/alz.13542), with a cut-point of 1.55% which again highlights the broader issue of variability and lack of universal agreement on plasma p-tau217 thresholds across cohorts. The authors should avoid making strong claims about the reliability and generalizability of their chosen cut-points.
3) Figure 2 does not display any of the data points (edges, arrowheads) that are described in the figure legend.
4) The authors state, “We conducted survival analyses across three groups categorized by baseline %pTau217 tertile (within the baseline A- subgroup only) and found that the survival probably (i.e., remaining A-) at 6 years from the baseline was 0.98 (95% CI 0.95 to 1.0) for participants in the first (lowest) %pTau217 tertile and 0.72 (95% CI 0.60 to 107 0.87) for those in the third (highest) %pTau217 tertile.” However, the use of arbitrary tertile-based categorization is unnecessary and statistically suboptimal. The authors have already defined an ad hoc cut-point for %pTau217; if the goal is to assess the association

between continuous %pTau217 levels and risk of progression to amyloid positivity, a Cox proportional hazards regression using %pTau217 as a continuous predictor would be a more appropriate and powerful approach. Trichotomization reduces statistical power, introduces artificial thresholds, and can obscure meaningful dose-response relationships. I recommend reanalyzing the data using a continuous model to more accurately capture the relationship between plasma %pTau217 levels and risk of Aβ-PET conversion.
5) In line with the previous comment, the authors state, "Then, we utilized paired measures of longitudinal plasma %pTau217 and Aβ PET (from the same study years) to evaluate their coordinated changes (Fig. 2a, n=223). Here, we used %pTau217 cut point 127 that optimally predicts Aβ progression (2.6%) to define low- versus high-%pTau217 subgroups." The repeated use of different ad hoc cut-points throughout the analyses raises concerns about the practical utility and robustness of plasma %pTau217 as a biomarker. Defining new cut-points optimized for each subset or analysis not only risks overfitting but also significantly undermines the generalizability of the results to independent populations. In clinical practice, a biomarker intended for early detection or risk stratification must rely on stable, validated thresholds that perform consistently across different cohorts and settings. The authors should directly address this limitation and discuss the extent to which their findings are likely to generalize beyond the current sample.
6) The authors state "Among the 117 participants with baseline %pTau217 less than 2.6 and longitudinal Aβ PET data, only 2 (<2%) were A+ at baseline, and 8 (7%) progressed to 117 A+ during the study (NPV=0.91 [107/117]). Thus, those below the %pTau217 cut point of 2.6 at study entry–which is much lower than the cut point used to predict Aβ positivity on PET– were unlikely to be baseline A+ and also had very low risk of progression to A+ during follow-up. Conversely, individuals with %pTau217 between 2.6 and 4.3% could represent a subset of CU older adults who are currently A- but at an elevated risk of progression to A+." This interpretation is based on an ad hoc definition of a new cut-point (2.6%) optimized for the current dataset, without reference to previously established thresholds from independent studies. If the authors choose to define new cut-points, they should at least systematically compare their findings with prior large studies, such as the JAMA study (cut-point ~3.26%) and the AHEAD study (cut-point ~1.55%), which demonstrate substantial variability across cohorts in %pTau217 thresholds. Please avoid presenting conclusions based on internally derived cut-points without considering and contextualizing variability observed in other studies.
7) The authors state, "To identify factors associated with A- to A+ progression in the A-/high %pTau217 subgroup (intermediate risk), we used logistic regression with A- to A+ progression as a binary outcome and baseline %pTau217, APOE ε4, age, and sex as predictors. We then examined a model including annual %pTau217 change ([last value – baseline value]/[interval]) as an additional predictor." However, logistic regression is not the appropriate modeling approach in this context. Logistic regression treats the outcome as a binary event without accounting for differences in follow-up time across participants, which could bias the results. A Cox proportional hazards model would be more appropriate, as it explicitly accounts for time-to-event data and variable follow-up durations, allowing for a more accurate estimation of risk factors associated with A- to A+ progression.
8) The authors state, "To examine the correlation between the longitudinal relationship between the paired measures of %pTau217 and PiB CL, we used a qualitative approach. First, we assigned subgroups based on their baseline measures (high/low %pTau217 by high/low PiB CL; 4 subgroups) and assessed how the participants moved across the subgroups by the time of their final measures." However, there is no need to rely solely on a qualitative assessment for this type of analysis. A more rigorous and informative approach would be to apply a multistate model, which is specifically designed to evaluate transitions between defined states over time. Multistate modeling would allow the authors to quantify transition probabilities, estimate transition intensities, and account for varying follow-up times, providing much richer and more interpretable metrics regarding biomarker dynamics.
9) "To examine the correlation between the longitudinal relationship between the paired measures of 349 %pTau217 and PiB CL, we used a qualitative approach." This sentence is a bit confusing, please consider rephrasing it.

Reviewer #2

(Remarks to the Author)
Review notes for Nature communications paper April 17 2025

Title: Plasma phosphorylated tau 217 and longitudinal trajectories of Aβ, tau, and cognition in cognitively unimpaired older adults

The authors have competed a thorough investigation into the %pTau217 against both AB and tau PET in a well characterised CU impaired cohort. Plasma measurements were taken at multiple collection visits along with AB and tau PET for well-planned statistical analyses. Results demonstrate that the %pTau217 biomarker was associated with both AB accumulation, and progression from AB-PET– to AB-PET+, as well as early tau accumulation and cognitive decline. Results are well presented, and this reviewer has only minor points for follow up.

1. Sample size: table 1 has a sample size at baseline of 317, yet in the results section (line 83) the text refers to 318 participants. Please confirm which is correct.
2. Sample size: It is clear that the sample sizes are different between those which were tested with the pTau217 assay and those which were scanned for AB, however it is unclear what the total sample size was for those which were scanned for tau (maybe this reviewer missed it). It would be helpful if A) in table 1 the number of participants who were T-/T+ at baseline was added, and B) a supplementary table which has the sample size for plasma, AB and tau PET at each time point, including how many were A-/A+ and T-/T+ at each visit.
3. Figure 2: It would appear that the data within the plots on Figure 2 has not shown up on this version. Please double check that there are supposed to be dots and or lines on these plots and not just empty quadrants.

Reviewer #3

(Remarks to the Author)
The manuscript by Yang et al report the associations between baseline plasma p-tau217 levels and longitudinal progression of amyloid and tau (as measured by PET) and cognitive decline in a cohort of cognitively unimpaired individuals at baseline. The authors show a good correlation between these plasma baseline levels and increases in amyloid and tau pathologies, even in subgroup of individuals with no pathology (as measured by PET) at baseline. Further, they showed that plasma levels seem to be increased before Ab-PET in these individuals at follow-up visits. Finally, they present a pathway analysis that suggests the direct and indirect associations between all these key changes during disease progression. This is a very nice study, using a very rich dataset which has the main biomarkers for Alzheimer's disease measured longitudinally for a relatively long follow-up. Further, the focus on cognitively unimpaired individuals makes it very relevant for the current situation in which the field is focusing on earlier stages of the disease. The manuscript is well-written and well structured.

The authors suggest that based on their results, longitudinal p-tau217 levels may be usefull to asess who is going to become Ab-positive. Nonetheless, no analyses on this regard are performed, although there seems to be the data necessary to support this claim. I'd suggest to include such analysis to strengthen this point.

Regarding the PPV and NPV values presented in the text, it would be worth mentioning that the cutoffs to extract those statistics were obtained from the same sample, which reduces the generalisability of the results.

The authors comment on the Ab-independent pathways of tau accumulation based on analyses done in Ab-negative individuals, but the association between ptau217 and tau accumulation could be also due to a larger dynamic ranges of p-tau217 levels compared to Ab-PET. This should be discussed.

Do the authors have any explanation on why some subjects (especially in the A-low/ptau high group) become p-tau217 low after having a high baseline level?

In figure 1a there are few individuals with high Ab-PET load (>50CL) but low p-tau217 levels. It would be good if the authors could give some details of these individuals (e.g., any specific genetic form, comorbidities, etc).

Figure 4b show positive trends between p-tau217 levels and PACC change. However, in the text the beta presented is negative. Could the authors explain this?

Minor suggestions:
-Can the authors clarify what does the second table in fig 1g refers to?
-The overall statistics of the path analysis should be presented somewhere.
-In the path analysis figure, it would be good to indicate that Ab and tau refer to longitudinal changes.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Responses to some of the author's comments below:

Comment: "Amyloid-β (Aβ) positivity (by PET) in cognitively unimpaired (CU) older adults, especially when combined with elevated tau PET signals in the mesial temporal region or neocortex, is a strong predictor of cognitive decline and clinical progression to MCI or dementia (e.g., references 12 and 24-26)."
Response: Amyloid PET positivity alone is a weak predictor of clinical progression in cognitively unimpaired (CU) individuals. In the Mayo Clinic Study of Aging (doi:10.1001/jamaneurol.2018.0629), fewer than 20% of A+ CU participants progressed to MCI or dementia over five years. By contrast, tau PET positivity—present in only ~25% of A+ CU—identifies the subgroup at highest risk and largely drives subsequent clinical decline (doi:10.1001/jama.2025.7817).

