

Beyond the p-Tau 217 Snapshot: Four-Axis ATN(I) Multi-Analyte Risk Score Resolves the ‘Shadow Zone’ of Leading-Edge Alzheimer’s Pathology

David Wilson,* Meenakshi Khare, Ann-Jeanette Vasko, Lindsey Mette, Jorge Marques Signes
Quanterix Corporation, Billerica, MA, USA; * Presenting/corresponding author

Contact: dwilson@quanterix.com



Background

Current blood-based biomarkers for Alzheimer’s primarily indicate the risk of amyloid pathology. However, single-protein snapshots of p-Tau 217 or p-Tau 217/Aβ42 are blind to the broader active biological environment and pace of disease progression. To move beyond this narrow detection paradigm, we evaluated a multi-analyte assay that interrogates four disease axes—amyloid, tau, neurodegeneration, and neuroinflammation [ATN(I)]—to move beyond a detection-only paradigm toward a more comprehensive assessment of biological activity in early Alzheimer’s disease.

Methods

Bio-Hermes-002 Interim Analysis. Analyses were performed using interim plasma and PET imaging data from the ongoing Bio-Hermes-002 study (NCT06584357), a community-based, multi-center cohort designed to evaluate blood biomarkers 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 includes substantial representation of amyloid-positive, tau-negative (A+/T-) individuals.

Methods. Using LucentAD Complete—which integrates p-Tau 217, Aβ42/40, GFAP, and NfL—we mapped biomarker Z-score trajectories across a continuous scale of composite Risk Scores. To visualize individual biologies, we generated multi-dimensional radar plots for p-Tau 217-matched ‘twins’ using Z-score scaling relative to control baselines. This allowed for population-level trajectory mapping and case-level identification of differences in underlying biological activity despite similar p-Tau 217 or p-Tau 217/Aβ42 levels.

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, a Janssen assay design [4]), 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. 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

