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

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Study objectives: Brain biomarkers have been used to predict intracranial injury in both adults and children following mild traumatic brain injury (mTBI). Several biomarkers have been evaluated, including S100B, NfL, Tau, glial fibrillary acidic protein (GFAP), and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1). The combined measurement of GFAP and UCH-L1 has recently been recommended by scientific societies, but no meta-analysis on the topic has been performed yet.

Methods: A meta-analysis was performed to assess the prognostic value of the association of GFAP and UCH-L1 blood levels in predicting intracerebral lesions in adults after mTBI. A protocol was designed and registered with PROSPERO (CRD42024562587). Studies were chosen if they included adults with mTBI who underwent GFAP and/or UCH-L1 measurement and cranial computed tomography scans. The quality of each study was assessed using the Quality Assessment of Diagnostic Accuracy Studies 2 criteria. Three databases (Medline, Embase, and the Cochrane Central Register of Controlled Trials) were consulted.

Results: Of the 379 articles screened, 16 were selected for inclusion. The overall pooled sensitivity (Se) and specificity (Spe) were 100% (95% confidence interval [CI] 99% to 100%) and 31% (95% CI 26% to 36%), respectively, for the association of GFAP and UCH-L1. For GFAP alone, the overall pooled Se and Spe were 94% (95% CI 91% to 97%) and 40% (95% CI 34% to 46%), respectively. For UCH-L1 alone, the overall pooled Se and Spe were 83% (95% CI 69% to 94%) and 51% (95% CI 40% to 63%), respectively. The areas under the curve were 88, 67, and 97%, respectively, for GFAP, UCH-L1, and the association GFAP/UCH-L1.

Conclusion: The combined measurement of GFAP and UCH-L1 allows the exclusion of intracranial injury after mTBI in adults with 100% Se and negative predictive value. Its routine use can theoretically reduce the number of cranial computed tomography scans by 31%. The different sampling times and techniques used in the studies did not allow us to make specific recommendations. [Ann Emerg Med. 2025;■:1-14.]

Please see page XX for the Editor's Capsule Summary of this article.

Keywords: CCT scan, Glial fibrillary acidic protein (GFAP), Mild traumatic brain injury (mTBI), Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1).

0196-0644/$-see front matter

https://doi.org/10.1016/j.annemergmed.2025.03.018

INTRODUCTION

Background

Traumatic brain injury (TBI) is a major cause of morbidity and mortality worldwide, with an incidence in Europe estimated to be 258 cases per 100,000 population.[1] More than 80% of TBI cases in adults are classified as mild (mTBI), with a Glasgow coma scale (GCS) score of 13 to 15.[2,3] The management of patients with mTBI involves cranial computed tomography (CCT) scans to rule out intracranial lesions, such as skull fracture, epidural hematoma, subdural hematoma, pneumocephalus, subarachnoid hemorrhage, hemorrhagic contusion, and otohematoma, which are observed only in a low proportion of patients (less than 10%).[4,5] Because of the organizational effect on managing patient flow in the emergency department (ED) when a CCT scan is ordered, the risks associated with radiation exposure, and the costs involved, clinical decision algorithms such as the Canadian CT Head Rule, the New

Editor's Capsule Summary

What is already known on this topic

Multiple blood markers are associated with intracranial findings on head computed tomography (CT) after mild traumatic brain injury (TBI).

What question this study addressed

Can measuring both glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) rule out intracranial abnormalities on head CT?

What this study adds to our knowledge

In a meta-analysis of 11 studies, combined measurement of GFAP and UCH-L1 had close to 100% sensitivity for detecting CT abnormalities.

How this is relevant to clinical practice

Patients with normal blood levels of both GFAP and UCH-L1 after mild TBI are unlikely to have intracranial lesions on CT scan.