Comment: "Our study shows that, in amyloid-negative (A-) CU older adults, plasma %pTau217 can predict future accumulation of both Aβ and mesial temporal (entorhinal) tau as measured by PET. These results support the use of plasma %pTau217 in CU older adults to predict the future emergence of high-risk Alzheimer's disease (AD) biomarker profiles even before amyloid PET abnormalities emerge."
Response: The reported effect sizes for tau accumulation in the entorhinal cortex are very small (4.6×10^{-3} SUVR/year per unit of %p-tau217). While the association may reach statistical significance, the effect size is so modest that it remains unclear whether %p-tau217 meaningfully predicts which individuals will transition into the high-risk state of being tau PET–positive, particularly when applying the relatively stringent cut-offs or visual reads used in the referenced studies.

Comment: "We believe this finding is significant for earlier risk stratification and the design of primary AD prevention trials (i.e., clinical trials testing interventions before AD pathology develops), especially given the clinical trial results suggesting that earlier intervention might lead to a greater clinical benefit (doi: 10.1001/jama.2023.13239 [donanemab phase 3])."
Response: All participants in the donanemab phase 3 trial were required to be tau PET–positive on visual interpretation with flortaucipir, representing primarily individuals with high AD neuropathologic change (ADNC). Therefore, citing this study to

argue for treating individuals with subthreshold amyloid levels is speculative and not supported by the available evidence. Moreover, with such a poor predictive power, it is unclear whether such prevention trials would be feasible to demonstrate a slowing of amyloid accumulation, let alone demonstrating clinical efficacy.

Comment: “Third, our findings do not yet support the widespread use of plasma pTau217 screening in CU older adults in routine clinical practice, because it remains unknown whether preventing or treating AD pathology in high-risk CU individuals would lead to slowing of clinical decline; this is actively being investigated through ongoing clinical trials”
Response: This is incorrect. The primary reason plasma p-tau217 is not appropriate for routine screening in CU older adults is its limited prognostic value: a positive plasma result, by itself, does not identify a high-risk state for clinical progression. This is independent of the current uncertainty about the clinical benefits of very early intervention.

Comment: “Notably, after additionally adjusting for baseline Aβ CL, baseline %pTau217 was no longer associated with Aβ progression (p=0.31), while baseline Aβ was a stronger predictor of Aβ progression in this model (HR per 1 CL increase in Aβ PET=1.22, 95% CI 1.13 to 1.32, p=3.9×10-7). This observation was likely driven by individuals with their baseline Aβ burden closer to the A+ threshold: after excluding 10 participants whose baseline Aβ was greater than 20 CL but less than cut point (24.1 CL), higher baseline %pTau217 predicted progression to A+ (n=178, HR=1.52, 95% CI 1.06 to 2.18, p=0.023), while the association between baseline Aβ CL and progression to A+ became numerically weaker (HR=1.17, 95% CI 1.06 to 1.28, p=1.4×10-3). Further, among those with baseline Aβ less than 10 CL, baseline %pTau217 predicted Aβ progression (n=HR=2.67, 95% CI 1.17 to 6.11, p=0.020) while baseline Aβ did not (p=0.19).”
Response: After incorporating baseline Centiloid (CL)—and confirming, consistent with prior studies, that baseline CL is the stronger predictor of progression to A+—the authors reanalyzed the data using a new a posteriori cut point (10 CL) that appears to align with their hypothesis. How many participants with baseline amyloid <10 CL actually progressed to A+? I suspect this number is small (likely fewer than 7–8). Given the ad hoc/exploratory nature of this analysis—and the likely low event count—I recommend caution in asserting that p-tau217 predicts progression to A+ beyond what is already provided by amyloid PET.

Comment: “This study defined the cut point (1.55%; using C2N v1 assay) as the value maximizing Youden’s index to identify those with Aβ CL > 20. Importantly, the values reported from the v1 and v2 assays are not interchangeable; 1.55% from the v1 assay corresponds to 3.9% in the v2 assay. Considering the different gold standards utilized (Aβ PET Centiloid >20 CL vs. >25 CL in reference 21), we expect this value to be lower than the 4.2% reported in reference 21. We note that the current v2 assay (used in our study) has been fully validated according to CLIA standards, with improved robustness compared to v1.”
Response: Thanks for clarifying this information.
In any case, it appears that the reporting of different cut-points does not limit to the aforementioned publications. For instance, in a previous publication in the WRAP study (doi: 10.1002/alz.14570), which focuses on CU individuals (see Supplementary Table 2), the reported cut-point for %p-tau217 was 2.66, which is significantly higher than cut-points reported for v1 and significantly lower than cut-points for v2.
Although the WRAP study does not report whether the assay version is v1 or v2 (as is common in publications employing this assay), the authors state that they applied conversion factors to update the results to their new platform, suggesting that v2 was most likely used:
“When C2N transitioned to the version of the p-tau217 assay that is used for the PrecivityAD2 test, an assay comparison study was performed which established conversion factors for converting the p-tau217, np-tau217, and %p-tau217 values from the prototype assay to the PrecivityAD2 assay. These conversion factors were applied to all the values from the prototype assay to allow comparison of values from this study to values obtained from the PrecivityAD2 assay.”
Considering that v2 was used, how is it possible that, using very different cut-points (2.66 vs 4.2), the sensitivity and specificity reported in WRAP (0.86 and 0.9) is very similar to that reported in the present study (0.89 and 0.9)? Maybe the results in WRAP used another version of the assay that we were not aware of? Could it be that standardization of the %p-tau217 results across different settings and cohorts remains a challenge?

In summary, the revision reiterates the claim that changes in %p-tau217 precede amyloid PET changes, but this remains insufficiently supported by the presented data. Although the authors now show that baseline Centiloid (CL) is the stronger predictor of progression to A+, this variable is omitted from the updated multistate analyses, making those models difficult to interpret in terms of the added value of %p-tau217. Overall, the manuscript overstates the prognostic value of %p-tau217 while downplaying its limitations, fostering hype and unrealistic expectations for this biomarker.

Reviewer #2

(Remarks to the Author)
The authors have taken time and careful consideration in responding to all reviewers comments, and have edited the manuscript according to the reviewers requests. The work now represents a more careful assessment of the important data and results from this work.

Reviewer #3

(Remarks to the Author)
The authors have successfully responded to all my comments.

Version 2:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
The authors have provided comprehensive updates to the manuscript based around reviewer 1's questions, yet a couple of things are yet to be adequately clarified.

For reviewer comments (and responses) 1 through 4, no further alterations are necessary.

For reviewer comment 5, the authors did not completely answer the reviewers questions. Mainly, how many participants exactly who started the study with a CL<10 actually converted to positive during the time of the study? It was clear from the survival figure using 188 participants that there were very few who became positive over the time of the study, thus the number which the sample size was reduced to 123 may have been smaller, which affects the confidence of any prediction. The actual sample sizeof the number of participants who converted needs to be added here for context, suggested to add directly after the result is stated. Furthermore, any reference to this result in the discussed needs to be moderated such that the inference is contained with respect to the sample size.

For reviewer comment 6: There seems to be some confusion around cut-off values previously used compared to conversion factors etc etc. Then the authors create a cohort specific cut off for some specific analysis, which seemed to "suit" their results quite well. The issue of different cut-off values is common across most biomarker assays, with differences in sample handling and pre-analytical factors, along with differences in the prevalence of Amyloid in the population all adding towards different cut off values per study population. This reviewer advises the authors to talk briefly in the discussion around the challenges of using different cut off values across studies, being clear about the use of assay version, any conversion factors which were/were not used in this data, and the benefits/drawbacks of using a study specific cut off.

For reviewer comment 7: This reviewer still has the opinion that the authors are overstating their opinion that changes in %pTau217 precede changes in Amyloid PET. The paragraph in the discussion beginning with: "The observation that the increase in %pTau217 can be seen even before “amyloid-positivity” suggests that the Aβ – tau interaction may begin well before Aβ plaque burden reaches the typical PET detection threshold. " The authors go on to say that their results that %pTau217 precede Amyloid and that this contradicts other works, is overstated as there is insufficient evidence from their work to support this claim, especially given the sample size of those which convert to AB positivity. This reviewer advises the authors to tone down/alter the statements, possibly adding a caveat as to the small sample size which they are deriving their findings from (especially when Figure 2C shows only 405 participants which move to AB+ from AB- with a %pTau217 value of >2.6).

Please note that Figure 4 has a typo on the word Bootstrap.

Reviewer #3

(Remarks to the Author)
I have no further comments.

We are grateful for the reviewers' thoughtful comments. We have thoroughly addressed all concerns raised, as documented in the following point-by-point response to the reviewer's comments. Our manuscript has been significantly improved with additional analyses (e.g., Cox proportional hazard ratio, multi-state modeling) and discussions (e.g., thorough discussion of the limitations of the presented cut points). We also made additional edits to conform to the editorial policy of the journal.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The present study investigated the associations between plasma p-tau217, measured using the C2N mass spectrometry method, and longitudinal changes in amyloid-PET, tau-PET, and cognitive decline in cognitively unimpaired individuals from the Harvard Aging Brain Study. Of particular relevance, the authors also examined whether plasma p-tau217 levels are associated with subsequent amyloid and tau accumulation among participants who were Aβ-PET negative at baseline (< 24 Centiloids), based on the hypothesis that plasma p-tau217 may serve as an earlier indicator of Alzheimer's disease pathology than amyloid-PET.

The study addresses an underexplored area, as little is currently known about predictors of amyloid-β positivity in cognitively normal individuals. However, given the uncertain clinical relevance of amyloid positivity alone, the potential impact of these findings for Alzheimer's disease therapeutics and patient care remains unclear.

