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RESEARCH ARTICLE



Biomarkers for acute mild brain injury (BAMBI): optimising GFAP/UCH-L1 thresholds to support CT stewardship in a NICE-guided mild-traumatic-brain-injury cohort

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ABSTRACT

Background: NICE head injury guidance prioritises sensitivity but results in high CT utilisation and low diagnostic yield, contributing to Emergency Department (ED) crowding, particularly among older adults after falls. GFAP/UCH-L1 blood biomarkers may support safer CT stewardship, although limited specificity in older adults may reduce clinical impact.

Objective: To evaluate plasma GFAP/UCH-L1 against CT findings in a Major Trauma Centre ED cohort and assess whether age-stratified interpretation improves clinical utility while preserving rule-out performance.

Methods: Adults with suspected mild traumatic brain injury (mTBI; GCS 13–15) who underwent non-contrast head CT and had plasma GFAP/UCH-L1 measured within 12 hours of injury were included (n=215). Manufacturer thresholds (GFAP 35 pg/mL; UCH-L1 400 pg/mL; OR rule) were compared with an age-stratified algorithm (GFAP 115 pg/mL; UCH-L1 335 pg/mL; OR rule) applying higher published thresholds in participants ≥ 65 years.

Results: Nineteen participants were CT-positive (8.8%). Manufacturer thresholds achieved 100% sensitivity and 29.6% specificity, with 27.0% biomarker-negative. Age-stratified interpretation preserved 100% sensitivity while improving specificity to 55.1% and increasing biomarker-negative results to 50.2%. In participants ≥ 65 years, specificity improved from 11.2% to 62.2%.

Conclusion: In this NICE-imaged ED cohort, GFAP/UCH-L1 showed strong CT rule-out performance. Age-stratified interpretation substantially improved specificity, supporting larger prospective validation and pathway evaluation.

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Introduction

Mild traumatic brain injury (mTBI) is a high-volume Emergency Department (ED) presentation, and computed tomography (CT) remains the dominant tool to exclude traumatic intracranial injury. In practice, the National Institute for Health and Care Excellence (NICE) head injury guidance and similar high-sensitivity decision rules can lead to high CT utilisation with low

diagnostic yield, contributing to ED crowding, prolonged length of stay, increased radiation exposure, and incidental findings.^{1,2} These pressures are amplified in ageing populations, where falls and antithrombotic use are common, lowering clinicians' tolerance for missed injury and increasing CT utilization.³

Blood-based biomarkers offer an objective adjunct to CT decision-making, with a primary 'rule-out' use: a

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negative test identifying patients at sufficiently low risk to safely forgo CT (or support observation/discharge decisions). Glial fibrillary acidic protein (GFAP: astroglial) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1: neuronal) have the strongest contemporary evidence base. In the pivotal 2018 ALERT-TBI Study, combined GFAP and UCH-L1 demonstrated very high sensitivity for CT-visible intracranial injury, supporting their use for rule-out within an early post-injury window.⁴ Subsequent work has supported high sensitivity in rapid and near-patient formats.⁵

However, clinical impact depends not only on rule-out safety but also on specificity: a test that is safe but frequently positive will not meaningfully reduce low-yield CT scanning. Systematic evidence indicates that biomarkers can achieve high sensitivity for CT abnormalities after mTBI, but specificity is often modest and variable across settings, emphasising the importance of threshold selection and pathway design.⁶

The specificity challenge is most problematic in older adults, where baseline biomarker concentrations can be higher due to age-related neurological change and physiological confounding. In an ageing cohort without suspected mTBI, GFAP and UCH-L1 varied with age and renal function, and the application of manufacturer-defined cut-offs yielded high 'positivity' rates, driven largely by GFAP.⁷ Large control cohorts demonstrate a nonlinear increase in blood GFAP with age, accelerating from around midlife; age-specific percentile curves confirm a progressive right-shift in the GFAP distribution with ageing, supporting age-aware interpretation strategies in ED populations.⁸ A recent study also demonstrated that elderly mTBI patients had significantly higher median GFAP levels even in CT-negative cases, with preserved sensitivity but reduced specificity when applying standard thresholds.⁹ These findings provide a plausible mechanism for reduced specificity in elderly ED populations.

A pragmatic strategy to preserve utility across a heterogeneous ED case mix is using age-stratified decision limits. A recent European cohort study reported that applying higher thresholds in patients ≥ 65 years improved specificity without compromising sensitivity, increasing the proportion of older patients who could potentially avoid CT.¹⁰ This is particularly relevant because many clinical decision rules and guidelines drive CT imaging based on age criteria, meaning older adults represent both a high-need subgroup and a potential barrier to implementation if specificity is too low.^{2,11}

The Biomarkers in Acute Mild Brain Injury (BAMBI) study was established to evaluate GFAP/UCH-L1 testing in a real-world Irish Major Trauma

Centre ED population undergoing CT for suspected mTBI, with a focus on clinically actionable endpoints: rule-out safety, specificity (and therefore potential CT avoidance), and age-stratified performance. Here, we present preliminary diagnostic accuracy and optimisation findings from the first 215 participants with complete biomarker and CT data. We intentionally position this paper as a clinical diagnostic accuracy and optimisation report, with implementation/health-economic outcomes reserved for planned next studies.