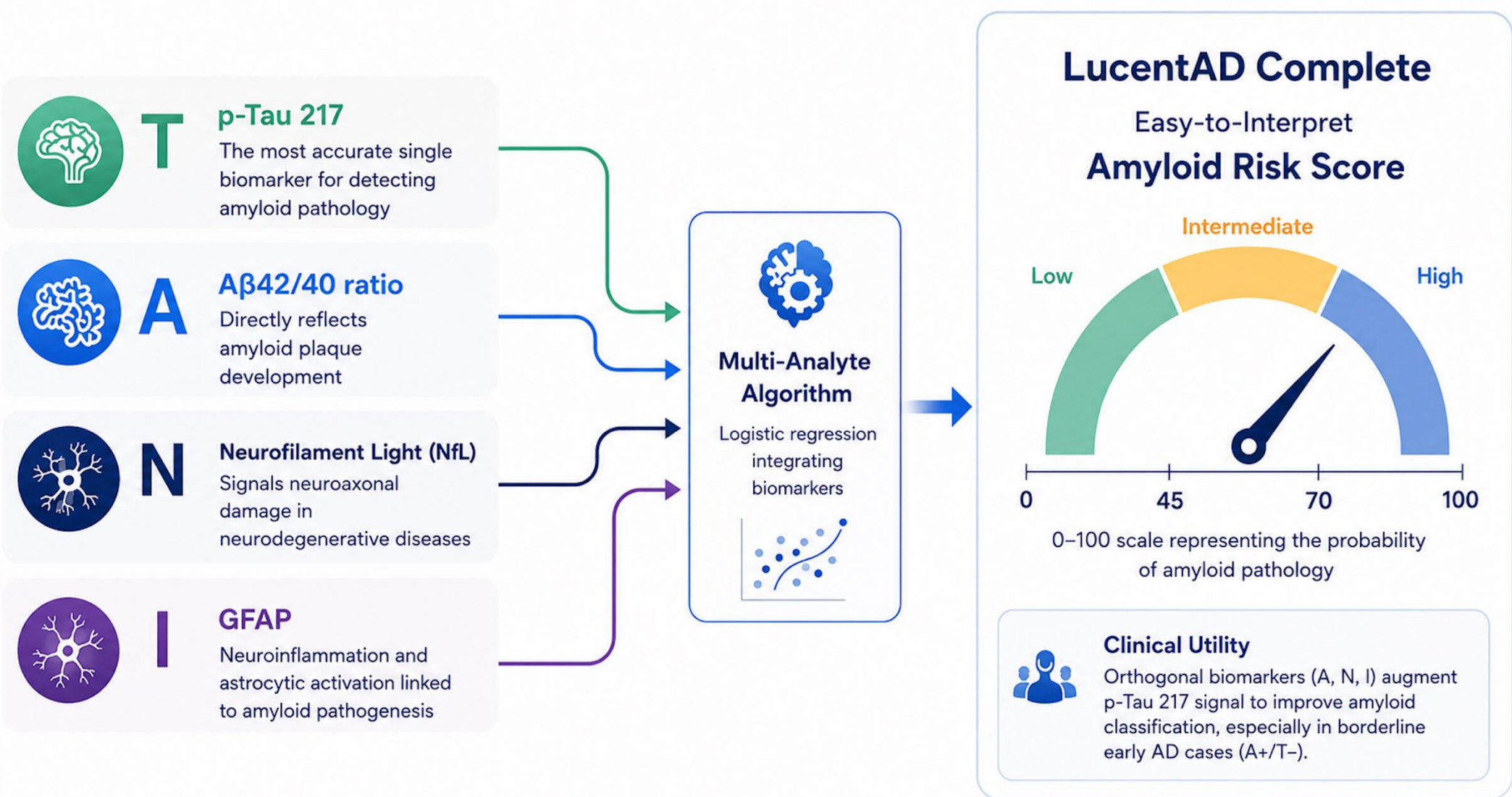


Figure 1. LucentAD Complete integrates four key Alzheimer’s-relevant biomarkers representing the **ATN(I) framework** (Jack *et al*², Imbimbo *et al*³) into a composite Amyloid Risk Score. p-Tau 217 is the backbone of the test, and the orthogonal biomarkers Aβ42/40 ratio, NfL, and GFAP augment the p-Tau 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.**

LucentAD Complete generally resolves ~2/3’s of borderline cases that cannot be classified by p-Tau 217 alone. This additional resolution of borderline intermediate cases is illustrated in **Figure 2**.