Response: Amyloid-β (Aβ) positivity (by PET) in cognitively unimpaired (CU) older adults, especially when combined with elevated tau PET signals in the mesial temporal region or neocortex, is a strong predictor of cognitive decline and clinical progression to MCI or dementia (e.g., references 12 and 24-26). Our study shows that, in amyloid-negative (A-) CU older adults, plasma %pTau217 can predict future accumulation of both Aβ and mesial temporal (entorhinal) tau as measured by PET. These results support the use of plasma %pTau217 in CU older adults to predict the future emergence of high-risk Alzheimer's disease (AD) biomarker profiles even before amyloid PET abnormalities emerge. We believe this finding is significant for earlier risk stratification and the design of primary AD prevention trials (i.e., clinical trials testing interventions before AD pathology develops), especially given the clinical trial results suggesting that earlier intervention might lead to a greater clinical benefit (doi: 10.1001/jama.2023.13239 [donanemab phase 3]).

Still, we agree with the reviewer that these findings cannot be directly applied to patient care and Alzheimer's therapeutics yet, because whether treating or preventing AD pathology in CU individuals would decrease the risk of MCI or dementia is actively being investigated through clinical trials (e.g., AHEAD 3-45, TRAILBLAZER-ALZ 3). We clarify these points in the revised manuscript as follows.

"These results suggest that changes in plasma %pTau217 likely precede changes in Aβ PET, and that very low levels of plasma %pTau217 indicate a low risk of substantial Aβ and tau accumulation in the following several years. Given that elevated Aβ and tau PET levels in CU older adults are strong predictors of future cognitive decline, MCI, and dementia,[24-26] our observations support the use of plasma pTau217 for early identification of at-risk individuals who can be enrolled in primary AD prevention studies."
(Discussion; page 11, lines 374 – 379)

"Third, our findings do not yet support the widespread use of plasma pTau217 screening in CU older adults in routine clinical practice, because it remains unknown whether preventing or treating AD

pathology in high-risk CU individuals would lead to slowing of clinical decline; this is actively being investigated through ongoing clinical trials.[13,31]"
(Discussion; page 13, lines 456 – 459)

Overall, the manuscript is well written and easy to follow, although some minor typos are present. The authors conclude that plasma p-tau217 changes early—earlier than amyloid-PET—and that baseline plasma p-tau217 levels could predict amyloid and tau accumulation among initially amyloid-negative unimpaired individuals.

However, this conclusion is not fully supported by their analyses, which, in my opinion, overlook other important predictors of amyloid-PET conversion that previous studies have found to be more strongly associated with progression to amyloid positivity.
1) Across all analyses, the authors do not consider what is likely the strongest predictor of future Aβ-PET positivity: baseline Centiloid values. Previous work by investigators at the Mayo Clinic, addressing this same research question, demonstrated that baseline Centiloid levels among amyloid-negative unimpaired individuals were actually a better predictor of future amyloid positivity than plasma %p-tau217 measured by mass spectrometry (doi: 10.1002/alz.14629). This raises an important and practical question: if readily available baseline information from the amyloid-PET scan can better predict progression risk, why should an additional, expensive mass-spectrometry plasma assay be performed? The authors should directly address this issue and clarify the potential incremental value, if any, of plasma p-tau217 over baseline PET metrics.

Response: We agree with the reviewer that the baseline Aβ (PiB tracer in our study) PET Centiloid (CL) value is a strong predictor of future amyloid positivity defined with the same modality (Aβ PET > 24.1 CL). Baseline Aβ CL value closer to the cut point (e.g., values between 20 and 24.1 CL) would be an especially strong predictor of progression to A+ in the next few years.

To address this important point, we performed Cox regression analyses with Aβ progression as an outcome, including models adjusting for baseline Aβ CL value. Then, we performed additional analyses limited to individuals with baseline Aβ<20 CL and <10 CL to examine the relative importance of plasma %pTau217 and baseline Aβ PET in predicting future progression to A+.

"Notably, after additionally adjusting for baseline Aβ CL, baseline %pTau217 was no longer associated with Aβ progression (p=0.31), while baseline Aβ was a stronger predictor of Aβ progression in this model (HR per 1 CL increase in Aβ PET=1.22, 95% CI 1.13 to 1.32, $p=3.9\times10^{-7}$). This observation was likely driven by individuals with their baseline Aβ burden closer to the A+ threshold: after excluding 10 participants whose baseline Aβ was greater than 20 CL but less than cut point (24.1 CL), higher baseline %pTau217 predicted progression to A+ (n=178, HR=1.52, 95% CI 1.06 to 2.18, p=0.023), while the association between baseline Aβ CL and progression to A+ became numerically weaker (HR=1.17, 95% CI 1.06 to 1.28, $p=1.4\times10^{-3}$). Further, among those with baseline Aβ less than 10 CL, baseline %pTau217 predicted Aβ progression (n=HR=2.67, 95% CI 1.17 to 6.11, p=0.020) while baseline Aβ did not (p=0.19). Thus, despite the same-modality advantage Aβ PET has in predicting progression to A+, plasma %pTau217 demonstrated additional value in predicting the progression to A+, especially in those with lower baseline Aβ levels (e.g., <20 CL)."
(Results; pages 5-6, lines 132 – 151)

2) Another important issue is the high number of ad-hoc points used in the study. In the Results section, the authors state, "These values were very close to previous reported AUC (0.94) and %pTau217 cut point (4.2%) to identify Aβ PET >25 CL using the same %pTau217 assay in a different cohort,21

demonstrating consistent performance of the plasma %pTau217 in detecting elevated cortical Aβ burden." However, this interpretation appears selective and potentially misleading. Different cut-points for the C2N assay have been reported across studies, reflecting variability in assay performance depending on the cohort and context. For example, in a recent publication in JAMA (doi: 10.1001/jama.2024.13855), a cut-point of 3.26% was proposed. Based on the distributions shown in Figure 1a of the present manuscript, applying a 3.26% threshold would likely lead to a substantial increase in false positive cases, significantly altering the author's results. Citing only studies that align with the authors' preferred cut-point may give a false sense of consistency. Another example of cut-point variability is the AHEAD study (doi: 10.1002/alz.13542), with a cut-point of 1.55% which again highlights the broader issue of variability and lack of universal agreement on plasma p-tau217 thresholds across cohorts. The authors should avoid making strong claims about the reliability and generalizability of their chosen cut-points.

Response: We thank the reviewer for bringing up this crucial point in biomarker research. To increase reliability and generalizability, in the revised manuscript, we now use 4.2% (C_2N's v2 assay cut-off recommended for clinical use)—instead of 4.3% we derived—as the cut point to differentiate A+ from A-. We chose to utilize the standard 4.2% cut point, not only because it is a widely used cut off in the clinics, but was also derived using the same assay version (C_2N %pTau217 v2 assay), a similar gold standard measure (Aβ PET Centiloid >25 CL), and the same approach (value maximizing Youden's index), even though the study population was different (MCI or dementia) (reference 21).

By contrast, other previously reported C_2N cut points (e.g., doi: 10.1001/jama.2024.13855 (Palmqvist et al., reference 5), doi: 10.1002/alz.13542 (Rissman et al., reference 16)) have important differences compared to the currently used clinical cut point.

1. Reference 5: This study defined the cut point (3.26%) as the value that achieves 90% specificity in identifying those with positive CSF AD biomarkers. This study was conducted among memory clinic patients. Given the different methods of deriving optimal cut points and the use of different gold standards (CSF vs. PET), this threshold is not directly comparable to reference 21.
2. Reference 16: This study defined the cut point (1.55%; using C_2N v1 assay) as the value maximizing Youden's index to identify those with Aβ CL > 20. Importantly, the values reported from the v1 and v2 assays are not interchangeable; 1.55% from the v1 assay corresponds to 3.9% in the v2 assay. Considering the different gold standards utilized (Aβ PET Centiloid >20 CL vs. >25 CL in reference 21), we expect this value to be lower than the 4.2% reported in reference 21. We note that the current v2 assay (used in our study) has been fully validated according to CLIA standards, with improved robustness compared to v1.

We have edited Fig. 1a-c (changed the 4.3% cut point to 4.2%), and edited the manuscript as follows.

"Receiver operating characteristics (ROC) area under the curve (AUC) to classify Aβ status (A+ vs. A-, with Aβ CL cut point of 24.1 Centiloid [CL]) with %pTau217 was 0.94 (**Fig. 1b-c**). Using the cut point of 4.2% recommended for clinical C_2N v2 assay interpretation,[21] sensitivity and specificity to detect A+ were 0.89 and 0.90, respectively, demonstrating consistent performance of the plasma %pTau217 in detecting elevated cortical Aβ burden."
(Results; pages 4 – 5, lines 101 – 108)

"Of note, among different %pTau217 cut points to detect AD pathology,[5,16,21] we chose to test the 4.2% cut point in our dataset because it is a manufacturer-recommended cut point for clinical use, and was derived using the same version of the assay (C_2N v2 assay) and gold standard (Aβ PET) as our study."
(Methods; page 14, lines 499 – 502)

Please also see our response to Reviewer #1 Comment 5 on the choice and limitations of the 2.6% cut point.

3) Figure 2 does not display any of the data points (edges, arrowheads) that are described in the figure legend.

Response: We apologize for the oversight in PDF format conversion errors. This was addressed in this revised submission.

