

Controlled Head-to-Head Comparison of p-Tau 217, p-Tau 217/Aβ42 Ratio, and Multi-Analyte Blood Tests in a Diverse Community-Based Cohort

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Background

Plasma p-Tau 217 is a high-performing biomarker for Alzheimer's, but single-marker and ratio-based approaches (p-Tau 217/Aβ42) can exhibit cohort-dependent diagnostic volatility and critical blind spots in early-stage pathology. A multi-analyte framework may mitigate these limitations by integrating adjacent pathological signals. We conducted a controlled head-to-head comparison of a multi-analyte algorithmic assay integrating biomarkers for amyloid, tau, neurodegeneration, and neuroinflammation [ATN(I)] against p-Tau 217 and p-Tau 217/Aβ42, utilizing a single assay platform and PET imaging as the reference standard.

Methods

Bio-Hermes-002 Interim Analyses. Preliminary analyses were performed using interim plasma and PET imaging data from the ongoing Bio-Hermes-002 study (NCT06584357), a landmark community-based, multi-center study designed to evaluate fluid biomarkers and digital cognitive assessments across the Alzheimer's disease continuum in a real-world population. As the architectural successor to the original Bio-Hermes-001 study [1], this protocol leverages a global network of research sites specializing in Alzheimer's disease therapeutic development. Unlike pathology-enriched memory clinic cohorts, Bio-Hermes-002 intentionally captures earlier disease stages and a diverse population, and includes substantial representation of amyloid-positive, tau-negative (A+/T-) individuals.

Methods. The present analysis included participants with available amyloid PET (N = 384) and tau PET (N = 284) data. Every participant underwent specialist evaluation to populate one of three clinical cohorts: Cognitively Unimpaired (CU), Mild Cognitive Impairment (MCI), and mild Alzheimer's disease (AD). Participants also underwent amyloid and tau PET imaging, providing definitive pathological classification. To evaluate the utility of multi-analyte profiling, we compared three Simoa p-Tau 217-based assay configurations using two-cutoff designs to assess performance across the clinical and pathological (imaging) spectrum through "mild AD" and established amyloid and tau pathology (A+/T+). The three assay configurations were standalone p-Tau 217 (Janssen design), p-Tau 217/Aβ42 ratio, and LucentAD Complete, which integrates markers of amyloid pathology (Aβ42/40), tau pathology (Janssen p-Tau 217), neurodegeneration (NFL), and neuroinflammation (GFAP) to generate a single composite Amyloid Risk Score. The p-Tau 217/Aβ42 ratio cutoffs were set within this study cohort to approximate the established performance of the p-Tau 217 assay and LucentAD Complete (90% sensitivity/90% specificity). **Table 1** summarizes the three assay configurations compared. Discordant results between the ratio and algorithmic models were forensically examined to adjudicate accuracy and identify contributing biological factors.

Table 1: Assay Configurations and Cutoffs

Assay Configuration	Components	Diagnostic Cutoffs (Low/High)
P-Tau 217	p-Tau 217	0.04 – 0.09 pg/mL (Previously validated ⁹)
P-Tau 217/Aβ42 Ratio	p-Tau 217 + Aβ42	0.010 – 0.015 (Modeled to achieve 90% sensitivity and 90% specificity)
LucentAD Complete	Algorithmic multi-analyte, p-Tau 217, Aβ42/40, GFAP, NFL	Risk Score 45 – 70 (Previously validated ⁷)

Multi-Analyte Algorithm. LucentAD Complete is a 5-analyte plasma algorithm based on **ATN(I) pathophysiological framework** [2,3] representing four distinct biological disease axes (**Figure 1**). These include p-Tau 217 ("T": LucentAD p-Tau 217), amyloid β-42/40 ratio ("A": Aβ42/40), neurofilament light chain ("N": NFL), and glial fibrillary acidic protein ("I": GFAP). The latter four biomarkers are quantified utilizing the multiplexed Simoa N4PE kit. (*Note that two distinct biomarkers, Aβ42 and Aβ40, are used to calculate the amyloid ratio.*) The LucentAD Complete algorithm integrates these biomarkers via a logistic regression to generate a unified Amyloid Risk Score. Development and clinical validation of LucentAD p-Tau 217 and LucentAD Complete are described elsewhere [4, 5].