Orleans Criteria or the National Emergency X-Radiography Utilization Study II have been proposed to limit CCT scan use.[4,6-11] These clinical rules helped reduce the number of CCT scans performed but were not sufficient, hence the use of blood biomarkers.[12,13]

Several blood biomarkers have been evaluated in the management of adult mTBI in EDs, particularly to rule out intracranial lesions. If the biomarker tested is positive, the patient undergoes a CCT scan. Otherwise, a CCT scan is no longer indicated if the biomarker test result is negative, and the patient can be discharged with instructions on what to do if symptoms occur. Thus, the primary value of biomarkers in the emergency setting is to reduce the number of unnecessary CCT scans. These include S100B, glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase-L1 (UCH-L1), neurofilament light chain (NFL), and Tau.[14-17] S100B has excellent sensitivity (Se) and negative predictive value (NPV) for excluding intracranial lesions on CCT scans in both adults and children and according to the guidelines, the S100B protein should be measured within 3 hours after mTBI.[18,19] However, its short half-life (30 to 90 minutes), the variation in blood concentrations according to skin pigmentation, and lack of neurospecificity led to the study and proposal of other biomarkers.[20-22] In the United States, the combination of GFAP and UCH-L1 has been used since its approval by the Food Drug Administration in 2018.[23] Their use has also been recommended by the French Society of Emergency Medicine since 2022.[24] GFAP is an intermediate filament primarily found in astrocytes, but also expressed in fibroblasts, lymphocytes, chondrocytes, and myoepithelial cells. GFAP is released by damaged astrocytes into the subarachnoid cerebrospinal fluid, then enters the peripheral circulation, either through the glymphatic pathway or by diffusion across the blood-brain barrier. Once in the bloodstream, GFAP is excreted by the kidneys with a half-life of 24 to 48 hours.[25-27] UCH-L1 is an enzyme expressed by neurons involved in the proteasome degradation pathway. It accounts for 5% of total neuronal proteins. UCH-L1 expression was also observed in kidney cells and gonads.[28] Following brain injury, UCH-L1 enters the bloodstream either through the blood-brain barrier or the glymphatic system, and has a half-life of 6 to 7 hours.[29,30] These 2 biomarkers, which are not influenced by skin pigmentation, should be measured within 12 hours after mTBI according to the recommendations.[24] Measurement of GFAP and UCH-L1, although not yet used in routine practice, is available for clinical use on 3 analyzers: a point-of-care i-STAT handheld device (electrochemiluminescence), a core lab Alinity platform (Chemiluminescent Microparticle Immunoassay [CMIA]) and on the Vidas platform (enzyme-linked fluorescent assay).[31] These 3 devices, suitable for routine use, allows results to be obtained in less than an hour.[32,33] All other techniques, such as enzyme-linked immunosorbent assays (ELISA) or single molecule array, are for research purposes only and cannot be used in routine practice. The cost of measuring GFAP and UCH-L1 is estimated to be between €30 and €50, making it less expensive than a CCT scan.[7] However, these costs need to be confirmed once the measurement is introduced into routine practice.

Importance

Although studies such as that by Bazarian et al[23] have reported data on adults with mTBI, to our knowledge, no meta-analysis has yet comprehensively summarized the use of blood GFAP and UCH-L1 in association as biomarkers.[32-48]

Goals of This Investigation

Therefore, the primary objective of our study was to perform a systematic review and meta-analysis to evaluate the effectiveness of determining serum levels of GFAP and UCH-L1 in detecting intracranial lesions in adult patients with mTBI. Another objective was to meta-analyze the utility of GFAP alone and UCH-L1 alone in ruling out intracranial injury after a mTBI and compare the results with those observed with other biomarkers such as S100B.[14,18,49]

MATERIALS AND METHODS

Study Design and Patients

In this systematic review and meta-analysis, we followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses.[50] A protocol was designed and registered with PROSPERO (CRD42024562587). Two retrospective studies and several prospective studies of adults with mTBI were included. Only one study was found that evaluated GFAP and UCH-L1 to rule out intracranial injury after mTBI in children.[51] All patients underwent a CCT scan along with measurement of the blood biomarker pair: GFAP and UCH-L1, or GFAP alone, or UCH-L1 alone. In 4 studies, a small proportion of patients with mTBI (GCS between 9 and 12) were included in addition to patients with moderate TBI (GSC between 13 and 15). Nevertheless, these studies were selected for inclusion in the meta-analysis due to the small number of patients with moderate TBI. Studies were eligible if they provided data on Se, Spe, NPV, and positive predictive value (PPV). All studies that included patients with severe TBI (CGS < 9) and where data could not be separated from those with mTBI were ineligible for inclusion.