4) The authors state, "We conducted survival analyses across three groups categorized by baseline %pTau217 tertile (within the baseline A- subgroup only) and found that the survival probably (i.e., remaining A-) at 6 years from the baseline was 0.98 (95% CI 0.95 to 1.0) for participants in the first (lowest) %pTau217 tertile and 0.72 (95% CI 0.60 to 107 0.87) for those in the third (highest) %pTau217 tertile." However, the use of arbitrary tertile-based categorization is unnecessary and statistically suboptimal. The authors have already defined an ad hoc cut-point for %pTau217; if the goal is to assess the association between continuous %pTau217 levels and risk of progression to amyloid positivity, a Cox proportional hazards regression using %pTau217 as a continuous predictor would be a more appropriate and powerful approach. Trichotomization reduces statistical power, introduces artificial thresholds, and can obscure meaningful dose-response relationships. I recommend reanalyzing the data using a continuous model to more accurately capture the relationship between plasma %pTau217 levels and risk of Aβ-PET conversion.

Response: We appreciate the reviewer's suggestion. We additionally presented Cox regression results as follows:

"We additionally used a Cox regression model adjusting for *APOE* ε4, age, and sex, and observed a consistent result: higher baseline %pTau217 was associated with higher risk of progression to A+ (n=188, hazard ratio [HR] per 1% increase in %pTau217= 1.40, 95% CI 1.13 to 1.72, $p=1.7\times10^{-3}$), while *APOE* ε4, age, and sex were not associated with the risk of progression to A+ (all $p>0.05$)."
(Results; pages 5, lines 128 – 132)

"To evaluate the continuous dose-response relationship between baseline %pTau217 and the likelihood of A+ progression while also considering potential confounders, we used the following Cox regression models (R "survival" package):
[Progression to A+ (event)] ~ [baseline %pTau217] + [baseline age, sex, *APOE* ε4 status]
[Progression to A+ (event)] ~ [baseline %pTau217] + [baseline Aβ CL] + [baseline age, sex, *APOE* ε4 status]"
(Methods; pages 16, lines 553 – 559)

We decided to leave the Kaplan-Meier curve (Fig. 1g) in the manuscript, as we believe it provides a helpful visualization, including event (A+ progression) counts at each given time point. However, we will remove the K-M curve if the editor or reviewer feels that this graph undermines the rigor of our study or can be misleading. We have clarified that the tertile was chosen for illustration purposes as follows.

"Second, the cut points we defined in this study to examine the A+ progression—%pTau217 tertiles and the 2.6% cut point—are cohort-specific cut points selected for illustration purposes and do not represent a biologically meaningful threshold. While cut points are useful tools for operationalizing biomarker interpretation and application, they do not always capture the continuous nature of disease biology, especially when the trait of interest varies continuously without a clear transition point."

(Discussion; page 13, lines 451 – 456)

5) In line with the previous comment, the authors state, "Then, we utilized paired measures of longitudinal plasma %pTau217 and Aβ PET (from the same study years) to evaluate their coordinated changes (Fig. 2a, n=223). Here, we used %pTau217 cut point 127 that optimally predicts Aβ progression (2.6%) to define low- versus high-%pTau217 subgroups." The repeated use of different ad hoc cut-points throughout the analyses raises concerns about the practical utility and robustness of plasma %pTau217 as a biomarker. Defining new cut-points optimized for each subset or analysis not only risks overfitting but also significantly undermines the generalizability of the results to independent populations. In clinical practice, a biomarker intended for early detection or risk stratification must rely on stable, validated thresholds that perform consistently across different cohorts and settings. The authors should directly address this limitation and discuss the extent to which their findings are likely to generalize beyond the current sample.

Response: We acknowledge that the ad hoc cut point of 2.6% we derived to predict A- to A+ progression in our dataset might not be generalizable. We toned down our interpretation of this numeric cut point throughout the manuscript.

For example, we have reorganized our manuscript so that the derivation and application of the 2.6% cut point only appear in the second section of Results ("Longitudinal increase in plasma %pTau217 appears to precede A- to A+ progression" and Fig. 2). We also clarified that this cut point is a cohort-specific threshold, as follows.

"We utilized paired measures of longitudinal plasma %pTau217 and Aβ CL (from the same study years; n=223) to evaluate whether changes in these biomarkers over time were correlated. The overall trajectory of %pTau217 (x-axis) and Aβ CL over time followed a sigmoid curve, suggesting that plasma %pTau217 increases before substantial cortical Aβ accumulation (**Fig. 2a**). To examine this possibility, we first defined subgroups based on their baseline Aβ status (A+ vs. A-) and %pTau217 level (low vs. intermediate-high %pTau217).

To define the subgroup with low %pTau217, we derived a cut point for %pTau217 that optimally predicts Aβ progression in our study. The ROC AUC for baseline %pTau217 in predicting Aβ progression among the baseline A- subgroup was 0.73 (95% CI 0.62 to 0.84), and a %pTau217 cut point of 2.6% maximized Youden's index (sensitivity 0.75, specificity 0.66) (**Supplementary Fig. 1**)."
(Results; page 6, lines 157 – 166)

We also clarified that the 2.6% cut point should be interpreted as a cohort-specific threshold instead of a generalizable value that captures a meaningful biological transition.

"It is important to note that the 2.6% cut point should be interpreted as a cohort-specific threshold optimized for our study, rather than a definite and generalizable value: this value was chosen to illustrate the longitudinal %pTau217 – Aβ trajectory of the A- participants with %pTau217 much lower than the clinical A+ cut point (4.2%[21])."
(Results; page 7, lines 174 – 177)

"Notably, the A-/intermediate-high %pTau217 state can still revert to the A-/low-%pTau217 state, with 15% annual probability. While it is unclear whether this is driven by true biological fluctuations or noise in measuring lower pTau217 levels, there is an important practical implication: the cohort-specific 2.6% threshold used in this study is not a definite indicator of irreversible brain pathology progression."
(Results; page 8, lines 284 – 288)

We also added an expanded discussion of the limitations of the cohort-specific cut points we used (also see Reviewer #1 Comment 4)

"Second, the cut points we defined in this study to examine the A+ progression—%pTau217 tertiles and the 2.6% cut point—are cohort-specific cut points selected for illustration purposes and do not represent a biologically meaningful threshold. While cut points are useful tools for operationalizing biomarker interpretation and application, they do not always capture the continuous nature of disease biology, especially when the trait of interest varies continuously without a clear transition point."
(Discussion; page 13, lines 451 – 456)

6) The authors state "Among the 117 participants with baseline %pTau217 less than 2.6 and longitudinal Aβ PET data, only 2 (<2%) were A+ at baseline, and 8 (7%) progressed to 117 A+ during the study (NPV=0.91 [107/117]). Thus, those below the %pTau217 cut point of 2.6 at study entry–which is much lower than the cut point used to predict Aβ positivity on PET–were unlikely to be baseline A+ and also had very low risk of progression to A+ during follow-up. Conversely, individuals with %pTau217 between 2.6 and 4.3% could represent a subset of CU older adults who are currently A- but at an elevated risk of progression to A+." This interpretation is based on an ad hoc definition of a new cut-point (2.6%) optimized for the current dataset, without reference to previously established thresholds from independent studies. If the authors choose to define new cut-points, they should at least systematically compare their findings with prior large studies, such as the JAMA study (cut-point ~3.26%) and the AHEAD study (cut-point ~1.55%), which demonstrate substantial variability across cohorts in %pTau217 thresholds. Please avoid presenting conclusions based on internally derived cut-points without considering and contextualizing variability observed in other studies.

Response: We appreciate this feedback, and we have toned down the interpretation of the ad hoc 2.6% threshold. More specifically related to this comment, we have removed the quoted sentences (NPV calculation and emphasis on the 2.6% - 4.3% category) to avoid overinterpretation. We also removed the emphasis of "2.6%" from Discussion; the updated paragraph reads as follows.

"Importantly, our results suggest that individuals with very low levels of plasma pTau217 may not need frequent blood-based screening or Aβ PET. In contrast, participants with modestly elevated %pTau217 (intermediate range, still below the clinical cut point of 4.2%) represent a group with an intermediate risk of future AD, and may require more frequent screening, as increasing %pTau217 on serial screening was found in most cases that progressed to A+."
(Discussion; page 12, lines 415 – 419)

Please also see our response to Reviewer #1 Comment 5.

7) The authors state, "To identify factors associated with A- to A+ progression in the A-/high %pTau217 subgroup (intermediate risk), we used logistic regression with A- to A+ progression as a binary outcome and baseline %pTau217, APOE ε4, age, and sex as predictors. We then examined a model including annual %pTau217 change ([last value – baseline value]/[interval]) as an additional predictor." However, logistic regression is not the appropriate modeling approach in this context. Logistic regression treats the outcome as a binary event without accounting for differences in follow-up time across participants, which could bias the results. A Cox proportional hazards model would be more appropriate, as it explicitly accounts for time-to-event data and variable follow-up durations, allowing for a more accurate estimation of risk factors associated with A- to A+ progression.

Response: We appreciate this very helpful suggestion. We replaced the logistic regression with Cox regression. Using Cox regression also enabled us to directly use time-varying (time-dependent) %pTau217 in the analysis.

"Lastly, we examined the predictors of A+ conversion in the intermediate-high %pTau217 state, given heterogeneous outcomes associated with this state. In a Cox regression model in this subgroup of participants who are A-/intermediate-high-%pTau217 at baseline (n=68), longitudinal (time-varying) %pTau217 predicted the risk of A+ progression (HR=1.53, 95% CI 1.20 to 1.96, $p=7.4\times10^{-4}$); by contrast, neither baseline %pTau217, *APOE* ε4, age, or sex could predict A+ progression (all p>0.05). Thus, in this subgroup, serial %pTau217 measurement may help identify those at the highest risk of A+ progression."
(Results; page 8, lines 289 – 294)

8) The authors state, "To examine the correlation between the longitudinal relationship between the paired measures of %pTau217 and PiB CL, we used a qualitative approach. First, we assigned subgroups based on their baseline measures (high/low %pTau217 by high/low PiB CL; 4 subgroups) and assessed how the participants moved across the subgroups by the time of their final measures." However, there is no need to rely solely on a qualitative assessment for this type of analysis. A more rigorous and informative approach would be to apply a multistate model, which is specifically designed to evaluate transitions between defined states over time. Multistate modeling would allow the authors to quantify transition probabilities, estimate transition intensities, and account for varying follow-up times, providing much richer and more interpretable metrics regarding biomarker dynamics.