Methods

Study design and setting

Prospective observational cohort study conducted at a trauma centre between 2 July 2025 and 16 January 2026. Preliminary complete-case diagnostic accuracy analysis of adults presenting to a major trauma ED with suspected mTBI who underwent non-contrast CT head imaging (ordered according to NICE guidance and clinician judgement/standard care) and had plasma GFAP and UCH-L1 measured.

Participants

Inclusion criteria: adults (≥ 18 years) with suspected mTBI (GCS 13–15), CT performed, plasma GFAP/UCH-L1 available within the protocol sampling window of ≤ 12 hours, and who gave consent. Exclusion criteria: Age < 18 years, transfer from another facility, known intracranial mass, recent neurosurgery (defined as cranial neurosurgical intervention within the preceding 30 days), and blood draw > 12 hours from injury.

Data collected: Demographic details, patient characteristics, radiology findings, and outcomes (management and disposition) were recorded on a case report form. Outcomes such as ED discharge, hospital or intensive care admission, intubation, and reattendance within 7 days of discharge were collected through chart reviews.

Reference standard

Non-contrast CT head scans were classified as CT-positive or CT-negative for traumatic intracranial abnormality using predefined radiological criteria (e.g. traumatic intracranial haemorrhage, contusion and traumatic subarachnoid haemorrhage). Patients were managed according to the standard of care, and physicians and radiologists were blinded to TBI biomarker results.

GFAP/UCH-L1 on Abbott Alinity i

Plasma GFAP and UCH-L1 were measured using the Abbott Chemiluminescent Microparticle Immunoassay (CMIA) on the Alinity i platform per manufacturer instructions.¹² The qualitative 'TBI test' interpretation is negative when both biomarkers are below the threshold and positive when either meets/exceeds the threshold (OR rule).

Biomarker testing was conducted solely for research purposes. Samples were processed, stored at -80°C , and analysed retrospectively on a single Abbott Alinity i random-access platform in scheduled analytical runs across four assay days (10, 11, 12 and 16 December 2025), according to laboratory workflow and sample availability. Analysis was performed by one experienced scientist. Samples were not grouped or scheduled by clinical category or CT status; because laboratory staff were blinded to CT findings and clinical categorisation at the time of analysis, CT-positive and CT-negative cases were not prospectively balanced across analytical runs. Biomarker analysis was performed independently of ED clinical assessment. Treating clinicians and radiologists were blinded to biomarker results. No formal statistical batch-effect correction was applied. Instead, between-run analytical variation was controlled prospectively within the laboratory's ISO 15189-accredited quality system, with all testing performed on the same platform by the same scientist using a single lot of reagents, calibrators, and quality control (QC) materials, and analytical runs accepted only when calibration and QC met predefined acceptability criteria.

Cut-offs evaluated

1. **Manufacturer thresholds:** GFAP 35 pg/mL and/or UCH-L1 400 pg/mL (OR rule).¹²
2. **Age-stratified thresholds (published):** for participants ≥ 65 years, GFAP 115 pg/mL and/or UCH-L1 335 pg/mL (OR rule), while retaining manufacturer thresholds for < 65 years.¹⁰

Covariates and eGFR handling

Age was analysed as < 65 vs ≥ 65 years. Renal function (eGFR, mL/min/ 1.73 m^2) was extracted where available. Because eGFR values are reported as '>60' above the laboratory reporting ceiling, '>60' values were arbitrarily recorded as 61 for statistical purposes; renal function was also evaluated categorically (≤ 60 vs > 60) in sensitivity analyses.

Analytical verification and trueness considerations

A local precision verification was performed prior to study sample analysis using a multi-day replicate design aligned with CLSI EP05-A3 principles.¹³ Three concentration levels per analyte met predefined imprecision targets ($\leq 5\%$ for within-laboratory coefficient of variation (CV)). At the time of validation, an external quality assessment (EQA) scheme for these analytes/platforms was not available; therefore, independent trueness verification was limited. As an interim mitigation, calibration and QC were performed per manufacturer recommendations, supplemented by periodic analysis of manufacturer calibrators/controls as unknowns to assess recovery against assigned values, recognising that non-commutable materials may not fully reflect patient matrix behaviour.