Search Strategy

The search for eligible publications was conducted with the assistance of a librarian from the Faculty of Medicine at Clermont-Ferrand. Three electronic databases were searched: Medline, Embase, and the Cochrane Central Register of Controlled Trials (CENTRAL). Customized search equations tailored to each database were developed to identify studies most likely to be included (Tables E1 and E2, available at http://www.annemergmed.com). The references of included studies were analyzed to enhance the bibliographic search.

Study Selection

The eligibility of all studies was assessed after reviewing the title and abstract of each article. This step was conducted by 2 different individuals (A.P. and S.K.), and a third person (D.B.) resolved any disagreements between the 2 reviewers. A total of 3 out of the 57 eligible articles were not reviewed because they were conference abstracts.

Data Extraction

The following information was extracted from each included article: study design (monocentric or multicentric, prospective or retrospective), number and characteristics of patients (age, skin pigmentation, GCS score), assay techniques and reference values for GFAP and UCH-L1, time interval between sampling and assay, comparison of CCT scan results with GFAP and UCH-L1 assay results (true positives, false positives, true negatives, false negatives, Se, Spe, PPV, NPV, area under the curve [AUC]).

The data were independently extracted by 2 authors (A.P. and C.O.) and verified by a third author (D.B.).

Methodological Quality Assessment

The quality of each study was assessed using criteria presented in the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2).[52] Assessments of study quality were conducted independently by 2 authors (A.P. and C.O.), and their discrepancies were resolved by a third author (D.B.).

Index test. Different analytical techniques have been used by the authors of the studies to perform the assays of GFAP and UCH-L1: ELISA, electrochemiluminescence (Abbott i-STAT), single molecule array, CMIA (Alinity i TBI), and enzyme-linked fluorescent assay (Vidas TBI). The reference values for the 2 blood biomarkers also varied between studies. We did not take these differences into account when interpreting the results in order to have a sufficient number of studies for comparison. A test was considered positive if 1 or 2 biomarkers were above their published and recommended cutoff values.

Reference test. To be included in this meta-analysis, patients had to have undergone a CCT scan. Therefore, all studies that focused on brain magnetic resonance imaging were excluded. In current practice, a CCT scan is performed after mTBI. If the scan is negative, the risk of developing an intracranial lesion is very low.[53]

Analysis

Statistical analyses were performed using Stata software, version 15 (StataCorp, College Station, Texas). For each study, patient characteristics were summarized and reported as mean and standard deviation or median and interquartile range. Se, Spe, NPV, and their 95% confidence intervals (CIs) were estimated using random-effects models assuming between-study and within-study variability (DerSimonian and Laird approach) using the *metaprop* Stata program. This routine provides procedures for pooling proportions in a meta-analysis of multiple studies and presents the results in a forest plot. CIs are based on score (Wilson) or exact binomial (Clopper-Pearson) procedures. As NPV is highly sensitive to prevalence, likelihood ratios (LR+ and LR−) were also estimated with 95% CIs. Statistical heterogeneity between results was assessed by examining forest plots, 95% CIs, and I^2: less than 25%—low, 25% to 50%—moderate, and >50%—high. However, as pointed out by Migliavaca et al,[54] meta-analyses of proportions (such as Se, Spe, and NPV) often yield high I^2 values, which may be biased. High I^2 values are not systematically synonymous with important

variability between studies and may not be discriminative. Therefore, prediction intervals may be an appropriate option for assessing heterogeneity in proportionate meta-analyses. Sensitivity analyses were then performed to check the robustness of the results by assessing the effect of inclusion and exclusion of studies on the Se, Spe, and NPV using a funnel plot and I^2. Using the estimated model coefficients and variance-covariance matrixes, summary operational Se and Spe with confidence and prediction contours in summary receiver operating characteristic space were estimated using the *midas* Stata program. Cells with a value of zero were adjusted with a continuity correction of 0.5, which is the default setting.