Response: We appreciate this excellent suggestion. We applied multistate modeling, and indeed, we were able to derive more interpretable metrics.

"Then, to quantitatively estimate the transition probability across the categories, we used multi-state modelling across three states: A-/low-%pTau217, A-/intermediate-high-%pTau217, and A+. There were three instances of A+ to A- regression (**Supplementary Table 3**). However, all three cases had their maximum Aβ just above the cut point (24.1<Aβ CL<26) before moving to A- states, making it difficult to rule out misclassification due to measurement errors. Thus, we excluded these three participants (leaving n=220) and set A+ as a terminal (absorbing) state. We calculated transition intensity (**Supplementary Table 4**) and annual transition probability (**Fig. 2f**) between the states. Most participants (>75%) were predicted to remain in their state within one year. The A-/low-%pTau217 state had a very low annual probability (<1%) of progressing to A+/high-%pTau217, while the A-/intermediate-high-%pTau217 state had a higher annual A+ progression risk (5.9%). Thus, plasma %pTau217 appears to rise before A+ progression. Notably, the intermediate-high %pTau217 state can still revert to the low-%pTau217 state, with 15% annual probability."
(Results; pages 7-8, lines 194 – 285)

"We then used multi-state modeling (R "msm" package) to quantitatively estimate transition intensity and annual transition probability between states. We examined three states: A-/low-%pTau217 ("state 1"), A-/intermediate-high-%pTau217 ("state 2"), and A+ ("state 3"); as A+/low-%pTau217 was rare, we combined it with A+/intermediate-high-%pTau217 to define a single A+ state. We excluded three individuals who transitioned from A+ to other states from the analyses, as their A+ classification was based on borderline-elevated values (all <26 CL) and misclassification due to measurement error could not be ruled out. Then, transition intensity and annual transition probability were estimated using bootstrapping confidence interval (n=1,000 simulations).
(Methods; page 17, lines 517 – 581)

9) "To examine the correlation between the longitudinal relationship between the paired measures of 349 %pTau217 and PiB CL, we used a qualitative approach." This sentence is a bit confusing, please consider rephrasing it.

Response: We rephrased the sentence as follows:
"To examine whether longitudinal changes in plasma %pTau217 and Aβ PET CL are correlated, we leveraged the paired measurements of %pTau217 and Aβ CL from the same study years."
(Methods; page 16, lines 563 – 564)

Reviewer #2 (Remarks to the Author):

Review notes for Nature communications paper April 17 2025

Title: Plasma phosphorylated tau 217 and longitudinal trajectories of Aβ, tau, and cognition in cognitively unimpaired older adults

The authors have competed a thorough investigation into the %pTau217 against both AB and tau PET in a well characterised CU impaired cohort. Plasma measurements were taken at multiple collection visits along with AB and tau PET for well-planned statistical analyses. Results demonstrate that the %pTau217 biomarker was associated with both AB accumulation, and progression from AB-PET– to AB-PET+, as well as early tau accumulation and cognitive decline. Results are well presented, and this reviewer has only minor points for follow up.

1. Sample size: table 1 has a sample size at baseline of 317, yet in the results section (line 83) the text refers to 318 participants. Please confirm which is correct.

Response: We apologize for this error. We examined 317 participants, and we corrected the main text.

"We examined 317 HABS participants who were CU at baseline and had plasma pTau217 and Aβ (^{11}C-Pittsburgh compound B [PiB]) PET data at baseline (**Table 1, Supplementary Table 1**)."
(Results; page 4, lines 98 – 99)

2. Sample size: It is clear that the sample sizes are different between those which were tested with the pTau217 assay and those which were scanned for AB, however it is unclear what the total sample size was for those which were scanned for tau (maybe this reviewer missed it). It would be helpful if A) in table 1 the number of participants who were T-/T+ at baseline was added, and B) a supplementary table which has the sample size for plasma, AB and tau PET at each time point, including how many were A-/A+ and T-/T+ at each visit.

Response: In HABS, Tau PET was introduced mid-study, and most participants (who agreed to get tau PET, n=233) had their first tau PET a few years after baseline (average of 2.6±1.7 years after baseline). The HABS does not use a uniform tau PET threshold to binarize T+ and T-. Unlike Aβ PET, which exhibits an intrinsic bimodal pattern, tau (FTP) PET measures in preclinical AD display a positively skewed unimodal distribution. Thus, tau PET cut points are highly dependent on the specific research questions and study population. Given Reviewer 1's comments related to ad hoc biomarker cut points, we decided not to define new T+ cut points for this study. Please note that all tau PET analyses in this manuscript use continuous tau PET SUVR and do not depend on a specific T+ threshold.

Nonetheless, we fully agree with the reviewer that it would be helpful for readers to see the overall tau PET characteristics of our study participants. We have added two lines to Table 1 (Italicized below), summarizing the tau PET characteristics of the study participants.

	All (n=317)	A- (N = 242)	A+ (N = 75)
Age, years (SD)	71.7 (8.0)	70.5 (8.1)	75.3 (6.2)
Females, n (%)	192 (61)	147 (61)	45 (60)
Education, yr, mean (SD)	15.9 (2.9)	15.8 (2.9)	16.1 (3.0)
White, n (%)	257 (81)	191 (79)	66 (88)
APOE ε4 carriers, n (%)	89 (28)	44 (18)	45 (60)
Mean length of follow-up, Years (SD)	8.0 (3.9)	8.2 (3.9)	7.5 (3.8)
Median MMSE (IQR)	29 (1)	29 (1)	29 (2)
Mean PiB cortical composite, Centiloid (SD)	*21.0 (26.9)*	*8.2 (7.0)*	*62.5 (25.6)*
Mean entorhinal cortex FTP SUVR[a] (SD)	*1.10 (0.12)*	*1.07 (0.088)*	*1.20 (0.17)*
Mean inferior temporal cortex FTP SUVR[a] (SD)	1.18 (0.097)	1.16 (0.066)	1.25 (0.15)
%pTau217 (SD)	3.6 (2.8)	2.5 (1.3)	7.30 (3.28)

Table 1. Baseline Participant Characteristics. PiB, Pittsburgh compound B (Aβ PET tracer); FTP, flortaucipir (tau PET tracer); SUVR, standardized uptake value ratio (cerebellar gray reference; partial volume corrected). [a]FTP PET was introduced mid-study, and the first available FTP PET data from each participant (available from n=233, with an average of 2.6±1.7 years after baseline) are summarized.

We also added a supplementary table summarizing the sample size for plasma, Aβ, and tau PET at each time point (including the number of A+ at each visit), as the reviewer suggested. We thank the reviewer for the excellent suggestions.

Study Year	Plasma %pTau217	PiB PET (A+)	FTP PET
1 (Baseline)	317	317 (75)	58
2	0	0 (0)	18
3	0	0 (0)	19
4	210	233 (70)	165
5	0	0 (0)	18
6	126	153 (47)	154
7	0	1 (1)	0
9	70	97 (31)	98
12	2	1 (0)	15

Supplementary Table 1. Sample size for plasma %pTau217, PiB PET, and FTP PET at each time point. The number of participants who were A+ at each time point was indicated in parentheses in the third column. Most PET and all blood sample collections were performed in study years 1, 4, 6, 9, and 12 ("imaging years"). FTP PET was introduced mid-study, and many participants had their first FTP PET in non-imaging years.

3. Figure 2: It would appear that the data within the plots on Figure 2 has not shown up on this version. Please double check that there are supposed to be dots and or lines on these plots and not just empty quadrants.

Response: We apologize for the error that occurred during the PDF conversion process during submission. The figure has been updated to show the dots and arrows.

Reviewer #3 (Remarks to the Author):

The manuscript by Yang et al report the associations between baseline plasma p-tau217 levels and longitudinal progression of amyloid and tau (as measured by PET) and cognitive decline in a cohort of cognitively unimpaired individuals at baseline. The authors show a good correlation between these plasma baseline levels and increases in amyloid and tau pathologies, even in subgroup of individuals with no pathology (as measured by PET) at baseline. Further, they showed that plasma levels seem to be increased before Ab-PET in these individuals at follow-up visits. Finally, they present a pathway analysis that suggests the direct and indirect associations between all these key changes during disease progression. This is a very nice study, using a very rich dataset which has the main biomarkers for Alzheimer's disease measured longitudinally for a relatively long follow-up. Further, the focus on cognitively unimpaired individuals makes it very relevant for the current situation in which the field is focusing on earlier stages of the disease. The manuscript is well-written and well structured.

The authors suggest that based on their results, longitudinal p-tau217 levels may be usefull to asess who is going to become Ab-positive. Nonetheless, no analyses on this regard are performed, although there seems to be the data necessary to support this claim. I'd suggest to include such analysis to strengthen this point.

Response: We have added multi-state modeling analyses to further analyze longitudinal dynamics of plasma %pTau217 and Aβ PET. The results indicate that plasma %pTau217 likely increases before A+ progression.