Sample size and planned study accrual

The BAMBI study is continuing with planned enrolment of approximately 1700 participants. Based on local CT-positive prevalence estimates, the study aims to accrue approximately 125 CT-positive cases to provide adequate power to estimate sensitivity precisely and to validate age-stratified thresholds and key subgroups.

Statistical analysis

Continuous variables were summarised as medians (interquartile ranges) and categorical variables as frequencies (%). Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate, and continuous non-parametric variables using the Mann-Whitney U test. Statistical significance was defined as a two-sided p -value < 0.05 .

Sensitivity, specificity, PPV, NPV, and likelihood ratios were calculated with 95% CIs, with performance reported overall and by age strata. ROC curves and AUC were estimated for GFAP and UCH-L1 (and combined models where applicable). Because the diagnostic rules represent binary classifiers (positive/negative) rather than continuous variables, ROC analysis yields single operating points, and the reported 'pooled AUC' represents the AUC-equivalent for binary classifiers, mathematically identical to balanced accuracy: $(\text{sensitivity} + \text{specificity})/2$.

Diagnostic accuracy metrics with 95% confidence intervals were calculated using the Wilson score method for sensitivity, specificity, and predictive values. For likelihood ratios, asymptotic confidence intervals were computed, with the rule of three method

applied to calculate the upper confidence limit for LR when no false negatives were observed. ROC curve analysis and comparison of AUCs between age-stratified approaches used the DeLong method. Statistical software: Analyse-IT version 6.16.

Results

Cohort characteristics and CT outcomes

Of 1112 eligible patients over the 6-month study period, 215 participants (19.3%) had complete biomarker and CT data; 19 (8.8%) were CT-positive, and 196 were CT-negative. Baseline characteristics (age, sex, antithrombotics, time from injury to sampling, eGFR band) are summarised in Tables 1, 2, and 3. The recruitment schematic is shown in Figure 1.

Pre-study analytical verification demonstrated acceptable assay precision (within-laboratory CV <5% across all levels of QC material for both GFAP and UCH-L1), as summarised in Supplementary Table S1.

Baseline characteristics by sex are summarised in Table 3. CT positivity did not differ by sex (male: 12/142 [8.5%]; female: 7/73 [9.6%]). In the overall cohort, median UCH-L1 was modestly higher in males than in females, whereas GFAP did not differ significantly. Within the CT-positive subgroup ($n=19$; 12 male, 7 female), no statistically significant sex differences in biomarker concentrations were observed. Median UCH-L1 was numerically higher in males than

females (448.5 [IQR: 295.6–548.1] vs 384.0 [218.8–427.5] pg/mL; $p=0.227$), whereas median GFAP was numerically higher in females than males (206.1 [120.7–307.9] vs 101.4 [78.9–247.2] pg/mL; $p=0.432$). Given the small number of CT-positive cases, these findings should be interpreted cautiously.

The median time from ED arrival to blood sampling was 3.1 hours (IQR: 1.4–6.6) and from injury to blood sampling was 5.2 hours (IQR: 3.3–8.9); the corresponding intervals to CT were 4.5 hours (IQR: 2.5–8.0) and 6.9 hours (IQR: 4.2–10.0), respectively (Table 2).

Diagnostic accuracy: manufacturer thresholds

Using manufacturer thresholds (GFAP: 35 pg/mL and/or UCH-L1: 400 pg/mL; OR rule), sensitivity was 100% (95% CI: 82.4–100) and specificity 29.6% (95% CI: 23.3–36.5). The biomarker-negative proportion was 27.0% (58/215), representing the theoretical maximum CT avoidance fraction under a biomarker-first pathway in this CT-selected cohort. Prediction outcome values were true positives (TP) $n=19$, true negatives (TN) $n=58$, false positives (FP) $n=138$, and false negatives (FN) $n=0$ (Table 4).

Diagnostic accuracy: age-stratified thresholds

Applying age-stratified thresholds (≥ 65 : GFAP 115 pg/mL and/or UCH-L1 335 pg/mL; < 65 : manufacturer

Table 1. Baseline characteristics overall and by CT status (CT+ vs CT–).

