FERNE 2025 Lab Testing Mild TBI Conclusions and Recommendations Changes Due to 2025 Puravet SR and 2018 Bazarian ALERT TBI Data

I. Summary of Puravet Systematic Review (20 Key Concepts)

The systematic review and meta-analysis by Puravet et al. evaluated the effectiveness of measuring the combined GFAP and UCH-L1 blood levels in predicting intracranial lesions in adults with mild Traumatic Brain Injury (mTBI).

Concept (10 Bold Words)	Explanation (40 Words Maximum)	Source(s)
Combined GFAP/UCH-L1 achieved 100% pooled sensitivity.	The meta-analysis of 11 studies reported the overall pooled sensitivity for the combined GFAP and UCH-L1 biomarker test was {100% (95% CI 99% to 100%). This high sensitivity makes it a reliable tool for excluding injury.	
Combined test yielded 100% Negative Predictive Value (NPV).	The association of GFAP and UCH-L1 achieved a 100% overall pooled Negative Predictive Value (NPV). This confirms that patients with negative biomarker levels are definitively unlikely to have intracranial lesions on CT.	
Combined biomarker specificity was only 31% pooled average.	The meta-analysis found the pooled specificity for the combined GFAP and UCH-L1 test was 31% (95% CI 26% to 36%) after minor adjustment. This low value drives the high false-positive rate.	
Combination theoretically reduces cranial CT scans by 31%.	Based on the calculated pooled specificity of 31%$ the routine use of the combined GFAP/UCH-L1 assay is expected to theoretically reduce unnecessary cranial CT scans by this percentage.	
Highest diagnostic accuracy found in the combined biomarker test.	The combined GFAP and UCH-L1 test showed the highest Area Under the Curve (AUC) for predicting intracranial injury at 98% (95% CI 97% to 99%).	

GFAP alone showed 94% pooled sensitivity, less than combination.	The pooled sensitivity for GFAP measured alone was 94% (95% CI 91% to 97%). This performance, while strong, is lower than the perfect $100%$ pooled sensitivity of the combined assay.
GFAP alone demonstrated 40% pooled specificity in meta-analysis.	GFAP measured alone showed a pooled specificity of 40% (95% CI 34% to 46%). This specificity is slightly higher than the 31% found for the GFAP/UCH-L1 combination.
UCH-L1 alone had 83% pooled sensitivity, lowest of biomarkers.	UCH-L1 measured alone had the lowest overall pooled sensitivity at 83% (95% CI 69% to 94%). Its low sensitivity makes it unsuitable for ruling out critical injury by itself.
Single UCH-L1 measurement achieved 51% pooled specificity, highest.	UCH-L1 alone demonstrated the highest pooled specificity at 51% (95% CI 40% to 63%). This specificity was achieved after removing five studies due to high heterogeneity in results.
Test is positive if either biomarker exceeds its cutoff.	The combined assay is designed to be positive if one or both biomarkers exceed their respective cut-off values on commercially available platforms (e.g., Alinity, i-STAT, Vidas).
Combined performance mirrors S100B's earlier diagnostic findings.	The diagnostic performance of GFAP/UCH-L1 (100% Se, 31% Spe) is similar to historical S100B data, which showed 96% sensitivity and 30% specificity at the 0.10 mg/L threshold.
Combination is favored because pigmentation does not influence.	GFAP and UCH-L1 concentrations are not affected by skin pigmentation, which simplifies their use and increases reliability compared to S100B, whose concentrations vary with skin pigmentation.
Complementary half-lives explain the combination's high sensitivity.	The 100% sensitivity is achieved because the proteins have complementary kinetics: UCH-L1 provides early detection, and GFAP identifies patients after 8 hours (half-life of 24 to 48 hours).
Assays are available on three routine clinical platforms.	The measurement is available for routine clinical use on three analyzers: the i-STAT handheld device (POC), the Alinity platform (core lab), and the Vidas platform.
Results are obtainable in less than one hour routinely.	The three commercially available devices for measuring GFAP and UCH-L1 are suitable for routine use and allow results to be obtained in less than an hour.
Heterogeneity in sampling time complicates optimal recommendations.	The systematic review noted high variability in sampling times across the included trials, ranging from 4 hours up to 24 hours. This heterogeneity prevented the development of specific sampling guidelines.