"Then, to quantitatively estimate the transition probability across the categories, we used multi-state modelling across three states: A-/low-%pTau217, A-/intermediate-high-%pTau217, and A+. There were three instances of A+ to A- regression (**Supplementary Table 3**). However, all three cases had their maximum Aβ just above the cut point (24.1<Aβ CL<26) before moving to A- states, making it difficult to rule out misclassification due to measurement errors. Thus, we excluded these three participants (leaving n=220) and set A+ as a terminal (absorbing) state. We calculated transition intensity (**Supplementary Table 4**) and annual transition probability (**Fig. 2f**) between the states. Most participants (>75%) were predicted to remain in their state within one year. The A-/low-%pTau217 state had a very low annual probability (<1%) of progressing to A+/high-%pTau217, while the A-/intermediate-high-%pTau217 state had a higher annual A+ progression risk (5.9%). Thus, plasma %pTau217 appears to rise before A+ progression. Notably, the intermediate-high %pTau217 state can still revert to the low-%pTau217 state, with 15% annual probability."
(Results; pages 7-8, lines 194 – 285)

Further, we have additionally performed Cox regression to assess the utility of longitudinal %pTau217 in predicting A+ progression.

"Lastly, we examined the predictors of A+ conversion in the intermediate-high %pTau217 state, given heterogeneous outcomes associated with this state. In a Cox regression model in this subgroup of participants who are A-/intermediate-high-%pTau217 at baseline (n=68), longitudinal (time-varying) %pTau217 predicted the risk of A+ progression (HR=1.53, 95% CI 1.20 to 1.96, $p=7.4\times10^{-4}$); by contrast, neither baseline %pTau217, *APOE* ε4, age, or sex could predict A+ progression (all p>0.05). Thus, in this subgroup, serial %pTau217 measurement may help identify those at the highest risk of A+ progression."
(Results; page 8, lines 289 – 294)

In the manuscript, we performed this analysis focused on the intermediate-high %pTau217 subgroup, as those with low %pTau217 had a very low chance of A+ progression. Nonetheless, we note that the results are similar when we include all A- individuals regardless of their baseline %pTau217 level: longitudinal (time-varying) %pTau217 predicted the risk of A+ progression (HR=1.47, 95% CI 1.26 to 1.73, $p=1.9\times10^{-6}$); neither baseline %pTau217, *APOE* ε4, age, or sex could predict A+ progression (all $p>0.05$).

Regarding the PPV and NPV values presented in the text, it would be worth mentioning that the cutoffs to extract those statistics were obtained from the same sample, which reduces the generalisability of the results.

Response: We agree with this point. To avoid overinterpretation, we removed the NPV calculation that relies on the 2.6% cut point derived from our dataset. We have also included extensive discussions on the limitations of the ad hoc cut point of 2.6% used in Fig. 2 and related analyses. Please see our response to Reviewer #1 Comments 5 and 6.

The authors comment on the Ab-independent pathways of tau accumulation based on analyses done in Ab-negative individuals, but the association between ptau217 and tau accumulation could be also due to a larger dynamic ranges of p-tau217 levels compared to Ab-PET. This should be discussed.

Response: We thank the reviewer for this point. We have added this possibility as an alternative explanation.

"Further, in those with low fibrillar Aβ burden, %pTau217 may also capture Aβ-independent pathways leading to early mesial temporal tau accumulation, although the larger dynamic range of %pTau217 compared to Aβ PET in this subgroup might have driven the results."
(Results; page 9, lines 335 – 337)

Do the authors have any explanation on why some subjects (especially in the A-low/ptau high group) become p-tau217 low after having a high baseline level?

Response: We appreciate the reviewer's point. We added our interpretation of this interesting observation as follows.

"Notably, the A-/intermediate-high %pTau217 state can still revert to the A-/low-%pTau217 state, with 15% annual probability. While it is unclear whether this is driven by true biological fluctuations or noise in measuring lower pTau217 levels, there is an important practical implication: the cohort-specific 2.6% threshold used in this study is not a definite indicator of irreversible brain pathology progression."
(Results; page 8, lines 284 – 288)

In figure 1a there are few individuals with high Ab-PET load (>50CL) but low p-tau217 levels. It would be good if the authors could give some details of these individuals (e.g., any specific genetic form, comorbidities, etc).

Response: We agree with the reviewer that these participants are intriguing. We included their information in **Supplementary Table 2**. Interestingly, all three of them were male and had normal kidney function, although we could not draw robust conclusions from only three cases. Further, all their follow-up %pTau217 values were higher, making it difficult to rule out false negatives at baseline rather than

biologically driven Aβ PET - %pTau217 mismatch. In the revised manuscript, we included the following information about these three interesting participants while intentionally leaving out any speculative interpretation, given the very small number of observations in this category.

"Notably, three participants had high Aβ PET signals (PiB CL>50 CL) but plasma %pTau217 levels below 4.2% at baseline (**Fig. 1b**); two of these participants had subsequent %pTau217 measures, all of which were greater than 4.2% (**Supplementary Table 2**)."
(Results; page 5, lines 108 – 111)

Participant	Age	Sex	*APOE*	Serum Creatinine	PiB CL	Baseline %pTau217	Follow-up %pTau217 (Study Year)
A	70.5	M	ε3/ε3	0.99	95.7	3.63	5.36 (4), 10.63 (6), 9.60 (9)
B	68.8	M	ε3/ε4	1.04	72.9	1.25	5.06 (4)
C	72.5	M	ε3/ε4	0.79	71.0	1.88	NA

Supplementary Table 2. Characteristics of baseline high-PiB/low-intermediate %pTau217 participants. Baseline characteristics of 3 outliers with high PiB CL (>50 CL) and low-intermediate %pTau217 (≤4.2%) are summarized. Notably, both participants with available follow-up %pTau217 data had high %pTau217 (>4.2%) on subsequent measures. The study year indicates the visit year (e.g., baseline = 1, 3 years from baseline = 4). NA=not applicable (missing data).

Figure 4b show positive trends between p-tau217 levels and PACC change. However, in the text the beta presented is negative. Could the authors explain this?

Response: We apologize that there was an error in the R script used for plotting Fig. 4b. We confirmed that the beta presented in the text is correct (negative; decreased slope with increasing baseline %pTau217). We have corrected this error and replaced Fig. 4b.

Minor suggestions:
-Can the authors clarify what does the second table in fig 1g refers to?

Response: We apologize that this was not clear in the original version. We added the description in the figure legends as follows:

"The number of at-risk (upper table) and cumulative events per group (lower table) at years 3, 6, 9 are noted below the plot."
(Fig. 1g legend)

-The overall statistics of the path analysis should be presented somewhere.

Response: We agree with the reviewer that the overall statistics (e.g., chi-square, model fit indices) could be informative in path analysis. However, the path analysis we performed in our manuscript used a just-identified (saturated) model, and thus, overall model fit indices are not interpretable. We appreciate this point, though, and we have clarified it in the Methods section.

"Since the model we specified is a just-identified (saturated) model, and the overall model fit indices are not informative, our focus was on the path coefficients."
(Results; page 17, lines 588 – 589)

-In the path analysis figure, it would be good to indicate that Ab and tau refer to longitudinal changes.

Response: We appreciate the reviewer's suggestion. The change was made in the revised Fig. 4.

Comment 1: "Amyloid-β (Aβ) positivity (by PET) in cognitively unimpaired (CU) older adults, especially when combined with elevated tau PET signals in the mesial temporal region or neocortex, is a strong predictor of cognitive decline and clinical progression to MCI or dementia (e.g., references 12 and 24-26)."
Reviewer Response 1: Amyloid PET positivity alone is a weak predictor of clinical progression in cognitively unimpaired (CU) individuals. In the Mayo Clinic Study of Aging (doi:10.1001/jamaneurol.2018.0629), fewer than 20% of A+ CU participants progressed to MCI or dementia over five years. By contrast, tau PET positivity—present in only ~25% of A+ CU—identifies the subgroup at highest risk and largely drives subsequent clinical decline (doi:10.1001/jama.2025.7817).

Author Response #1: We appreciate the reviewer's concern and, more broadly, the ongoing controversy in the field regarding the extent to which PET-based amyloid positivity (A+) alone is a significant predictor of clinical progression. We would note that a recent multi-center study reported MCI/dementia progression rates for A+ CU of > 30% over 5 years (e.g., doi: 10.1056/NEJMoa2305032), and >50% progression in people with very high levels of amyloid (e.g., doi: 10.14283/jpad.2024.122). Also, according to the cited JAMA study (doi:10.1001/jama.2025.7817), A+/T- CUs have a higher risk of MCI or dementia compared to A-/T- CUs (HR=2.1, 95% CI 1.5 – 3.2). Nevertheless, we agree that the combination of amyloid and tau pathology is the strongest predictor of imminent decline. We agree that the language needs to be better qualified in our discussion, and that it is advisable not to refer to A+ as "strong" predictor given the scope of this paper and the data presented.

Therefore, we have removed the following potentially problematic sentence from our discussion: "Given that elevated Aβ and tau PET levels in CU older adults are strong predictors of future cognitive decline, MCI, and dementia,[24-26] our observations support the use of plasma pTau217 for early identification of at-risk individuals who can be enrolled in primary AD prevention studies." (Discussion, Lines 253-255)

Comment 2: "Our study shows that, in amyloid-negative (A-) CU older adults, plasma %pTau217 can predict future accumulation of both Aβ and mesial temporal (entorhinal) tau as measured by PET. These results support the use of plasma %pTau217 in CU older adults to predict the future emergence of high-risk Alzheimer's disease (AD) biomarker profiles even before amyloid PET abnormalities emerge."
Reviewer Response 2: The reported effect sizes for tau accumulation in the entorhinal cortex are very small (4.6×10^{-3} SUVR/year per unit of %p-tau217). While the association may reach statistical significance, the effect size is so modest that it remains unclear whether %p-tau217 meaningfully predicts which individuals will transition into the high-risk state of being tau PET–positive, particularly when applying the relatively stringent cut-offs or visual reads used in the referenced studies.