Figure 1. LucentAD Complete: Four-Axis "ATN(I)" Plasma Test

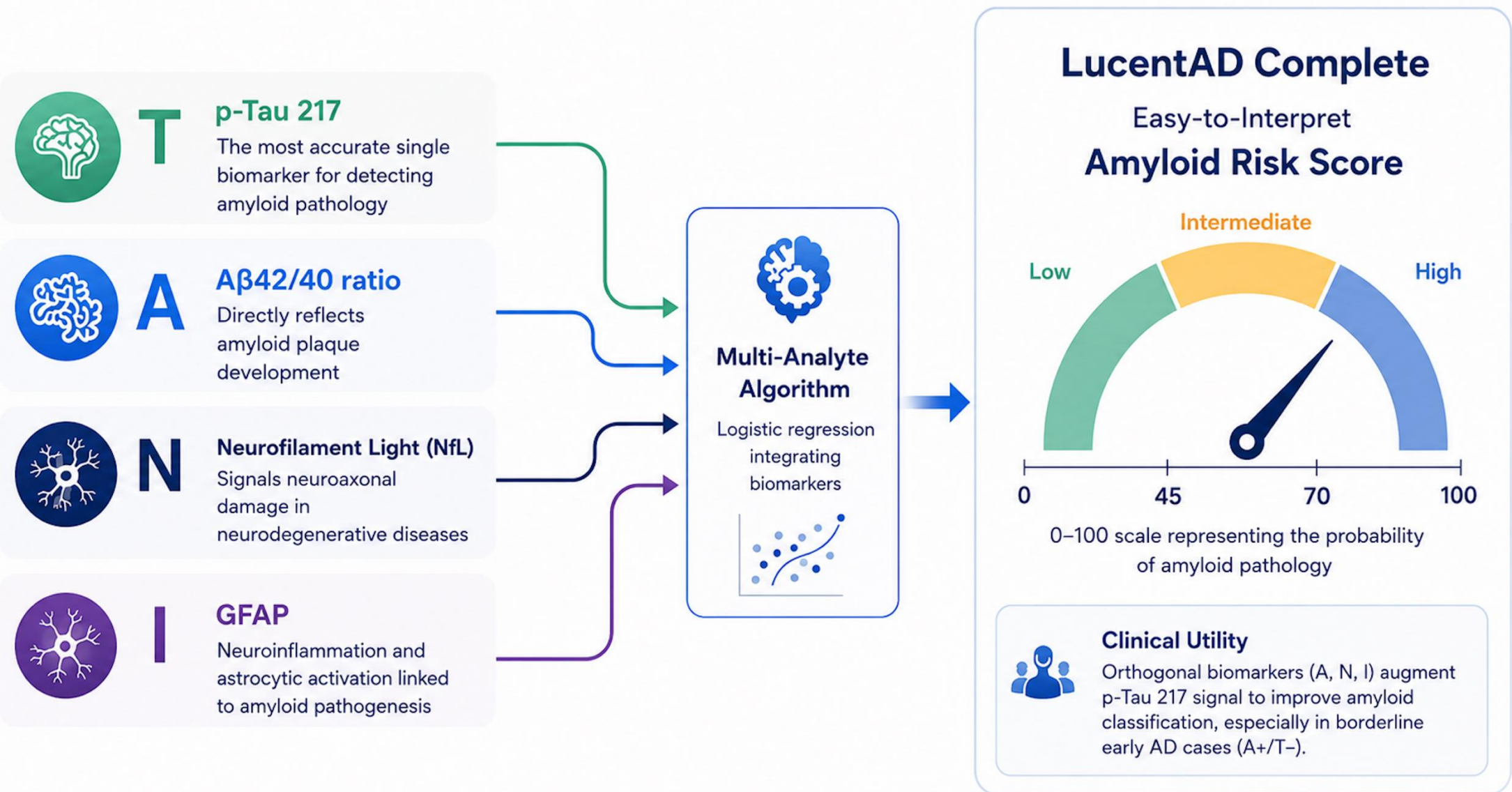


Figure 1. LucentAD Complete integrates four key Alzheimer's-relevant biomarkers representing the **ATN(I) framework** (Jack *et al*., Imbimbo *et al*) into a composite Amyloid Risk Score. p-Tau 217 is the backbone of the test, and the orthogonal biomarkers Aβ42/40 ratio, NFL, and GFAP augment the p-Tau 217 signal for amyloid classification in difficult borderline early AD cases (amyloid+, tau-, A+/T-). **The LucentAD Complete test report includes the Amyloid Risk Score and the individual ATN(I) biomarkers values alongside interpretive normative ranges for additional potential clinical insights.**

Results

Table 2: Comparative Diagnostic Performance (AUC) for Amyloid and Tau PET Status

Target Pathology	Method	AUC, [95% CI]	AUC, [95% CI]
Amyloid PET (A+ vs. A-)		Evaluable Cohort (N=384)	Symptomatic Only (N=233)
	LucentAD Complete	0.918 [0.894 – 0.942]	0.903 [0.864 – 0.942]
	p-Tau 217/Aβ42 Ratio	0.892 [0.864 – 0.920]	0.868 [0.825 – 0.911]
Tau PET (T+ vs. T-)		Evaluable Cohort (N=284)	Symptomatic Only N=184
	LucentAD Complete	0.902 [0.874 – 0.930]	0.914 [0.877 – 0.951]
	p-Tau 217/Aβ42 Ratio	0.873 [0.841 – 0.905]	0.882 [0.841 – 0.923]
		p-Tau 217	0.838 [0.803 – 0.873]
			0.852 [0.805 – 0.899]

Table 2: Areas Under the Curve, AUC across the full Bio-Hermes-002 interim cohort and symptomatic sub-populations. The 4-axis LucentAD Complete algorithm demonstrated higher classification accuracy for both amyloid and tau pathology compared to p-Tau 217 or p-Tau 217/Aβ42 ratio. *Note:* The reduced sample size for Tau PET analysis reflects the subset of the interim cohort that had completed both plasma collection and [18F]flortaucipir PET imaging at the time of data cutoff.

As depicted in **Figure 2**, the diagnostic performance of the three assay configurations was evaluated across the **clinical** sub-populations of the Bio-Hermes-002 study. These cohorts were classified by specialist assessment **prior** to PET imaging as "cognitively unimpaired", "mild cognitive impairment" (MCI), and "mild AD" based on NIH/clinical and cognitive assessment criteria.