Figure 2: LucentAD Complete - Resolution of Intermediate Borderline Cases

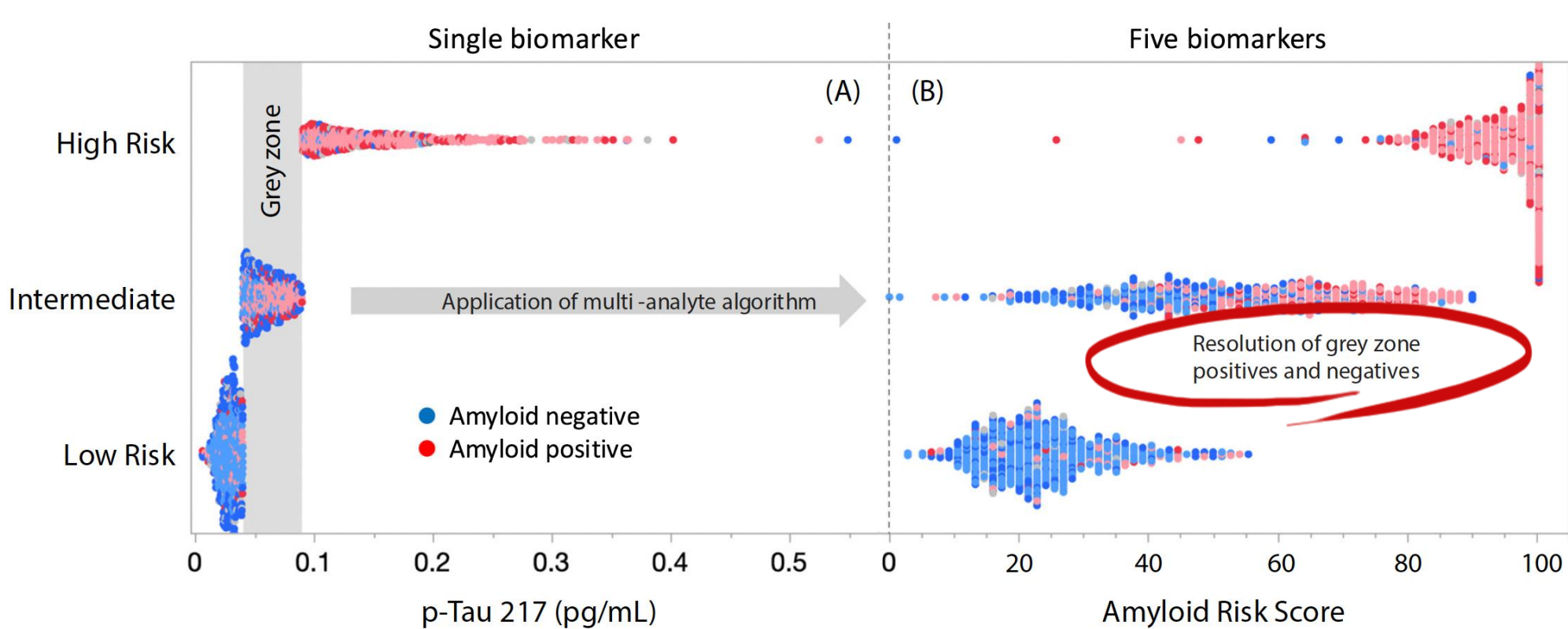


Figure 2. Multi-analyte interrogation of plasma samples for amyloid status. **A.** p-Tau 217 classifies samples with low and high amyloid burden, identifying intermediate risk cases. **B.** Multivariate analysis with all four of the ATN(I) biomarkers resolves majority of intermediate cases. All results are converted to an Amyloid Risk Score for final interpretation.

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[3] Imbimbo BJ, 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.13084.
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[6] Treu C, et al. Longitudinal Blood-Based Biomarkers and Clinical Progression in Subjective Cognitive Decline. *JAMA Netw Open*. 2025 Dec 1;8(12):e2545862. doi: 10.1001/jamanetworkopen.2025.45862.
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[8] Guillén N, Esteller D, Sarto J, et al. Plasma p-Tau217 and GFAP predict widespread cognitive decline in Alzheimer’s disease. *J Neurol*. 2025 Dec 17;273(1):28. doi: 10.1007/s00415-025-13556-5.

Results

Figure 2: Trajectories of LucentAD Complete Biomarkers Across Alzheimer’s Spectrum