Author Response #2: We agree with the reviewer that the annualized effect size for the association between baseline %pTau217 and longitudinal entorhinal tau PET is modest, despite being statistically significant. We apologize that our interpretation or discussion might not have made this clear enough, and we will edit these sentences as follows.

We will change the following: “Together, these results demonstrate that greater plasma %pTau217 is associated with fibrillar tau accumulation visible via FTP tau PET even in individuals with sub-threshold levels of Aβ PET.” (Lines 196-198)

To: “Together, these results demonstrate that greater plasma %pTau217 is modestly associated with fibrillar tau accumulation, even in individuals with sub-threshold levels of Aβ PET.”

We will remove the following:
“Given that elevated Aβ and tau PET levels in CU older adults are strong predictors of future cognitive decline, MCI, and dementia,[24-26] our observations support the use of plasma pTau217 for early identification of at-risk individuals who can be enrolled in primary AD prevention studies.” (Discussion, Lines 253-255)

Comment 3: “We believe this finding is significant for earlier risk stratification and the design of primary AD prevention trials (i.e., clinical trials testing interventions before AD pathology develops), especially given the clinical trial results suggesting that earlier intervention might lead to a greater clinical benefit (doi: 10.1001/jama.2023.13239 [donanemab phase 3]).”
Reviewer Response 3: All participants in the donanemab phase 3 trial were required to be tau PET–positive on visual interpretation with flortaucipir, representing primarily individuals with high AD neuropathologic change (ADNC). Therefore, citing this study to argue for treating individuals with subthreshold amyloid levels is speculative and not supported by the available evidence. Moreover, with such a poor predictive power, it is unclear whether such prevention trials would be feasible to demonstrate a slowing of amyloid accumulation, let alone demonstrating clinical efficacy.

Author Response #3: We agree with the reviewer that the passage cited could be misinterpreted in the way the reviewer suggests, which was not our intention. We will make the following changes to address this important concern:

We will change the following: “AD includes up to two decades of asymptomatic or preclinical stage when the pathophysiology progresses before clinical symptom onset,[9-11] and clinical trial results suggest that earlier intervention might lead to greater clinical benefit.[3] Thus, preclinical AD represents a unique window of opportunity to prevent irreversible neurodegeneration and progression to symptomatic AD. Accordingly, prevention trials targeting AD pathophysiology in cognitively unimpaired (CU) participants have been completed or are ongoing.[12,13]” (Introduction, lines 54-59)

To: “AD includes up to two decades of asymptomatic or preclinical stage when the pathophysiology progresses before clinical symptom onset.[9-11] As a successful disease-modifying intervention in the preclinical stage of AD might prevent irreversible neurodegeneration and progression to symptomatic AD, prevention trials targeting AD pathophysiology in cognitively unimpaired (CU) participants have been completed or are ongoing.[12,13]”

Comment 4: "Third, our findings do not yet support the widespread use of plasma pTau217 screening in CU older adults in routine clinical practice, because it remains unknown whether preventing or treating AD pathology in high-risk CU individuals would lead to slowing of clinical decline; this is actively being investigated through ongoing clinical trials"
Reviewer Response 4: This is incorrect. The primary reason plasma p-tau217 is not appropriate for routine screening in CU older adults is its limited prognostic value: a positive plasma result, by itself, does not identify a high-risk state for clinical progression. This is independent of the current uncertainty about the clinical benefits of very early intervention.

Author Response #4: We agree with the reviewer that pTau217 should not be used for screening in clinical practices. While we think there is sufficient room for multiple interpretations for why pTau217 should not be used in this way, we fully recognize that the data we present here are not well-suited to fully address this controversial question. In this context, we will revise our manuscript as follows in order to address the point the reviewer raises (about the unclear prognostic value of pTau217 alone):

We will change the following: "Third, our findings do not yet support the widespread use of plasma pTau217 screening in CU older adults in routine clinical practice, because it remains unknown whether preventing or treating AD pathology in high-risk CU individuals would lead to slowing of clinical decline; this is actively being investigated through ongoing clinical trials.[13,31]" (Discussion, Lines 302-305)

To: "Third, our findings do not support the widespread use of plasma pTau217 screening in CU older adults, as further clinical and observational studies are needed to fully understand the prognostic utility of pTau217 alone, and how it may be combined with further confirmatory testing (e.g., amyloid PET) to best identify individuals at high-risk of progression."

Comment 5: "Notably, after additionally adjusting for baseline Aβ CL, baseline %pTau217 was no longer associated with Aβ progression (p=0.31), while baseline Aβ was a stronger predictor of Aβ progression in this model (HR per 1 CL increase in Aβ PET=1.22, 95% CI 1.13 to 1.32, p=3.9×10-7). This observation was likely driven by individuals with their baseline Aβ burden closer to the A+ threshold: after excluding 10 participants whose baseline Aβ was greater than 20 CL but less than cut point (24.1 CL), higher baseline %pTau217 predicted progression to A+ (n=178, HR=1.52, 95% CI 1.06 to 2.18, p=0.023), while the association between baseline Aβ CL and progression to A+ became numerically weaker (HR=1.17, 95% CI 1.06 to 1.28, p=1.4×10-3). Further, among those with baseline Aβ less than 10 CL, baseline %pTau217 predicted Aβ progression (n=HR=2.67, 95% CI 1.17 to 6.11, p=0.020) while baseline Aβ did not (p=0.19)."
Reviewer Response5: After incorporating baseline Centiloid (CL)—and confirming, consistent with prior studies, that baseline CL is the stronger predictor of progression to A+—the authors reanalyzed the data using a new a posteriori cut point (10 CL) that appears to align with their hypothesis. How many

participants with baseline amyloid <10 CL actually progressed to A+? I suspect this number is small (likely fewer than 7–8). Given the ad hoc/exploratory nature of this analysis—and the likely low event count—I recommend caution in asserting that p-tau217 predicts progression to A+ beyond what is already provided by amyloid PET.

Author Response #5: We agree with the reviewer that baseline amyloid PET is the strongest predictor of future increases in amyloid PET, as may be reasonably expected. As noted in the quoted comment above, we observed that the strong association between baseline amyloid PET and future progression to supra-threshold amyloid burden (A+) was partially driven by those whose initial amyloid PET was on the cusp of A+ transition (>20 CL). After excluding 10 participants whose baseline Aβ was greater than 20 CL but less than cut point (24.1 CL), higher baseline %pTau217 was associated with progression to A+ (n=178, HR=1.52, 95% CI 1.06 to 2.18, p=0.023). We agree with the reviewer that this association is weak, although it was nominally significant.

The <10 CL threshold was chosen to test the association of plasma pTau217 with PET biomarkers among those who are clearly in the A- range, as <10 CL would be considered "A-" by most study groups. We have included the analyses of the <10 CL subgroup in both the initial and revised submissions, and it is not a new post-hoc threshold that we added to fit the data. Nonetheless, we will remove the <10 CL subgroup result for this analysis if the reviewer and/or editor feels the results can be misleading.

In accordance with the reviewer's concerns, we will make the following changes to properly contextualize the data presented:

We will change the following: "Further, among those with baseline Aβ less than 10 CL, baseline %pTau217 predicted Aβ progression (n=HR=2.67, 95% CI 1.17 to 6.11, p=0.020) while baseline Aβ PET CL did not (p=0.19). (Lines 120-122)"

To: "Though the proportion of participants who progressed to amyloid PET positivity from low initial levels of amyloid PET signal was small, we did observe that greater baseline pTau217% was associated with greater risk of progression from baseline Aβ PET of less than 10 CL to amyloid positivity (>24.1 CL) during longitudinal follow-up (n=123, HR=2.67, 95% CI 1.17 to 6.11, p=0.020)."

And we will remove the following:
"Thus, despite the same-modality advantage Aβ PET has in predicting progression to A+, plasma %pTau217 demonstrated additional value in predicting the progression to A+, especially in those with lower baseline Aβ levels (e.g., <20 CL)." (Lines 122-124)

Comment 6: "This study defined the cut point (1.55%; using C2N v1 assay) as the value maximizing Youden's index to identify those with Aβ CL > 20. Importantly, the values reported from the v1 and v2 assays are not interchangeable; 1.55% from the v1 assay corresponds to 3.9% in the v2 assay. Considering the different gold standards utilized (Aβ PET Centiloid >20 CL vs. >25 CL in reference 21), we expect this value to be lower than the 4.2% reported in reference 21. We note that the current v2 assay (used in our study) has been fully validated according to CLIA standards, with improved robustness

compared to v1."
Reviewer Response 6: Thanks for clarifying this information.
In any case, it appears that the reporting of different cut-points does not limit to the aforementioned publications. For instance, in a previous publication in the WRAP study (doi: 10.1002/alz.14570), which focuses on CU individuals (see Supplementary Table 2), the reported cut-point for %p-tau217 was 2.66, which is significantly higher than cut-points reported for v1 and significantly lower than cut-points for v2.
Although the WRAP study does not report whether the assay version is v1 or v2 (as is common in publications employing this assay), the authors state that they applied conversion factors to update the results to their new platform, suggesting that v2 was most likely used:
"When C2N transitioned to the version of the p-tau217 assay that is used for the PrecivityAD2 test, an assay comparison study was performed which established conversion factors for converting the p-tau217, np-tau217, and %p-tau217 values from the prototype assay to the PrecivityAD2 assay. These conversion factors were applied to all the values from the prototype assay to allow comparison of values from this study to values obtained from the PrecivityAD2 assay."
Considering that v2 was used, how is it possible that, using very different cut-points (2.66 vs 4.2), the sensitivity and specificity reported in WRAP (0.86 and 0.9) is very similar to that reported in the present study (0.89 and 0.9)? Maybe the results in WRAP used another version of the assay that we were not aware of? Could it be that standardization of the %p-tau217 results across different settings and cohorts remains a challenge?