Figure 2: Comparative Diagnostic Performance Across *Clinical* Sub-Cohorts

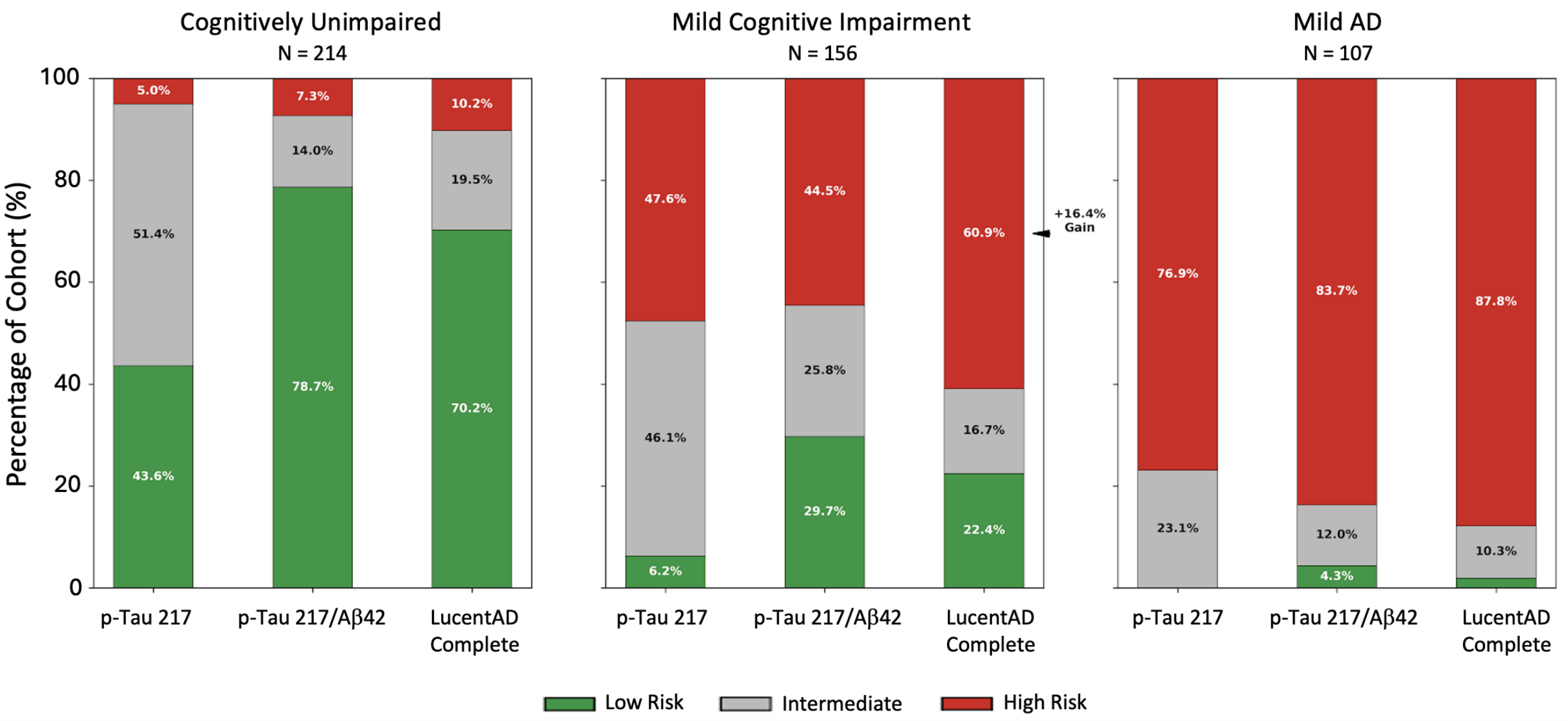


Figure 2. Distributions of Low (Green), Intermediate (Grey), and High Risk (Red) amyloid classifications across three p-Tau 217-based assay configurations. In the MCI cohort, the LucentAD 4-axis panel classified a much higher proportion of cases as High Risk (60.9%) while significantly reducing the Intermediate zone to 16.7%. For clinicians, this translates into **definitive Low or High-Risk classifications in 83.3% of MCI cases**, substantially reducing the fraction of patients left in the Intermediate zone. As patients progress into the Mild AD cohort, where cognitive symptoms are more pronounced, all three assays show improved performance; however, LucentAD Complete provided the highest resolution, with approximately 90% of participants receiving a definitive (Low or High Risk) classification.