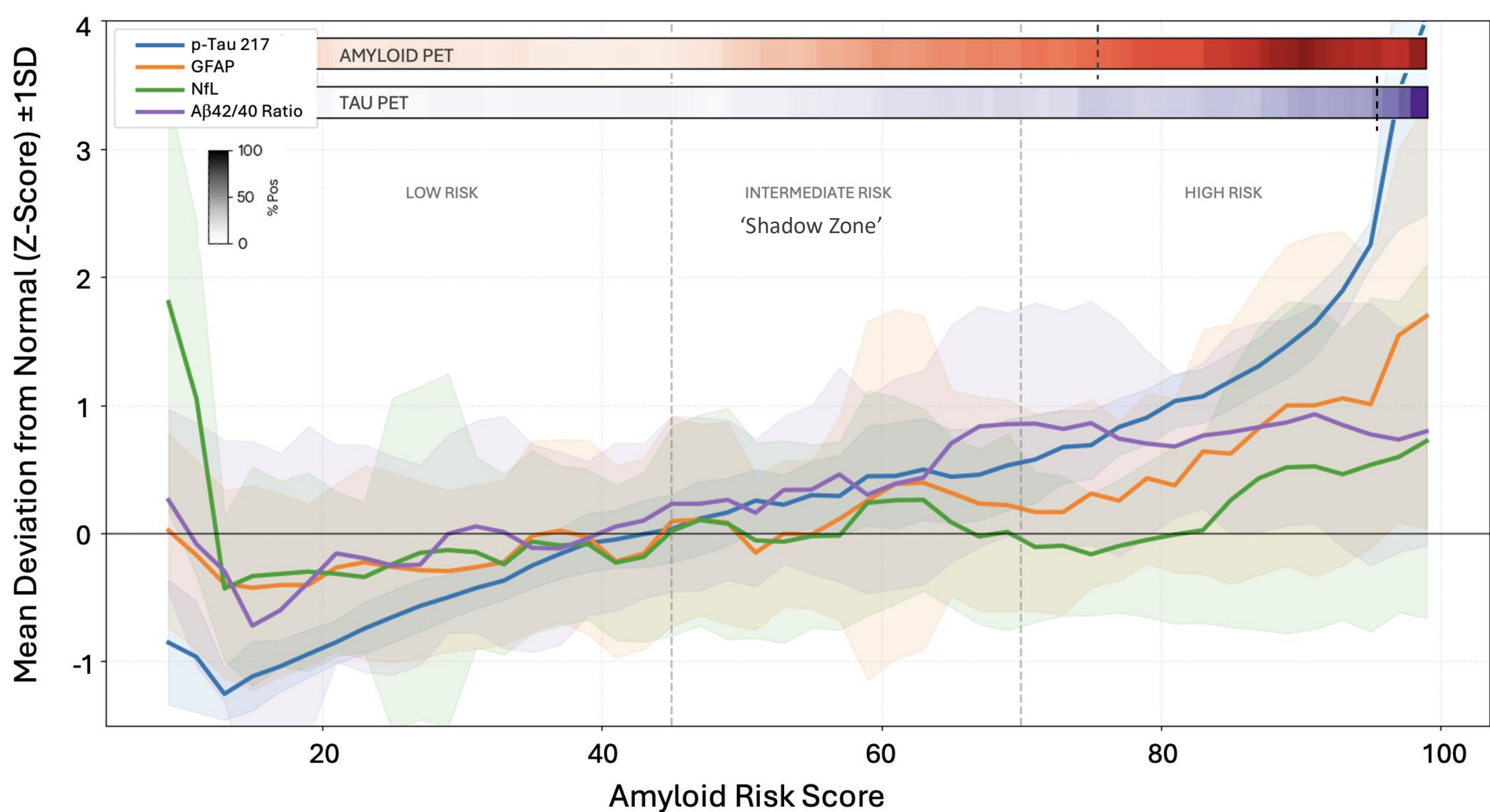


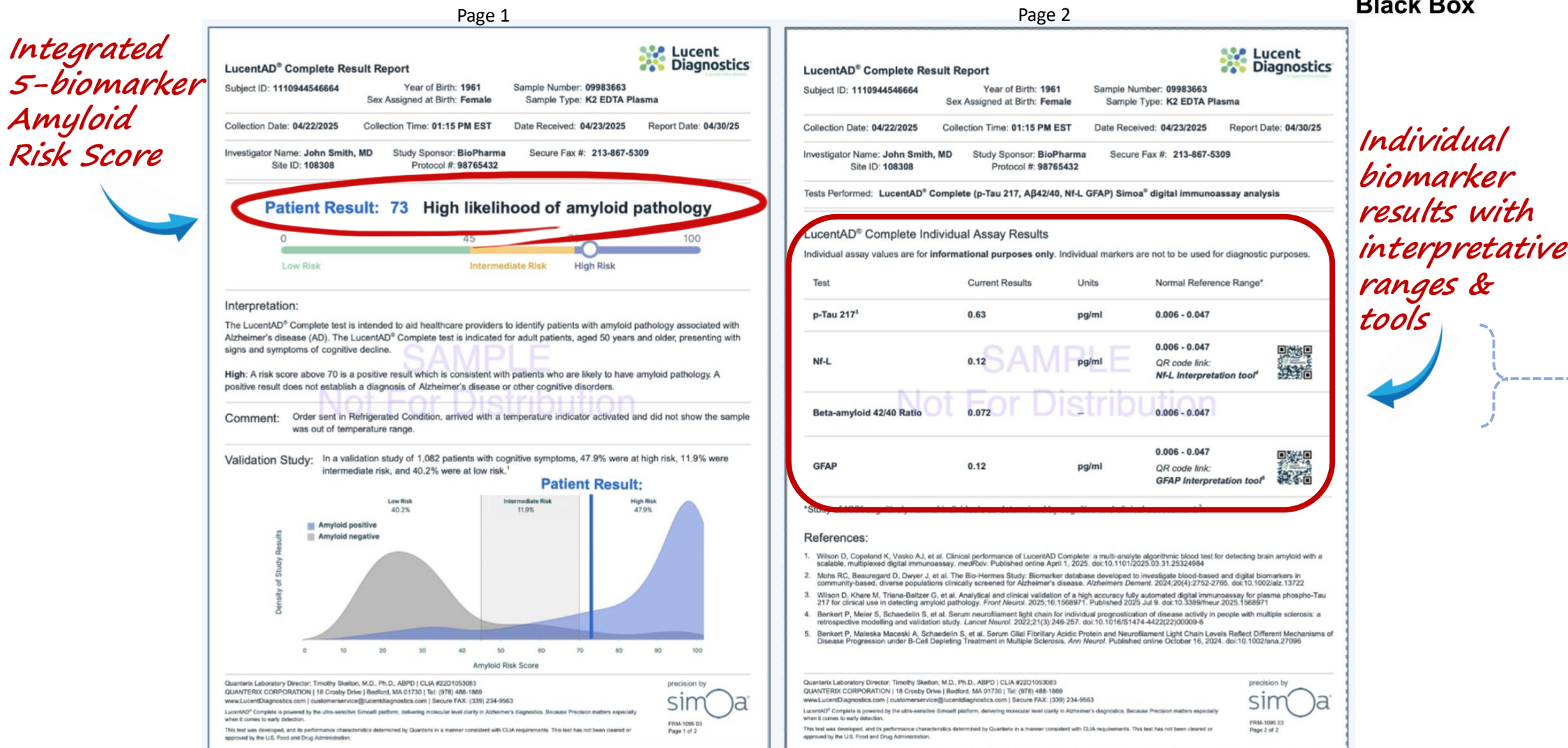
Figure 2: Leading Indicators. Trajectory analysis reveals the Intermediate zone (45–70) is a discrete biological stage, not a region of ambiguity. While PET imaging remains largely negative, plasma GFAP and Aβ42/40 show significant departure from baseline, capturing a shadow period of pathology accumulation (see **Table 1 below**). The accelerative rise of p-Tau 217 in the A+/T+ stage explains the high accuracy of single-analyte p-Tau 217 tests in advanced AD (see **Figure 3**); **however, in the early A+/T- stage, no single biomarker dominates—all four axes are required to provide the most reliable and sensitive diagnostic.** **Figure 2** highlights a key finding: accurately classifying the complex A+/T- population requires a multi-faceted biological lens because no single biomarker dominates this transition. This aligns with forensic analysis in of discordant A+/T- samples in **abstract/poster #13875** where the composite algorithm Risk Score outperformed p-tau 217/Aβ42 ratio by a factor of 4:1. **Figure 2** also illustrates why p-tau 217 and ratio tests can be volatile across different clinical cohorts. While the p-tau 217 signal is present in the early A+/T- stage, it only reaches its maximum, vertical trajectory once the pathology has advanced into Tau PET positivity. Because the p-Tau 217 signal is so disproportionately strong in these A+/T+ patients, even minor variations in the proportion of T- vs. T+ individuals within a study cohort can lead to significantly different performance metrics for standalone tests. By integrating biomarkers across all four pathological axes, the composite Risk Score provides a more stabilized and reliable assessment that is less sensitive to the specific tau-status mix of a given population.

Table 1: ‘Shadow Zone’ Biomarker Accumulation Trajectories—Low-Risk through Intermediate Zone