RESULTS

Identification of Trials

The search equations applied to the 3 databases identified 379 studies. After removing duplicates, 261 studies remained. After reviewing the title and abstract of each study, 54 articles were selected for full-text review. Finally, 16 articles were included in the meta-analysis (Figure 1).[23,32,33,35-44,46-48]

Characteristics of Included Trials

In all included studies, the total number of patients was 7,828, and the median number was 252 (minimum: 62, maximum: 1,920). The mean age of the patients ranged from 39 to 79 years. CCT scans were consistently used as inclusion criteria, and blood samples were taken between 4 and 24 hours, depending on the study. The matrix was serum in 10 studies and plasma in the remaining 6 studies.[33,36,42,44,46,48] The median time of sampling after the head injury ranged from 1 to 11 hours. For GFAP and UCH-L1, more than half of the studies used the ELISA technique.[23,36-42,44] The cutoff values for both GFAP and UCH-L1 varied due to differences in sample type and analytical techniques.

Regarding the biomarkers' (GFAP and UCH-L1 in combination) diagnostic performance, the Se ranged from 97.0% to 100%. The Spe results were more heterogeneous. In the majority of cases, it was between 25% and 40%. In one study, it was very low—at 11%. All study characteristics are shown in the Table.

Study Quality

Of the 16 studies, 11 met at least 6 criteria of the QUADAS-2 tool. The remaining 5 studies met 5 criteria each (Figure 2).[33,36-38,47] In one study, there was a risk of bias regarding flow time because patients with an S100B protein level less than 0.1 μg/L were classified as a "negative CCT scan" group due to the biomarker's sensitivity.[43] Therefore, 2 reference tests were used. In 4 studies, the index test was not blinded.[33,37,47,48] In 4 studies, the CCT scan results were not blinded to laboratory personnel.[33,36,46,47] In 5 studies, only 1 biomarker (GFAP or UCH-L1) was evaluated, which raises concerns about the applicability of the index test.[35-38,44] In one study, only patients aged more than 60 years were included, raising concerns about applicability regarding patient selection.[39] All the data are summarized in Figure 2.

Main Results

GFAP alone. The diagnostic performance of GFAP alone was evaluated in 13 studies. The overall pooled Se (Figure 3A) was 94% (95% CI 91% to 97%) with an I^2 value of 67.22%. The overall pooled NPV was 97% (95% CI 95% to 99%), with an I^2 value of 67.32%. A funnel plot was performed and did not identify one or more studies responsible for the observed heterogeneity by the I^2 value. The overall pooled Spe (Figure 3B) was 48% (95% CI 39% to 57%). After performing a funnel plot (Figure 3C), 3 studies were removed, resulting in an overall pooled specificity of 40% (95% CI 34% to 46%).[40,41,44] Despite performing the funnel plot, the I^2 value remained high, decreasing from 96.52% to 89.77%. The overall pooled positive and LR− were 1.85 (95% CI 1.56 to 2.18) and 0.14 (95% CI 0.10 to 0.19), respectively.

The AUC, as assessed by summary receiver operating characteristic, was 88% (95% CI 85% to 91%) (Figure 3D).