Figure 3: Comparative Diagnostic Performance Across *Imaging* Sub-Cohorts

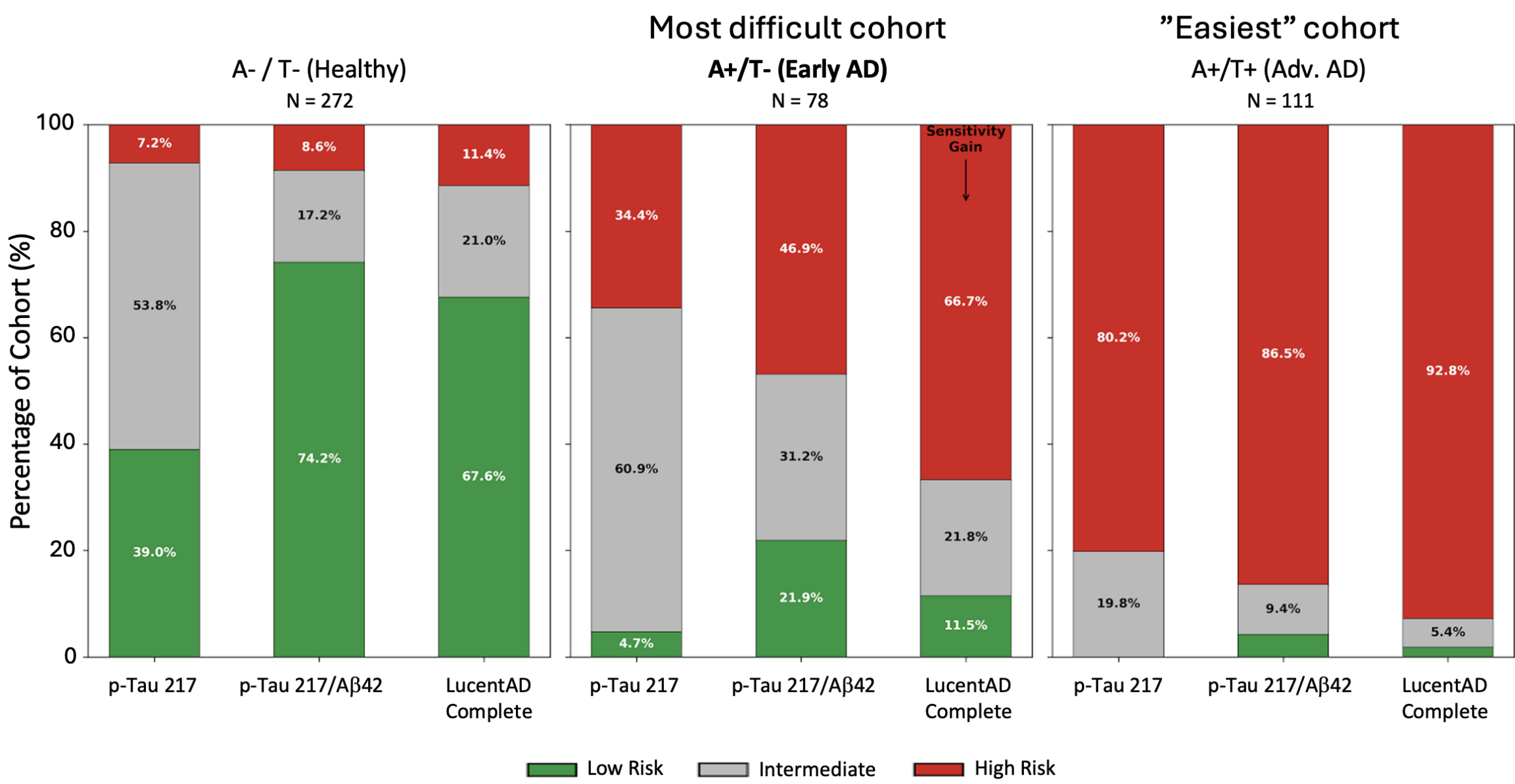


Figure 3. Distributions of Low (Green), Intermediate (Grey), and High Risk (Red) amyloid classifications. The **A+/T-** cohort (early Alzheimer's) represents the most challenging class of patients for plasma-based assays due to a weaker amyloid/p-Tau 217 signal vs. more advanced A+/T+ patients. In the A+/T- cohort, **LucentAD Complete correctly classified two-thirds of these patients vs. ~one-half for the p-Tau 217/Aβ42 ratio configuration.** The intermediate zone was also reduced from 31.2% to 21.8% vs. p-Tau 217/Aβ42 ratio.

Results (cont.)

Mechanism: Why LucentAD Complete Outperforms in Early AD

Since all three assay configurations utilize identical p-Tau 217 and Aβ42 values, and the diagnostic cutoffs represent ~90% sensitivity/90% specificity-level performance, the **higher performance of LucentAD Complete is driven by the integration of the orthogonal biomarkers for neuroinflammation (GFAP) and neurodegeneration (NFL).** **Figure 4** provides a case-by-case forensic breakdown of the four-axis 'rescue' mechanism. In this analysis of the challenging A+/T- cohort, there were 16 discordant cases among amyloid PET positives where LucentAD Complete outperformed the p-Tau 217/Aβ42 ratio by correctly classifying cases as a higher risk.