Pathological Axis	Biomarker	Low Risk Zone Mean ± SD	Intermediate “Shadow” Zone Mean ± SD	p-value (Mann-Whitney U)	Linear Slope (Δ Z-Score per Risk Unit)	p-value (2-sided t-test)	Clinical Real-World Takeaway
Tau Phosphorylation	p-Tau 217	0.0369 ± 0.0091 pg/mL	0.0590 ± 0.0101 pg/mL	p < 0.0001 (9.0 x 10 ⁻⁷²)	+0.0742	p < 0.0001 (1.5 x 10 ⁻¹⁹⁹)	Tight, rapid, highly predictable continuous climb across the early window.
Amyloid Accumulation	Aβ42/40 ratio	0.0640 ± 0.0103	0.0575 ± 0.0099	p < 0.0001 (2.7 x 10 ⁻¹³)	+0.0218	p < 0.0001 (3.4 x 10 ⁻²²)	Steady, progressive depletion of soluble amyloid occurring linearly (Note: depletion inverted for visualization)
Astroglisis / Inflammation	GFAP	107.31 ± 47.69 pg/mL	131.95 ± 62.90 pg/mL	p < 0.0001 (3.9 x 10 ⁻⁹)	+0.0157	p < 0.0001 (9.7 x 10 ⁻⁹)	Active neuroinflammatory scaling; glial activation ramps smoothly up into the Shadow Zone.
Neuro-degeneration	NfL	16.41 ± 13.03 pg/mL	19.42 ± 9.55 pg/mL	p < 0.0001 (3.6 x 10 ⁻⁷)	+0.0054	p = 0.0105	Outside of non-AD noise (Risk Score < 20), a significant linear progression.

Table 1. Statistical profiling of plasma biomarkers tracking the transition from low-risk baselines into the clinical intermediate zone (see **Figure 2**). Group comparisons show highly significant elevations in concentrations across all four pathological axes (p < 0.0001). Standardized linear slopes illustrate that biological escalation is not uniform: p-Tau 217 drives the primary continuous climb, followed by progressive amyloid depletion and smooth neuroinflammatory scaling (GFAP). NfL exhibits significant linear progression (p = 0.0105) once isolated from low-risk non-AD noise evident below Risk Score < 20 (see **Figure 2**). **These distinct accumulation rates mathematically substantiate the continuous accumulation of pathology prior to confirmed amyloid positivity by either amyloid PET or immunoassay, as well as the clinical importance of a fuller, multi-axis assessment of patients suspected of Alzheimer’s.**

Figure 4: LucentAD Complete Test Report



Amyloid status with prognostic and differential diagnostic insights

Figure 3: Dynamic Shifts in Biomarker Predictive Strength Across Alzheimer’s Progression