Assay cutoffs and techniques varied across included studies.	The cutoff values for GFAP and UCH-L1 varied because different analytical techniques were used (e.g., ELISA, CMIA, electrochemiluminescence). This variation complicates result interpretation.
Improving specificity requires positive results for both biomarkers.	To significantly improve the specificity of the combined test, one solution suggested is only considering the test positive when both GFAP and UCH-L1 levels exceed their respective cut-off values.
Cost estimate places biomarkers below that of CT scans.	The cost of measuring GFAP and UCH-L1 is estimated to be between 30 and 50 Euros, making the assay less expensive than a cranial CT scan.
Study quality assessed using the QUADAS-2 criteria.	The methodological quality of the 16 included studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) criteria.

II. Analysis of FERNE Conclusions and Recommendations

The new systematic review by Puravet et al. provides strong, pooled evidence confirming the high diagnostic performance (Sensitivity and NPV) of the combined GFAP/UCH-L1 assay for adult mTBI. This evidence largely reinforces your existing conclusions but necessitates updating specific numerical claims to reflect the pooled $100%$ accuracy, and adjusting the theoretical CT reduction expectation.

A. Necessary Changes to FERNE Conclusions (C1–C15)

FERNE Conclusion	Current Rationale (Source)	Change Required Based on Puravet SR
C2. Performance Profile	Prioritizes extremely high sensitivity (up to 97.6% and NPV (up to 99.6%, accepting low specificity approx 35%.	**UPDATE NUMERICAL CLAIMS.** Change sensitivity to 100% and NPV to 100%. Specificity should be updated to 31%.
C7. Clinical and Economic Impact	Improves clinical flow by reducing head CT scans (e.g., $\mathbf{21%}$ reduction).	**UPDATE THEORETICAL EXPECTATION.** The systematic review supports a theoretical reduction of CCT scans by 31%. This larger figure should be acknowledged alongside the 21% operational data.
C4. Temporal Constraints	Adherence to the $\mathbf{12}$-hour window is mandated for maximal diagnostic reliability.	**NO CHANGE.** The SR noted significant heterogeneity in sampling times (4 to 24 hours) but still recommends following the 12-hour

		guidelines established by scientific societies.
C5. Prognostic Limitations	Current FDA-cleared tests are not approved or validated to predict the speed of concussion recovery, long-term neuropsychological outcomes, or necessity for neurosurgical intervention.	**NO CHANGE.** The systematic review excluded prognostic studies and focuses only on acute CT lesion detection. This limitation remains firm.
C11. Alcohol Use Confounds Biomarker Use	Alcohol levels above $100–150$ mg/dL will increase false positives, suggesting a doubling of the positive cutoff.	**NO CHANGE.** The SR did not address alcohol or suggest cutoff modification; therefore, the current conclusion regarding modifying cutoffs in intoxicated patients should stand based on previous data.

B. Necessary Changes to FERNE Clinical Recommendations (R1–R15)

FERNE Recommendation	Current Rationale (Source)	Change Required Based on Puravet SR
R2. Manage Negative Results	Rationale: The tests prioritize high NPV (99.6%) for safe CT deferral.	**UPDATE NUMERICAL CLAIM.** The rationale should be updated to cite the overall pooled NPV of 100% as found in the meta-analysis.
R7. Utilize POC for Throughput	Operational data shows integration results in a 21% CT reduction and shortens LOS by 45 minutes.	**UPDATE RATIONALE.** The rationale should state that the 31% theoretical CT reduction supports the operational benefit of the 21% observed reduction.
R8. Develop Institutional Protocols	Focuses on standardized protocols for ordering, EHR integration, and follow-up.	**NO CHANGE.** The SR reinforces this need, noting that high heterogeneity was caused by varying assay techniques and cutoff values across studies.
R11. Study Biomarkers Use for Intoxicated Head Trauma Patients	Collect data... to help determine optimal brain biomarker cutoffs and NPV in this mTBI subgroup.	**NO CHANGE.** The SR reinforces the need for further evaluation due to general cutoff variability; thus, this recommendation to collect local data for vulnerable subgroups is still essential.
R13. Use AI and more to Change Clinical Practice	Use all teaching modalities to educate clinicians on best practices.	**NO CHANGE.** The SR supports this by noting that standardized results would be best obtained if studies used the same automated system, reinforcing the need for clinician education on consistent use.