Figure 4: Four-Axis 'Rescue' Mechanism — LucentAD Complete vs. p-Tau 217/Aβ42 Ratio in Discordant, Amyloid PET-Positive Cases

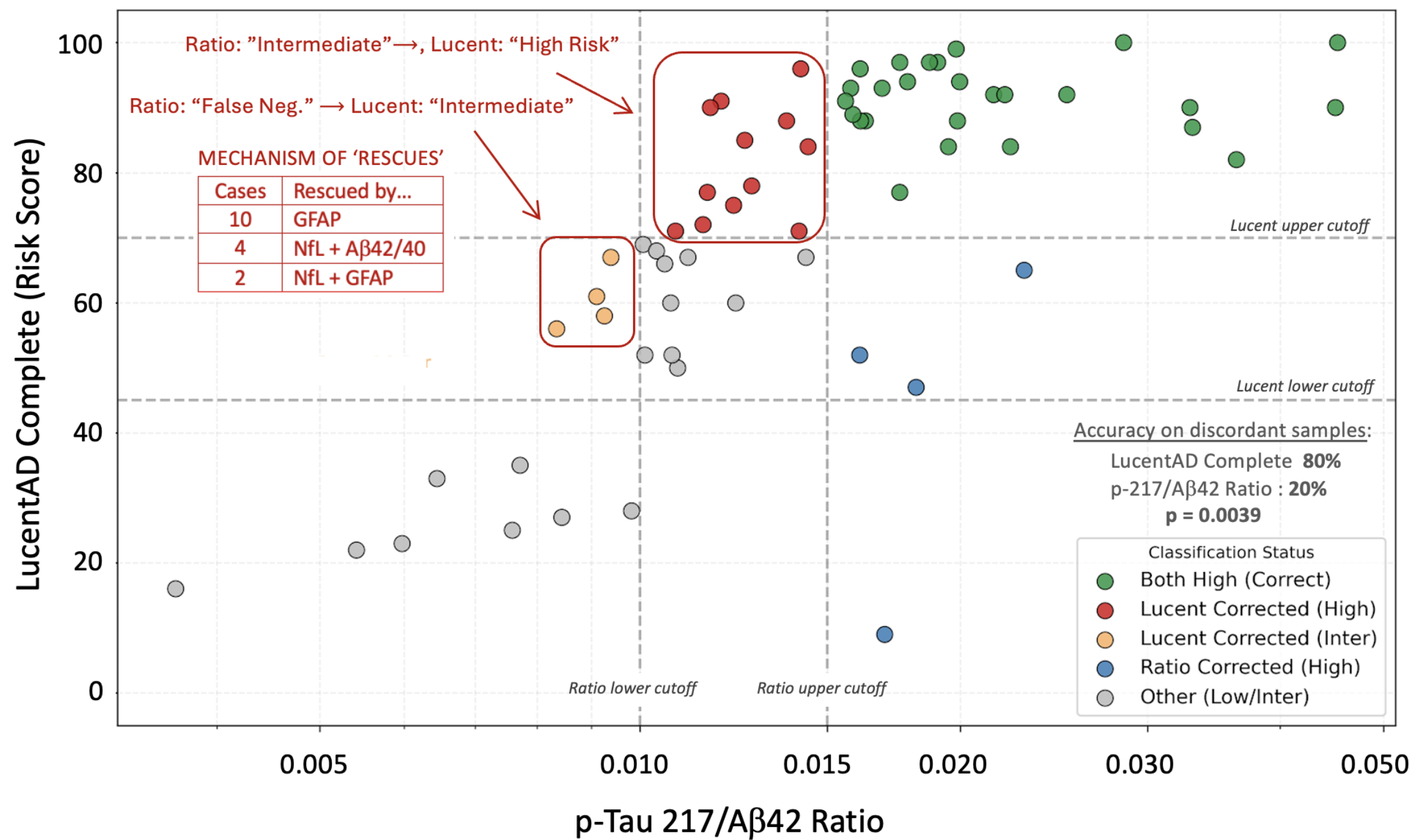


Figure 4: Head-to-head comparison of the 4-axis LucentAD Complete Risk Score versus the 2-axis p-Tau 217/Aβ42 ratio across amyloid PET-positive individuals. Dashed lines represent established clinical cutoffs for both assay configurations. While both tests successfully identified more advanced amyloid pathology (green dots), they frequently diverged on earlier-stage, borderline samples. On the 20 samples where the assays generated conflicting classifications (red, orange, and blue dots), LucentAD Complete achieves an 80% accuracy rate (correctly classifying 16/20 cases) compared to 20% for the ratio (p = 0.0039), which missed or under-classified them. Samples missed by both blood-based methods are shown in grey. The inset table outlines the specific biological pathways responsible for the 16 LucentAD re-classifications: 10 cases were corrected via astrogliosis tracking (GFAP), 4 cases by neurodegeneration combined with amyloid deposition (NFL + Aβ42/40) and 2 cases via elevation NFL + GFAP.

Table 3: The 'Rescuers': Elevation of GFAP and Nfl

Biomarker	"Rescued 16" (Mean Z-score)	vs. A-/T- Controls (p-value)	vs. other A+/T- (p-value)
GFAP	+3.18	< 0.001	< 0.001
NFL	+2.04	< 0.001	0.019
Aβ42/40 Ratio	+1.28	0.012	0.18 (NS)
p-Tau 217	+1.01	0.048	0.49 (NS)

Table 3: Evidence for the Four-Axis Rescue Mechanism. Analysis of the discordant cases revealed that GFAP and NFL were the primary statistical drivers of improved diagnostic accuracy with LucentAD Complete vs. the p-Tau 217/Aβ42 ratio. Among 'rescued' cases, concentration elevations—expressed as Z-scores relative to A-/T- baselines—were significantly higher than both the healthy A-/T- cohort and the remaining amyloid-positive A+/T- population. These elevated orthogonal biomarkers influenced the LucentAD Complete Amyloid Risk Score toward greater accuracy, pulling