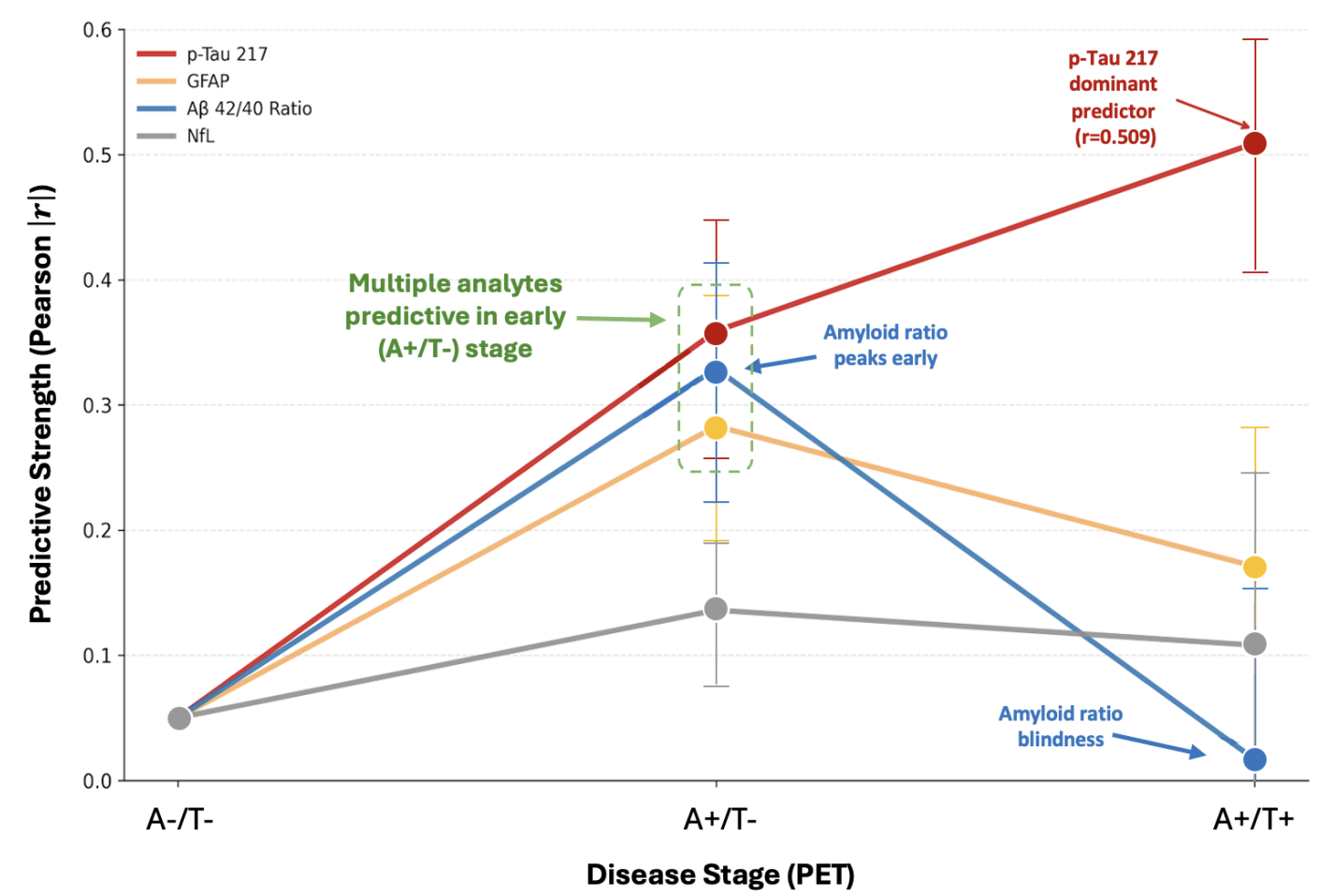


Figure 3. Points represent point-biserial correlation coefficients (|r|) measuring the diagnostic signal strength of individual plasma biomarkers during discrete pathological transitions. Error bars represent asymmetric 95% CIs calculated via Fisher z-transformation based on transition sub-cohorts (N=287 upstream; N=160 downstream). In the early A+/T- stage, a multi-analyte cluster (green box) provides superior diagnostic context, augmenting the relative latency of p-Tau 217. Conversely, the Aβ42/40 ratio diminishes to statistical blindness (r=0.017) during the transition to tau positivity, where p-Tau 217 emerges as the dominant, solitary predictor.

Figure 5: Four-Axis Multi-Analyte Interpretation Engine*
Two Patients, Same p-Tau 217, Very Different Progression Risks