Parameters	Total cohort	CT negative	CT positive	<i>p</i> -Value
Number	215	196 (91.2%)	19 (8.8%)	
Age, years: median (range)	64 (18–96)	64 (18–96)	58 (20–94)	0.966
Biological sex: <i>n</i> (%)				0.803
Female	73 (34.0%)	66 (33.7%)	7 (36.8%)	
Male	142 (66.0%)	130 (66.3%)	12 (63.2%)	
Mechanism				0.575
Fall <i>n</i> (%)	132 (61.4%)	122 (62.2%)	10 (52.6%)	
Assault <i>n</i> (%)	37 (17.2%)	33 (16.8%)	4 (21.1%)	
RTA/MVA <i>n</i> (%)	11 (5.1%)	9 (4.6%)	2 (10.5%)	
Head impact/object <i>n</i> (%)	13 (6.0%)	13 (6.6%)	0 (0.0%)	
Collapse/syncope <i>n</i> (%)	10 (4.7%)	9 (4.6%)	1 (5.3%)	
Other <i>n</i> (%)	12 (5.6%)	10 (5.1%)	2 (10.5%)	
GCS: median (range)	15 (13–15)	15 (13–15)	15 (13–15)	0.102
GCS 15 <i>n</i> (%)	162 (75.3%)	149 (76%)	13 (68.4%)	
GCS 14 <i>n</i> (%)	47 (21.9%)	43 (21.9%)	4 (21.1%)	
GCS 13 <i>n</i> (%)	6 (2.8%)	4 (2.0%)	2 (10.5%)	
eGFR (MDRD): median (range)	61 (17–61)	61 (17–61)	61 (18–61)	0.932
TBI biomarkers:				
GFAP (pg/mL) median (IQR)	50.0 (27.8–96.2)	44.4 (25.6–84.7)	140.5 (80.3–307.9)	<0.001
UCH-L1 (pg/mL) median (IQR)	236.1 (147.1–413.6)	226.1 (143.1–373.5)	437.0 (283.8–481.9)	0.005
Meds: prescriber/illicit				0.339
None reported <i>n</i> (%)	168 (78.1%)	153 (78.1%)	15 (78.9%)	
Anticoagulant/antiplatelet <i>n</i> (%)	18 (8.4%)	18 (9.2%)	0 (0.0%)	
Intoxication (alcohol/drugs) <i>n</i> (%)	19 (8.8%)	17 (8.7%)	2 (10.5%)	
Other <i>n</i> (%)	10 (4.7%)	8 (4.1%)	2 (10.5%)	

Tests used: Fisher's exact for sex; chi-square for multi-category variables; Mann-Whitney *U* for continuous variables.

RTA: road traffic accident; MVA: motor vehicle accident; GCS: Glasgow coma scale; eGFR: estimated glomerular filtration rate; TBI: traumatic brain injury; GFAP: glial fibrillary acidic protein; UCH-L1: ubiquitin carboxy-terminal hydrolase L1; Meds: medications.

Table 2. Baseline characteristics by age.

Variable	<65 years	≥65 years	p-Value
Number	108 (50.2%)	107 (49.8%)	
Biological sex			0.001
Female	25 (23.2%)	48 (44.9%)	
Male	83 (76.8%)	59 (54.1%)	
Mechanism of injury			<0.001
Assault	37 (34.3%)	3 (2.8%)	
Fall	37 (34.3%)	93 (87.0%)	
RTA	12 (11.1%)	1 (0.9%)	
Other	22 (20.4%)	10 (9.3%)	
GCS on arrival: median (IQR)	15 (15–15)	15 (15–15)	0.315
Alcohol intoxication			<0.001
No	63 (58.3%)	89 (83.2%)	
Yes	45 (41.7%)	18 (16.8%)	
Antiplatelets			<0.001
No	105 (97.2%)	73 (68.2%)	
Yes	3 (2.8%)	34 (31.8%)	
Anticoagulation			<0.001
No	104 (96.3%)	77 (72.0%)	
Yes	4 (3.7%)	30 (28.0%)	
Time from injury to bloods (h): median (IQR)	6 (4–9)	6 (4–9)	0.570
Time from arrival to bloods (h): (median, IQR)	4 (2–8)	4 (2–8)	0.286
Time from arrival to scan (h): median (IQR)	6 (3–8)	5 (3–8)	0.771
Stayed in department overnight for a scan			
No	74 (68.5%)	79 (73.8%)	0.452
Yes	34 (31.5%)	28 (26.2%)	
CT results			1.000
Positive	10 (9.3%)	9 (8.4%)	
Negative	98 (90.7%)	98 (91.6%)	
Discharge			0.048
No	23 (21.3%)	36 (33.7%)	
Yes	85 (78.7%)	71 (66.3%)	
7-day reattendance			0.369
No	104 (96.3%)	106 (99.1)	
Yes	4 (3.7%)	1 (0.9%)	
ED LOS-(h) : median (IQR)	13 (9–16)	13 (9–16)	0.998
TBI biomarkers: median (IQR)			
GFAP (pg/mL)	36.4 (20.6–76.3)	59.9 (39.9–100.0)	<0.001
UCH-L1 (pg/mL)	187.4 (115.1–402.8)	256.7 (192.9–425.9)	0.003

Tests used: Fisher's exact for 2×2 categorical variables, chi-square for multi-category variables, and Mann-Whitney *U* for continuous variables.

RTA: road traffic accident; GCS: Glasgow coma scale; CT: computed tomography; ED LOS: emergency department length of stay; TBI: traumatic brain injury; GFAP: glial fibrillary acidic protein; UCH-L1: ubiquitin carboxy-terminal hydrolase L1.