UCH-L1 alone. The diagnostic performance of UCH-L1 alone was evaluated in 10 studies. The overall pooled Se (Figure 4A) was 83% (95% CI 69% to 94%) with an I^2 value of 92.84%. The overall pooled NPV was 94% (95% CI 90% to 97%), with an I^2 value of 80.30%. Performing a funnel plot for Se and NPV did not indicate any studies to be removed for analysis of the results. The overall pooled Spe (Figure 4B) was 46% (95% CI 33% to 59%). After performing a funnel plot (Figure 4C) and removing 5 studies, the overall specificity remained stable at 51% (95% CI 40% to 63%).[37,40,42,43,48] The I^2 value showed slight improvement, decreasing from 97.91% to 92.62%. The overall pooled LR+ and LR− were 1.57 (95% CI 1.32 to 1.86) and 0.31 (95% CI 0.16 to 0.62), respectively.

For UCH-L1, the AUC was 67% (95% CI 62% to 71%) (Figure 4D).

GFAP and UCH-L1. The diagnostic performance of the GFAP and UCH-L1 biomarker combination was evaluated in 11 studies. The overall pooled Se (Figure 5A) and NPV were 100% (95% CI 99% to 100% and 100% to 100%, respectively). I^2 values were 0%. The overall pooled Spe (Figure 5B) was 29% (95% CI 23% to 35%). The I^2 value was 93.91%. After performing a funnel plot