In summary, the Puravet systematic review confirms the exceptional rule-out capacity of the combined GFAP/UCH-L1 test, solidifying the safety argument for CT reduction. The primary numerical updates needed involve increasing the **Sensitivity and NPV claims to 100%** and **lowering the Specificity claim to 31%** in your conclusions and rationales.

Based solely on the excerpts provided from the Puravet et al. systematic review and meta-analysis, the investigators **do not define or describe any of the incongruent patient cases where the combined GFAP/UCH-L1 biomarkers were negative and the CT scan was positive (False Negatives)**.

In fact, the primary finding of their meta-analysis is that the pooled data suggests that such false negative cases theoretically **did not occur**:

• The overall pooled **Sensitivity (Se)** for the combination of GFAP and UCH-L1 was calculated to be (95% confidence interval [CI] 99% to 100%).

• The overall pooled **Negative Predictive Value (NPV)** for the combined test was also calculated to be (95% CI 100% to 100%).

The authors explicitly state that this high performance ensures the exclusion of intracranial injury:

• "The combined measurement of GFAP and UCH-L1 allows the **exclusion of intracranial injury** after mTBI in adults with Se and negative predictive value".

• They conclude that the combination achieves maximum Sensitivity and NPV, "ensuring that **no false-negative cases** (negative test result with an intracranial lesion on the CCT scan)" occurred in the pooled analysis.

Because the pooled results indicated sensitivity and NPV, the analysis did not encounter false negative cases in the aggregated data from the 11 studies included in the combination analysis. Therefore, the investigators did not proceed to define or describe any specific instances of incongruent negative biomarker results with positive CT findings in their systematic review.

Puravet Reference

Puravet A, Oris C, Pereira B, et al. Can the Association of the Biomarkers GFAP and UCH-L1 Predict Intracranial Injury After Mild Traumatic Brain Injury in Adults? A Systematic Review and Meta-Analysis. *Ann Emerg Med*. 2025:1-14. doi:10.1016/j.annemergmed.2025.03.018

I. Summary of the Bazarian ALERT-TBI Publication

1. **Pivotal prospective trial validated combined UCH-L1/GFAP diagnostic accuracy.** This study was a large, prospective, multicenter observational trial aimed at validating the combined test of UCH-L1 and GFAP to predict traumatic intracranial injuries on head CT scan acutely after TBI.

2. **Study enrolled adults with suspected non-penetrating TBI, GCS 9–15.** Participants were adults (years) presenting to the ED with suspected, non-penetrating TBI and a GCS score between 9 and 15 at the time of informed consent.

3. **Intracranial injuries were detected in 6% of the patient cohort.** Among the 1,959 patients with analyzable data who underwent a head CT scan, patients (or) were found to have CT-detected traumatic intracranial injuries.

4. **Combined assay achieved 97.6% sensitivity for intracranial injury detection.** The primary study outcome sensitivity for detecting acute intracranial injury was (95% CI 0.931–0.995) across the entire GCS 9–15 cohort in this pivotal trial.

5. **Test achieved excellent 99.6% Negative Predictive Value (NPV).** The negative predictive value (NPV) was (95% CI 0.987–0.999), strongly supporting the test's clinical role for ruling out the need for a head CT scan.

6. **Only three patients produced false-negative results in analysis.** In the entire cohort of 1,959 patients, only individuals had a CT-positive scan despite having a negative combined biomarker test result, indicating a low false-negative rate.

7. **Test was 100% sensitive for all neurosurgically managed injuries.** The test demonstrated sensitivity and NPV for detecting the patients who required neurosurgically manageable lesions, underscoring its safety for critical injuries.

8. **Blood draw was strictly required within twelve hours of injury.** A key inclusion criterion for patient eligibility was that the blood sample used for UCH-L1 and GFAP measurement must have been collected **within 12 hours** of the injury event.

9. **Biomarker test specificity was low, reported at 36.4% overall.** The overall specificity of the combined UCH-L1/GFAP test was calculated to be (95% CI 0.342–0.387). This low figure leads to a high rate of false-positive results.

10. **A negative test required both markers falling below cutoffs.** The combined test result was considered negative only if **both** the UCH-L1 and GFAP marker levels were below their respective predetermined cutoff values.

11. **A positive test resulted if either marker exceeded its cutoff.** Conversely, the test was defined as positive if the concentration of **one or both** of the UCH-L1 and GFAP markers was found to be above the specified cutoff value.