Table 3. Baseline characteristics stratified by biological sex.

Variable	Male	Female	p-Value
Total number (n)	142	73	(Male vs female)
Age, years: median (IQR)	55.0 [35.2, 76.0]	74.0 [49.0, 82.0]	<0.001
Age ≥65 years, n (%)	59 (41.5%)	48 (65.8%)	<0.001
GCS: median (IQR)	15.0 [14.0, 15.0]	15.0 [15.0, 15.0]	0.187
Intracranial injury positive (non-CTHPOS), n (%)	12 (8.5%)	7 (9.6%)	0.803
TBI biomarkers:			
GFAP (pg/mL), median (IQR)	47.0 [27.8, 85.8]	59.9 [28.5, 111.1]	0.242
UCH-L1 (pg/mL), median (IQR)	256.1 [155.8, 451.6]	213.1 [131.5, 313.6]	0.042
GFAP ≥35 pg/mL, n (%)	92 (64.8%)	52 (71.2%)	0.362
UCH-L1 ≥400 pg/mL, n (%)	41 (28.9%)	15 (20.5%)	0.251

Tests used: Fisher's exact for 2×2 categorical variables and Mann-Whitney *U* for continuous variables.

GCS: Glasgow coma scale; non-CTHPOS: non-contrast head computed tomography positive; TBI: traumatic brain injury; GFAP: glial fibrillary acidic protein; UCH-L1: ubiquitin carboxy-terminal hydrolase L1.

Age-stratified clinical utility

thresholds), observed sensitivity remained 100% (95% CI: 82.4–100) while specificity improved to 55.1% (95% CI: 47.9–62.2). The biomarker-negative proportion increased to 50.2% (108/215), with TP $n = 19$, FN $n = 0$, FP $n = 88$, TN $n = 108$ (Table 4).

Clinical utility was evident in both age strata (Table 5).

- <65 years: $n = 108$; CT+ $n = 10$. Under the clinical algorithm, 43.5% were biomarker-negative (47/

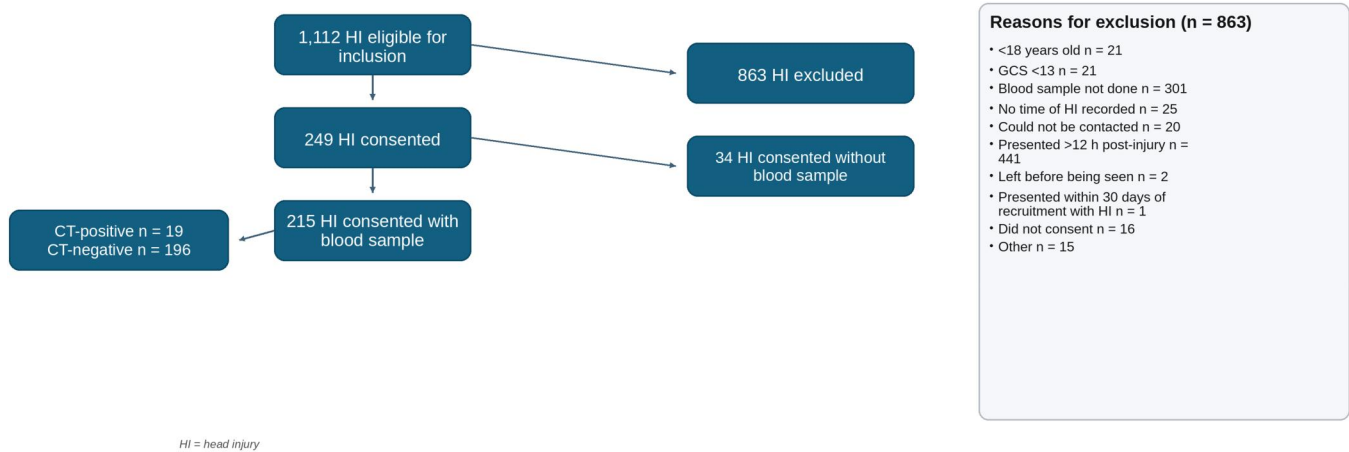


Figure 1. Recruitment flow diagram.

Table 4. Diagnostic accuracy of manufacturer thresholds vs age-stratified thresholds (overall and by age strata).