the score into higher risk categories. **Because the cutoffs for both assay configurations were optimized for 90%/90% performance, and p-Tau 217 and Aβ42 values were identical between the two assay configurations, these results indicate that the orthogonal axes (GFAP and NFL) were critical for identifying the 20% of early-stage patients missed by amyloid/tau-only configurations in this cohort (Figure 3).** *Note:* For consistent visualization, the sign of Aβ42/40 Z-scores has been inverted to appear positive.

Conclusions

- Resolving Early-Stage Blind Spots:** Incorporating neuroinflammatory (GFAP) and neurodegenerative (NFL) pathways provides a safety net for early-stage A+/T- patients who can be missed by conventional amyloid/tau-only ratios. By capturing this broader physiological landscape, LucentAD Complete significantly enhances diagnostic utility within diverse, community-based clinical settings.
- Unmasking Early Pathology:** In the highly challenging **A+/T- (Early AD)** cohort, the multi-analyte approach reduced the clinically ambiguous intermediate zone from **31-61% to 21.8%.** This demonstrates that integrating neuroinflammation and neurodegeneration markers provides greater clinical clarity required to more safely evaluate borderline patients. In **A+/T+** participants, the intermediate zone dropped to **5.4%.**
- Scalable Community Validation:** High diagnostic performance was maintained across a diverse, community-based cohort, demonstrating that LucentAD Complete is robust to performance loss seen when transferring biomarker models from homogeneous, academic research centers into heterogeneous, real-world clinical environments.

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[3] Imbimbo BP, Walling M, Imbimbo C, et al. Plasma ATN(I) classification and precision pharmacology in Alzheimer's disease. *Alzheimers Dement*. 2023 Oct;19(10):4729-4734. doi: 10.1002/alz.133084. Epub 2023 Apr 20.
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