Author Response #6: We appreciate the reviewer's thorough comments on this important matter of assay version and test threshold selection.

From the WRAP study (doi: 10.1002/alz.14570)'s eMethods:
"C_2N plasma biomarker procedures and analysis
Concentration of p-tau217 and np-tau217 were measured using the prototype p-tau217 assay developed at C_2N Diagnostics, as described by Rissman and colleagues.[6] When C_2N transitioned to the version of the p-tau217 assay that is used for the PrecivityAD2 test, an assay comparison study was performed which established conversion factors for converting the p-tau217, np-tau217, and %p-tau217 values from the prototype assay to the PrecivityAD2 assay. These conversion factors were applied to all the values from the prototype assay to allow comparison of values from this study to values obtained from the PrecivityAD2 assay. Converted %p-tau217 values were also used to calculate the amyloid probability score 2 (APS2) the output of the PrecivityAD2 test. Development of the PrecivityAD algorithm is described elsewhere.[7]"

As the concentrations of pTau217 and npTau217 in this previous study were measured with the prototype pTau217 assay (v1), the values and cut points derived using these assays may differ from the v2 assay used here —even after conversion factors are applied. Our understanding is that the v2 assay uses improved protein standards compared to the v1 assay.

At the reviewer and/or editor's discretion, we are happy to revise the paper to include a discussion of this methodological point and its impact on the resulting threshold values obtained.

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Reviewer Response 7: In summary, the revision reiterates the claim that changes in %p-tau217 precede amyloid PET changes, but this remains insufficiently supported by the presented data. Although the authors now show that baseline Centiloid (CL) is the stronger predictor of progression to A+, this variable is omitted from the updated multistate analyses, making those models difficult to interpret in terms of the added value of %p-tau217. Overall, the manuscript overstates the prognostic value of %p-tau217 while downplaying its limitations, fostering hype and unrealistic expectations for this biomarker.

Author Response #7: The multi-state models referred to here were included in response to an earlier reviewer comment, and it was not our intent to generate unrealistic expectations for this or any other marker. As the states defined in the multi-state model are dependent on amyloid PET positivity, the inclusion of amyloid PET itself as a covariate generates models that are unstable and difficult to interpret. After discussion with the biostatistician for our study (Dr. Healy), we decided to remove the multi-state models that have led to this confusion or overstatement of the results. Importantly, these multi-state analyses were not part of the initial manuscript, and their inclusion was to address prior Reviewer 1 comments.

REVIEWER COMMENTS

Reviewer #2 (Remarks to the Author):

The authors have provided comprehensive updates to the manuscript based around reviewer 1's questions, yet a couple of things are yet to be adequately clarified.

For reviewer comments (and responses) 1 through 4, no further alterations are necessary.

For reviewer comment 5, the authors did not completely answer the reviewers questions. Mainly, how many participants exactly who started the study with a CL<10 actually converted to positive during the time of the study? It was clear from the survival figure using 188 participants that there were very few who became positive over the time of the study, thus the number which the sample size was reduced to 123 may have been smaller, which affects the confidence of any prediction. The actual sample sizeof the number of participants who converted needs to be added here for context, suggested to add directly after the result is stated. Furthermore, any reference to this result in the discussed needs to be moderated such that the inference is contained with respect to the sample size.

Author Response: We thank the reviewer for this valid point and have added the actual number of A- to A+ progression events (n=6 among 123 participants).

We edited the following:
"Though the proportion of participants who progressed to amyloid PET positivity from low initial levels of amyloid PET signal was small, we did observe that greater baseline %pTau217 was associated with a greater risk of progression from baseline Aβ PET of less than 10 CL to Aβ positivity (<24.1 CL) during longitudinal follow-up (n=123, HR=2.67, 95% CI 1.17 to 6.11, p=0.020)."

To:
"Moreover, greater baseline %pTau217 was associated with a higher risk of progression from baseline Aβ PET of less than 10 CL to Aβ positivity (n=123, HR=2.67, 95% CI 1.17 to 6.11, p=0.020); however, only 6 participants from this subgroup with very low initial Aβ PET progressed to amyloid PET positivity within the study period, and this result should be interpreted cautiously." (Lines 120 - 124 of the updated manuscript ["No Markup" version])

We have also confirmed that our Discussion does not include a separate interpretation of the <10 CL subgroup result.

For reviewer comment 6: There seems to be some confusion around cut-off values previously used compared to conversion factors etc etc. Then the authors create a cohort specific cut off for some specific analysis, which seemed to "suit" their results quite well. The issue of different cut-off values is common across most biomarker assays, with differences in sample handling and pre-analytical factors, along with differences in the prevalence of Amyloid in the population all adding towards different cut off values per study population. This reviewer advises the authors to talk briefly in the discussion around the challenges

of using different cut off values across studies, being clear about the use of assay version, any conversion factors which were/were not used in this data, and the benefits/drawbacks of using a study specific cut off.

Author Response: We thank the reviewer for this advice and have expanded the discussion of the limitations of the biomarker cut points.

We edited the following:
"Second, the cut points we defined in this study to examine the A+ progression—%pTau217 tertiles and the 2.6% cut point—are cohort- and assay-specific, selected for illustration purposes, and do not represent a biologically meaningful threshold. While cut points are useful tools for operationalizing biomarker interpretation and application, they do not always capture the continuous nature of disease biology, especially when the trait of interest varies continuously without a clear transition point."

To: "Second, the cut points we used are specific to the C_2N v2 %pTau217 assay (which is a component of the PrecivityAD2™ test with intended use in individuals with cognitive impairment). The manufacturer-established clinical cut point of 4.2%[21] is applicable to the assay used in this study, but cannot be directly applied to other assay platforms[6] or to earlier versions of the C_2N assay.[16] The lower cut points we used here to examine the A+ progression—%pTau217 tertiles and the 2.6% cut point—are assay- and cohort-specific cut points selected primarily for illustration purposes, and may not represent biologically meaningful thresholds. While cut points are useful tools for operationalizing biomarker interpretation and application, they do not always capture the continuous nature of disease biology, especially when the trait of interest varies continuously without a clear transition point." (Lines 273 - 282 of the updated manuscript ["No Markup" version])

For reviewer comment 7: This reviewer still has the opinion that the authors are overstating their opinion that changes in %pTau217 precede changes in Amyloid PET. The paragraph in the discussion beginning with: "The observation that the increase in %pTau217 can be seen even before "amyloid-positivity" suggests that the Aβ – tau interaction may begin well before Aβ plaque burden reaches the typical PET detection threshold. " The authors go on to say that their results that %pTau217 precede Amyloid and that this contradicts other works, is overstated as there is insufficient evidence from their work to support this claim, especially given the sample size of those which convert to AB positivity. This reviewer advises the authors to tone down/alter the statements, possibly adding a caveat as to the small sample size which they are deriving their findings from (especially when Figure 2C shows only 405 participants which move to AB+ from AB- with a %pTau217 value of >2.6).

Author Response: We appreciate the reviewer's comment and acknowledge that the discussion paragraph mentioned by the reviewer could be seen as overstating the knowledge that can be directly deduced from our observation. We have decided to remove the paragraph because it included a speculative hypothesis not directly supported by the current observational biomarker study's findings (e.g., "the early increase in plasma %pTau217 may reflect soluble Aβ (e.g., oligomers) related processes"). Removal of this paragraph should not change the overall impact of this study, as the key interpretation directly based on the study's results is discussed in the preceding paragraphs.

Thus, we have removed the following paragraph:
"The observation that the increase in %pTau217 can be seen even before "amyloid-positivity" suggests that the Aβ – tau interaction may begin well before Aβ plaque burden reaches the typical PET detection threshold. Our results showing that the %pTau217 increase may precede Aβ PET signal increase might seem to contradict a previous report of plasma pTau217 mediating Aβ PET – tau PET association[25] or even the amyloid hypothesis.[26] However, recent phase 3 clinical trials suggest that elevations of plasma pTau are likely causally downstream of brain Aβ, as removal of Aβ with anti-Aβ monoclonal antibodies was sufficient to decrease (normalize) plasma pTau181 and pTau217 levels[2,3]. This is consistent with our finding that higher %pTau217 might explain the association between higher baseline Aβ PET signal and subsequent tau PET signal increase (Table 2). Thus, based on previous model system studies,[26,27] we hypothesize that the early increase in plasma %pTau217 may reflect soluble Aβ (e.g., oligomers) related processes (e.g., tau phosphorylation in the neuropils) that underlie the accumulation of both fibrillar Aβ and tau pathology."

Please note that Figure 4 has a typo on the word Bootstrap.

Author Response: We apologize for the oversight, and the error has been corrected in the updated manuscript.

Reviewer #3 (Remarks to the Author):

I have no further comments.