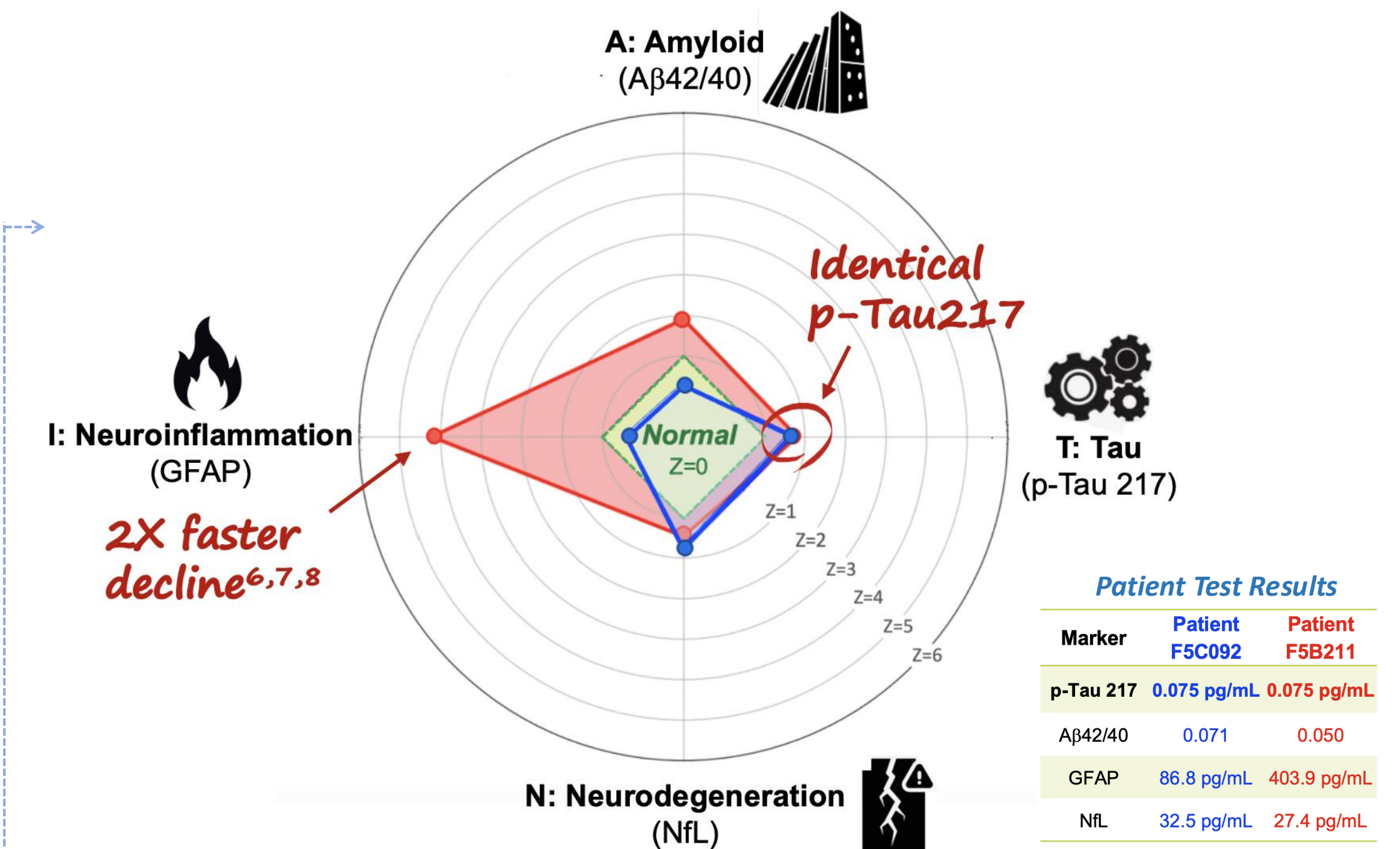


Figure 5: Two Bio-Hermes participants with identical p-Tau 217 signals present **vastly different biological trajectories**. While p-Tau 217 or p-Tau 217/Aβ42 assays may bucket them with similar risk, a 4-axis assessment reveals very different risk profiles. Emerging evidence shows that elevated GFAP is predictive of disease velocity and more rapid onset of cognitive decline[6-8]. Moving beyond a univariate “Tau clock” to a more comprehensive “Four Axis Velocity” assessment can provide the resolution to distinguish between simply ‘being at risk’ and being in a **state of active, rapid decline**. This provides a framework for both more personalized clinical management and more decisive, high-power therapeutic trials.

*Concept, not currently part of the LucentAD Complete test report

Conclusions

- Multi-Axis Necessity Over Single-Marker Standalones:** Progression across the Alzheimer’s continuum is a dynamic biological relay. While p-Tau 217 is highly effective in later stages as tau pathology sets in, it lags significantly during early pre-tau disease incubation (A+/T-) where multiple ATN(I) biomarkers exhibit predictive signals. In this therapeutically critical early stage, a simultaneous multi-analyte approach that utilizes all these upstream signals is needed to maximize diagnostic performance.
- The ‘Shadow Zone’ is Highly Active, Not Ambiguous:** The intermediate risk window represents a distinct, statistically significant stage of biological escalation (p < 0.0001). During this phase, critical pathological accumulation—characterized by accelerated p-Tau 217 deposition, progressive soluble amyloid depletion, and neuroinflammatory scaling (GFAP)—occurs long before confirmation by binary amyloid PET thresholds.
- Resolving Heterogeneity via the 4-Axis Interpretation Engine:** Relying on univariate biomarker assessments masks profound clinical heterogeneity. As demonstrated by the multi-axis engine, patients presenting with identical p-Tau 217 concentrations can possess radically divergent neuroinflammatory (GFAP) and neurodegenerative (NfL) profiles. Revealing this hidden risk enables precise classification between indolent biology and rapid clinical decline. This shifts the clinical framework from a narrow “presence/absence” binary paradigm to actionable, personalized management guided by the full ATN(I) pathophysiological continuum to better inform therapeutic decisions.