Decision thresholds	
Abbott	Age-stratified
UCH-L1 ≥ 400 pg/mL OR GFAP ≥ 35 pg/mL	<65 years (Abbott): UCH-L1 ≥ 400 pg/mL, GFAP ≥ 35 pg/mL ≥ 65 years (Ladang et al): UCH-L1 ≥ 335 pg/mL, GFAP ≥ 115 pg/mL
Prediction outcomes	
TP 19 TN 58 FP 138 FN 0	TP 19 TN 108 FP 88 FN 0
Sensitivity: 100.0%	Sensitivity: 100.0%
Specificity: 29.6%	Specificity: 55.1%
PPV: 12.1%	PPV: 17.8%
NPV: 100.0%	NPV: 100.0%
LR+: 1.42	LR+: 2.23
LR-: 0.00	LR-: 0.00

TP: true positives; TN: true negatives; FP: false positives; FN: false negatives; PPV: positive predictive value; NPR: negative predictive value; LR: likelihood ratio.

Table 5. Age-adjusted rule: superior clinical tool compared to the standard Abbott rule.

	Standard Abbott rule	Age-adjusted rule	Difference	Clinical benefit
Younger specificity (n = 108)	48.0%	48.0%	No change	
Elderly specificity (n = 107)	11.2%	62.2%	+51.0% improvement	~5.5× higher specificity
Pooled AUC (binary rule)	0.648	0.776	+0.128	Higher AUC
Total false positives	138 patients	88 patients	50 fewer false positives	
Renal correlation	Not modelled	Not modelled	No formal update	Planned in full cohort

AUC: Area under the curve; Pooled AUC represents balanced accuracy for binary diagnostic rules.

108), and diagnostic specificity was 48.0% (95% CI: 37.8–58.3).

- ≥ 65 years: n = 107; CT+ n = 9. With age-adjusted thresholds, specificity improved from 11.2% to 62.2% (95% CI: 51.9–71.8), with 57.0% biomarker-negative (61/107).

ROC analysis

In this population, GFAP demonstrated stronger discrimination for CT-positive injury than UCH-L1 in this preliminary dataset (Figure 2). GFAP and UCH-L1 concentrations were substantially higher in CT-positive than CT-negative participants (GFAP: 140.5

[80.3–307.9] vs 44.4 [25.6–84.7] pg/mL, $p < 0.001$; UCH-L1: 437.0 [283.8–481.9] vs 226.1 [143.1–373.5] pg/mL, $p = 0.005$; Table 1).

Discussion

This preliminary BAMBI analysis from a Major Trauma Centre ED shows that the GFAP/UCH-L1 assay maintains a clinically significant rule-out signal for CT-positive traumatic intracranial abnormalities. In this dataset, no biomarker-negative, CT-positive cases were observed, achieving 100% sensitivity for intracranial injury. While these results are promising, the key question for real-world clinical impact is