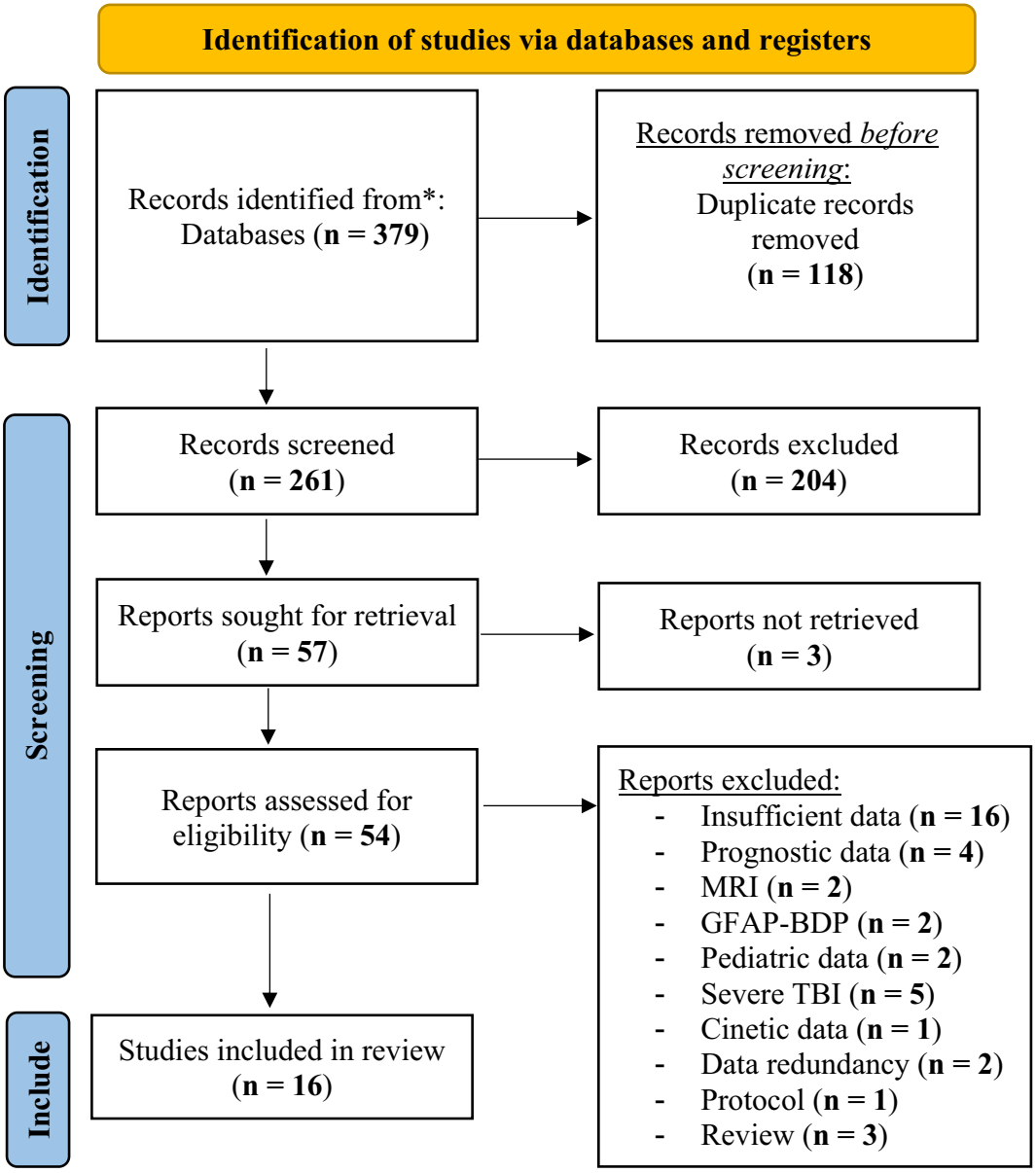

Figure 1. PRISMA flowchart explaining article screening and selection. *BDP,* breakdown products; *MRI,* magnetic resonance imaging; *PRISMA,* Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

(Figure 5C), one study was excluded from the analysis.[42] The overall specificity increased to 31% (95% CI 26% to 36%), and I^2 improved slightly to 89.43%. The overall pooled LR+ and LR− were 1.34 (95% CI 1.23 to 1.45) and 0.14 (95% CI 0.08 to 0.27), respectively.

For the association of GFAP and UCH-L1, the AUC was 98% (95% CI 97% to 99%) (Figure 5D).

LIMITATIONS

This meta-analysis has several limitations. First, the maximum inclusion time for patients after mTBI varied between trials, with up to 4 hours in 3 of the 16 trials and up to 24 hours in 4 other trials. This heterogeneity in sampling times complicates the confirmation of the 12-hour window recommended by scientific societies, with only 7 of the 16 studies adhering to this timeframe.[23,32,33,39,43,47,48] Second, the cutoff values for GFAP and UCH-L1 differed between studies. Although Bazarian et al[23] prospectively evaluated cut-off values, which were subsequently used by other researchers, most studies derived cut-offs directly from ROC curves.[35-38,40,42,44] Third, the assays used different analytical techniques and instruments, explaining variations of the cutoff values. Ideally, consistent results would be best obtained if all studies used the same automated system

Table. Characteristics of included studies.