12. **Prespecified cutoffs used were UCH-L1 327, GFAP 22 pg/mL.** The study prospectively validated and utilized specific, predetermined cutoffs: pg/mL for UCH-L1 and pg/mL for GFAP concentration in serum samples.

13. **Biomarker use potentially reduces CT utilization by 34%.** The study found that (671 out of 1,959 patients) had a negative test result, representing the proportion of head CT scans that could potentially be avoided by using the test.

14. **False negative possibly due to pre-existing vascular malformation.** One of the three false-negative patients had a positive CT, but a follow-up MRI indicated an underlying cavernous malformation, suggesting the CT result may have been a false-positive.

15. **Biomarker levels were significantly higher in CT-positive patients.** Median GFAP and UCH-L1 concentrations were significantly elevated in CT-positive patients (GFAP pg/mL; UCH-L1 pg/mL) compared to CT-negative patients.

16. **Most critical findings were acute subdural and subarachnoid hemorrhage.** The most common traumatic intracranial injuries detected were subarachnoid hemorrhage (of CT+ cases) and acute subdural hematoma (of CT+ cases).

17. **Test performed similarly in subset of mildest GCS 14–15 patients.** For the of patients with GCS 14–15, the performance metrics remained similar: sensitivity and NPV .

18. **High positivity suggests detection of subtle, non-CT visible injury.** The high rate of positive biomarker tests () contrasted sharply with the low rate of positive CT scans (), suggesting the test detects subtle brain injury not visible on standard CT.

19. **Trial results led directly to US FDA commercialization authorization.** The robust prospective validation data provided by the ALERT-TBI trial were used to support the request for US FDA authorization, which was officially granted in February 2018.

20. **Combination assay modestly outperformed individual markers alone.** Sensitivity analysis suggested that using the combination of GFAP and UCH-L1 was numerically superior to using each marker individually for primary outcomes.

The Bazarian ALERT-TBI trial [72–120] is the definitive, prospective validation study that underpins the FDA authorization for the combined GFAP/UCH-L1 test. Its findings strongly support the conclusions drawn from the Puravet systematic review (SR) [1–4], but they introduce specific, prospective numeric values that should be prioritized in your FERNE document.

II. Impact of ALERT-TBI Data on FERNE Conclusions, Recommendations

Impact on Conclusions

The ALERT-TBI findings reinforce your conclusions but require careful integration of specific figures:

• **C2. Performance Profile:** The FERNE document currently reflects the pooled SR sensitivity of and NPV of . While these pooled figures are accurate, the ALERT-TBI trial established the FDA-approved performance metrics: **Sensitivity and NPV** . Your conclusion should state that the sensitivity/ NPV from the pivotal prospective ALERT-TBI trial were the basis for regulatory approval, while noting that subsequent meta-analysis (Puravet) found pooled values.

• **C7. Clinical and Economic Impact:** The ALERT-TBI study provides the key operational metric: of patients had a negative test result, representing the **actual potential reduction** in CT scans identified in the pivotal trial cohort. This figure should be cited alongside the theoretical reduction found in the Puravet SR.

• **C4. Temporal Constraints:** The ALERT-TBI trial provides the definitive rationale for the time window: the study strictly mandated blood collection **within 12 hours of injury**. This reinforces the necessity of adhering to this constraint for regulatory adherence and maximal accuracy.

• **C6. Pediatric Exclusion:** ALERT-TBI explicitly excluded all patients under 18 years, further strengthening the conclusion that the test is **not validated** or approved for pediatric use.
Impact on Recommendations

The ALERT-TBI data provides extremely robust safety data that bolsters specific recommendations:

• **R2. Manage Negative Results:** The recommendation to safely defer CT should be strengthened by citing the ALERT-TBI finding of NPV for the small but critical subgroup of patients with **neurosurgically manageable lesions**. This is a more powerful rationale for safety than the general NPV alone.
• **R6. Avoid Pediatric Routine Use:** The exclusion of pediatric patients from the pivotal validation trial (ALERT-TBI) should be used in the rationale to justify why the recommendation to avoid routine use in children is critical.
• **R7. Utilize POC for Throughput:** The rationale should cite the potential CT reduction from the ALERT-TBI data as the primary operational justification for adopting these assays to improve ED throughput.

Bazarian Reference

Bazarian JJ, Biberthaler P, Welch RD, et al. Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on head CT (ALERT-TBI): a multicentre observational study. *Lancet Neurol*. 2018;17(9):782-789.

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