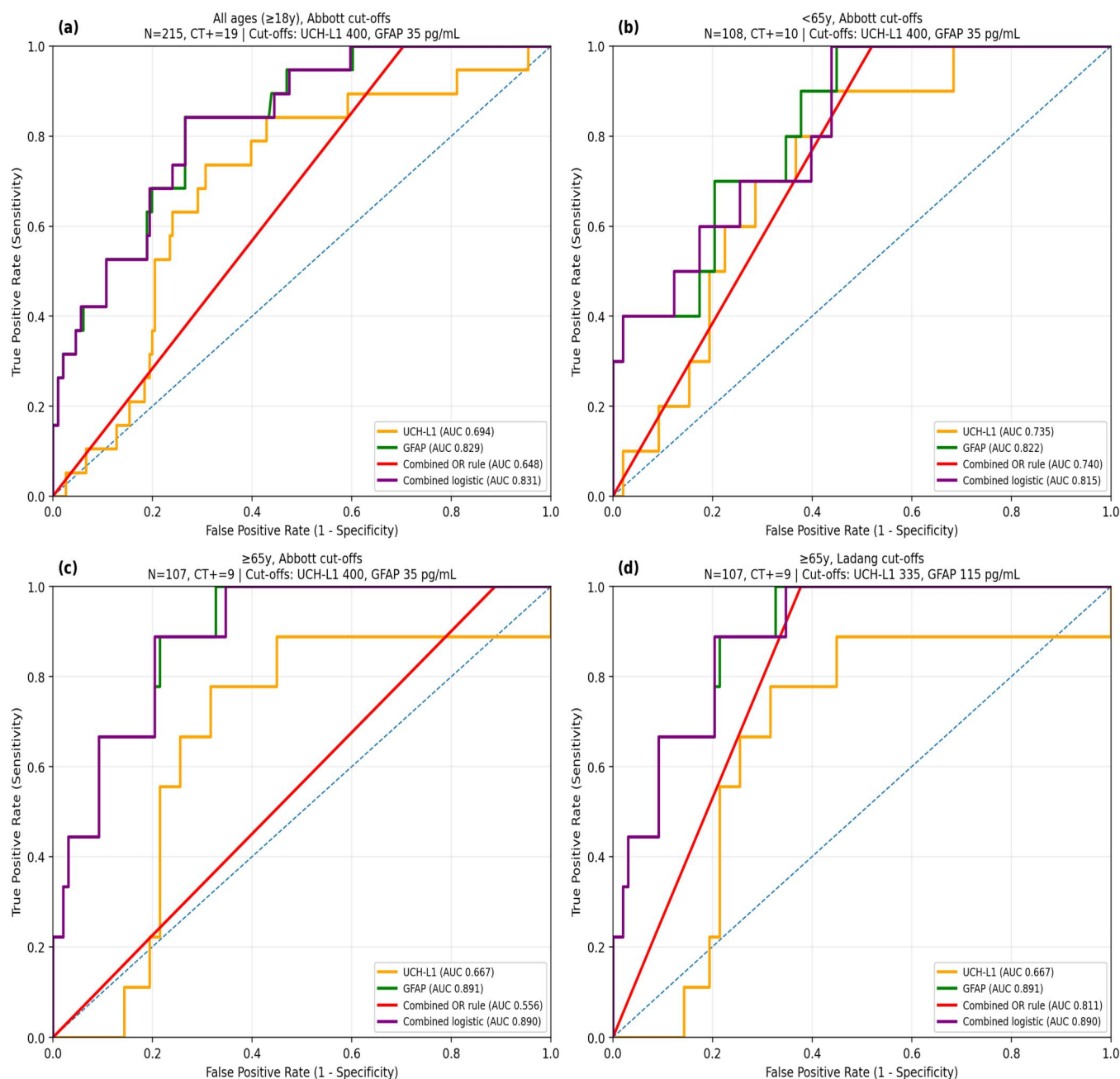


Figure 2. ROC curves for GFAP, UCH-L1, and combined model (all ages and age-stratified analyses). Decision thresholds evaluated: Abbott (UCH-L1 ≥ 400 pg/mL OR GFAP ≥ 35 pg/mL) and age-adjusted thresholds for those ≥ 65 years (UCH-L1 ≥ 335 pg/mL OR GFAP ≥ 115 pg/mL).

whether specificity is high enough to meaningfully reduce low-yield CT scans, enabling quicker decision-making in a busy ED. Using manufacturer thresholds, specificity was low (29.6%), limiting the proportion of biomarker-negative cases (27.0%) and, consequently, the potential to avoid CT scans in a biomarker-first approach.

Importantly, the clinical value of GFAP/UCH-L1 testing in this cohort extends beyond older adults. Among participants < 65 years, a substantial proportion (43.5%) were biomarker-negative, highlighting an

opportunity for CT stewardship in a large, generally lower-risk group. Conversely, older adults pose a distinct implementation challenge. These are the patients whom NICE guidance typically directs toward imaging based on age criteria alone, representing a high-volume, low-yield subgroup where traditional clinical decision rules are most aggressive.¹ Baseline biomarker elevations and comorbidities can undermine specificity when universal cut-offs are used, reducing the usefulness of testing in a subgroup that already influences CT imaging decisions under most

guidelines.^{7,9,11} Age-related increases in blood GFAP are well documented, likely reflecting astroglial changes and decreased blood–brain barrier integrity rather than acute trauma.⁸ In our study, applying universal thresholds to those aged ≥ 65 years resulted in a specificity of 11.2%.

The main finding of this study is that age-specific action limits for those aged 65 years and older significantly enhance diagnostic utility without sacrificing safety. Implementing these cut-offs increased overall specificity from 29.6% to 55.1%, and the biomarker-negative proportion rose from 27.0% to 50.2%. Notably, the greatest benefit was observed in the older group, where specificity improved to 62.2%. This calibration serves as a practical tool to maintain the test's usefulness across the full ED case mix. Importantly, utility extends beyond the elderly; standard thresholds already provide meaningful CT avoidance potential in younger adults, while age-adjusted cut-offs specifically address the specificity challenges in older patients.

The BAMBI age-stratified approach represents an important evolution in the implementation debate currently reflected in international guidelines. The 2023 French guidelines (SFMU/SFAR),¹⁴ the 2024 Spanish guidelines (SEMES),¹⁵ and the 2024 American College of Surgeons (ACS) guidelines¹⁶ all endorse GFAP/UCH-L1 testing to rule out CT imaging in intermediate-risk patients. However, these guidelines generally advocate universal thresholds across all ages (GFAP: 22–35 pg/mL and UCH-L1: 327–400 pg/mL, depending on the platform).

While the ACS guidelines recognise that biomarker testing should be *'integrated with clinical assessment and decision rules, not used in isolation, due to modest specificity and potential confounders such as age'*, they do not yet specify adjusted thresholds.¹⁶ The French and Spanish approaches use an age ≥ 65 as a criterion to determine who undergoes testing, but they do not adjust the interpretation of that test. The BAMBI data suggest that threshold calibration is as important as risk stratification; without it, those who qualify for testing under these guidelines may be more likely to receive a 'false-positive' result because of physiological baseline elevations.