Study	Design Prospective Study	n	Age, y Mean	GCS	Brain Injury /Total CCT Scan (Neurosurgery)	Reference test	Assay Technique	Cut-off (pg/mL) GFAP UCH-L1	Sampling Time (Hours) Max	Sampling Time (Hours) Median	Results	Se (%) (95% CI)	Spe (%) (95% CI)	AUC ROC (95% CI)
Papa et al[37] 2012	Multicentric cohort	105	39	G9-12: 10 G13-14: 18 G15: 77	28/105 (14)	CCT	ELISA	/ 90	4	2.7	UCH-L1	100 (88-100)	21 (13-32)	0.73 (0.62-0.83)
Papa et al[38] 2014	Multicentric cohort	262	40	G9-12: 3 G13: 2 G14: 27 G15: 230	20/262 (/)	CCT	ELISA	67 /	4	3.1	GFAP	100 (63-100)	55 (43-66)	0.84 (0.73-0.95)
Welch et al[41] 2016	Multicentric cohort.	251	45.6	G9-12: 4 G13: 5 G14: 17 G15: 225	36/251 (/)	CCT	ELISA	15 41	6	/	UCH-L1 and GFAP	100 (90-100)	39 (33-46)	/
Bazarian et al[23] 2018	Multicentric cohort	1,920	48.8	G13: 0 G14: 92 G15: 1,828	113/1,920 (5)	CCT	ELISA	22 327	12	3.2	UCH-L1 and GFAP	97.3 (92.4-99.4)	36.7 (34.5-39.0)	/
Gardner et al[44] 2018	Multicentric cohort	169	41.9	G13: 2 G14: 28 G15: 139	56/169 (/)	CCT	ELISA	430 /	24	11	GFAP	80.4 (68.0-89.0)	79.6 (71.0-86.0)	/
Posti et al[36] 2019	Monocentric cohort	93	42.8	G13: / G14: / G15: /	37/93 (/)	CCT	ELISA digital (Simoa)	132 /	24	/	GFAP	97.3 (86.0-100)	32.1 (21.0-45.0)	0.72 (0.62-0.82)
Okonkwo et al[35] 2020	Multicentric cohort	1,137	/	G13-15: 1,137	358/1,137 (/)	CCT	Abbott i-STAT	37.8 /	24	/	GFAP	96.4 (94.4-98.0)	30.3 (27.1-34.0)	0.85 (0.83-0.87)
Iverson et al[39] 2022	Monocentric cohort	83	79	G13: 0 G14: 5 G15: 78	22/83 (2)	CCT	ELISA digital (Simoa)	323 42	12	3.1	UCH-L1 and GFAP	100 (85.0-100)	36.1 (25-49)	/
Papa et al[40] 2022	Monocentric cohort	349	40	G13: 2 G14: 33 G15: 314	23/349 (/)	CCT	ELISA	67 189	4	3.0	UCH-L1 and GFAP	100 (82-100)	25 (20-30)	0.83 (0.73-0.93)
Li et al[42] 2023	Monocentric cohort	441	50.8	G<13: 37 G13-14: 93 G15: 333	122/441 (/)	CCT	ELISA	22 327	6	1.0	UCH-L1 and GFAP	100 (97.0-100)	11 (8.0-15.0)	/
Oris et al[43] 2023	Monocentric cohort	239	59.1	G13: 0 G14: 10 G15: 139	12/239 (/)	CCT	Abbott i-STAT	30 360	12	1.8	UCH-L1 and GFAP	100 (73.5-100)	31.7 (25.7-38.2)	/
Lagares et al[32] 2024	Multicentric cohort	1,438	/	G13: 9 G14: 67 G15: 1,362	179/1,438 (/)	CCT	ELFA	22 327	12	4.5	UCH-L1 and GFAP	98.3 (95.0-99.7)	24.9 (22.6-27.4)	/
Lapić et al[33] 2024	Monocentric cohort	62	/	G13: 0 G14-15: 62	7/62 (/)	CCT	CMIA	35 400	12	2.5	UCH-L1 and GFAP	100 (59-100)	30.9 (19.1-44.8)	0.66 (0.52-0.77)

Chayoua et al[46] 2024	Multicentric cohort	253	/	G13: 7 G14: 94 G15: 152	59/253 (/)	CCT	Abbott i-STAT	30 360	24	/	UCH-L1 and GFAP	97 (89-99)	19 (14-25)	0.82 (Unknown)
Legramante et al[47] 2024	Monocentric cohort	130	54	G13: 0 G14: / G15: /	7/130 (/)	CCT	CMIA	35 400	12	/	UCH-L1 and GFAP	100 (64.5-100)	27.6 (20.0-36.4)	/
Ladang et al[48] 2024	Multicentric cohort	362	/	G13: / G14: / G15: /	113/362 (/)	CCT	CMIA	35 400	12	/	UCH-L1 and GFAP	99.1 (95.0-100)	40.6 (35-47)	/

AUC, area under the curve; *CCT*, cranial computer tomography; *CMIA*, chemiluminescent microparticle immunoassay; *ELISA*, enzyme-linked immunosorbent assay; *GFAP*, glial fibrillary acidic protein; *Max*, maximum; *Se*, sensitivity; *Spe*, specificity; UCH-L1, ubiquitin C-terminal hydrolase-L1.
A "/" means the data were not available.

suitable for routine clinical use. Standardization of assays would enhance the comparability and reliability of future studies. Although high heterogeneity was observed, it is important to interpret these results with caution, as meta-analyses of proportions often produce high I^2 values without necessarily reflecting meaningful variability between studies.