From a neurosurgical and service perspective, the most relevant implication is CT stewardship while maintaining patient safety. The increase in potential CT avoidance from 27.0% to 50.2% is likely to have important clinical, service, and economic implications if confirmed in prospective implementation work.

Real-world studies on the same platform have explicitly shown that assay analysis can be rapid—15–

18 minutes—and that CT turnaround times can exceed 60–120 minutes for many scans in some systems, indicating that faster biomarker availability could feasibly support earlier decision-making and reduce throughput bottlenecks.³ Because samples were biobanked and analysed retrospectively, real-time biomarker turnaround time could not be measured in this interim study. However, if implemented clinically, we anticipate a laboratory turnaround time of <60 minutes from sample receipt to result authorisation, based on manufacturer workflow requirements and local operational experience with the assay platform; this will be confirmed in a prospective implementation study and health-economic evaluation.

This study has limitations. As a preliminary proof-of-concept complete-case analysis, the CT-positive event count is small ($n = 19$). Although no false negatives were observed and the 100% sensitivity is promising, the wide confidence intervals around sensitivity require confirmation in a larger cohort. Because samples were biobanked and analysed retrospectively across multiple assay days, residual run-related variation cannot be entirely excluded, although testing was performed under tightly standardised analytical conditions on a single random-access platform by one experienced scientist using a single lot of reagents, calibrators, and QC materials with predefined calibration and QC acceptance criteria. BAMBI recruitment is ongoing, with plans to enrol approximately 1400–1700 participants. Initial sample size calculations based on the landmark ALERT-TBI study prevalence (5.3%)⁴ have been updated to reflect our observed CT-positive rate of 8.8%, targeting 125–150 CT-positive events—comparable to or exceeding the 106 CT-positive events in ALERT-TBI—for robust validation of age-stratified thresholds and other clinically relevant subgroups, including those with renal impairment and receiving antithrombotic therapy.

Consistent with this study's diagnostic focus, neurosurgical outcomes were not formally evaluated; no patients required neurosurgical intervention. The significant elevation of both biomarkers in CT-positive participants nonetheless supports their biological association with radiologically evident intracranial injury. Additionally, CT-positive findings vary in clinical significance; planned analyses will assess relevant injury endpoints where feasible, including those requiring neurosurgical intervention, neurological deterioration, and re-presentations. A dedicated health economics assessment is also planned to translate diagnostic performance into policy-relevant outcomes (avoided CT scans, impacts on ED flow, and opportunity costs).

using local service parameters, building on existing models that suggest biomarker-first approaches may be cost-effective when specificity and operational factors support significant CT reduction.¹⁷

Although 1112 patients met NICE imaging criteria during the study period, only 215 (19.3%) were included in this interim analysis because inclusion required prospective consent and complete paired biomarker and CT data within the protocol sampling window in a real-world ED workflow. As this proof-of-concept phase was conducted without a dedicated research assistant, recruitment was additionally subject to pragmatic staffing constraints. This may have introduced selection bias and may limit generalisability. Certain clinically important subgroups also remained small at this interim stage; for example, only 34 participants were anticoagulated and only 4 were CT-positive, precluding robust subgroup-specific threshold evaluation despite all CT-positive anticoagulated cases being biomarker-positive under both diagnostic rules.

Conclusions

In this preliminary NICE-imaged ED cohort, the GFAP/UCH-L1 assay demonstrated strong rule-out performance for CT-positive traumatic intracranial injury. However, its clinical utility is limited by the low specificity of universal thresholds. Age-stratified decision thresholds directly address physiological baseline biomarker elevations in older adults, significantly increasing the potential to avoid CT imaging while maintaining safety. These findings support the inclusion of age-aware interpretation in future guidelines to optimise the health system impact of mTBI biomarkers.

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Author contributions

Paula M. O'Shea, Etimbuk Umana and Philip J. O'Halloran had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

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All authors read and approved the final manuscript.

Declarations

Ethics approval and consent to participate

Ethical approval was granted by the Mater Misericordiae University Hospital Research Ethics Committee (REC ref: 1/378/2482). The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from all participants prior to enrolment.

Health and safety

This was a non-interventional observational study conducted within routine emergency and laboratory workflows. Patient assessment and CT imaging were performed according to standard clinical care. Blood sample collection, handling, processing and storage were undertaken by trained staff in accordance with Mater Misericordiae University Hospital policies, standard operating procedures, and applicable laboratory biosafety requirements. No study-specific procedures introduced additional material risk beyond standard care and venepuncture.

Disclosure statement

The authors report there are no competing interests to declare.

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Data availability statement

The anonymised datasets used and/or analysed during the current study will be available from the corresponding author on reasonable request.

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