DISCUSSION

This meta-analysis evaluates the diagnostic accuracy of GFAP and UCH-L1 in ruling out intracranial injury in adults following mTBI, thereby aiming to reduce unnecessary CCT scans. The studies included in this analysis compared the diagnostic performance of GFAP and UCH-L1, both individually or in combination, against CCT scans. Our findings indicate that the combined use of GFAP and UCH-L1 yields an overall pooled Se of 100% and an NPV of 100%, although with a Spe of 31%. For GFAP alone, the Se were 94% and the Spe 40%, whereas UCH-L1 alone had a Se of 83% and a Spe of 51%. The combination of GFAP and UCH-L1 achieves maximum Se and NPV, ensuring that no false-negative cases (negative test result with an intracranial lesion on the CCT scan). However, this combination results in decreased Spe. Therefore, there will be some patients with false-positive results (positive test result without intracranial lesion on CCT scan). Few studies have examined the outcomes of these patients with false-positive results, but those that have reported favorable clinical outcomes.[32] In current clinical practice, the priority is to avoid false negatives. Therefore, biomarkers with high sensitivity are essential. Consequently, the superior diagnostic performance of the combined biomarkers justifies their preferred use in routine practice. This approach has been approved by the Food Drug Administration in 2018 and by the French Society of Emergency Medicine since 2022.

The 100% sensitivity for the combination of GFAP and UCH-L1 is due to the different and complementary half-lives of the 2 proteins.[27,30] UCH-L1 allows for early detection (peak concentration immediately after mTBI) of patients with intracranial injury, whereas GFAP identifies patients after 8 hours, hence the recommendation of measurement within 12 hours of trauma.[55,56] One solution to significantly improve specificity would be to consider the test positive only when both GFAP and UCH-L1 levels exceed their respective cut-off values. Currently, the assay is available on the Alinity i, i-Stat Alinity (Abbott), and Vidas (Biomerieux) platforms, where it is considered positive if either biomarker exceeds its cut-off value.[57]

In evaluating the biomarker pair GFAP and UCH-L1, one study was excluded based on the funnel plot results.[42] This study reported a Spe of 11%, significantly lower than the other studies. After its removal, the overall Spe increased

Studies	Risk of Bias				Applicability Concerns		
	Patient Selection	Index Test	Reference Standard	Flow Timing	Patient Selection	Index Test	Reference Standard
Iverson 2022	✅	✅	✅	✅	❌	✅	✅
Papa 2022	✅	✅	✅	✅	✅	✅	✅
Bazarian 2018	✅	✅	✅	✅	✅	✅	✅
Papa 2014	✅	✅	✅	✅	❌	❌	✅
Welch 2016	✅	✅	✅	✅	✅	✅	✅
Gardner 2018	✅	✅	✅	✅	✅	❌	✅
Posti 2019	✅	✅	❓	✅	✅	❌	✅
Okonkwo 2020	✅	✅	✅	✅	✅	❌	✅
Papa 2012	✅	❓	✅	✅	✅	❌	✅
Li 2023	✅	✅	✅	✅	✅	✅	✅
Oris 2023	✅	✅	✅	❓	✅	✅	✅
Lagares 2024	✅	✅	✅	✅	✅	✅	✅
Lapić 2024	✅	❓	❓	✅	✅	✅	✅
Chayoua 2024	✅	✅	❓	✅	✅	✅	✅
Legramante 2024	✅	❓	❓	✅	✅	✅	✅
Ladang 2024	✅	❓	✅	✅	✅	✅	✅

Figure 2. Risk of bias summary, QUADAS 2011. ✅ = Low risk. ❓ = Unclear risk. ❌ = High risk.

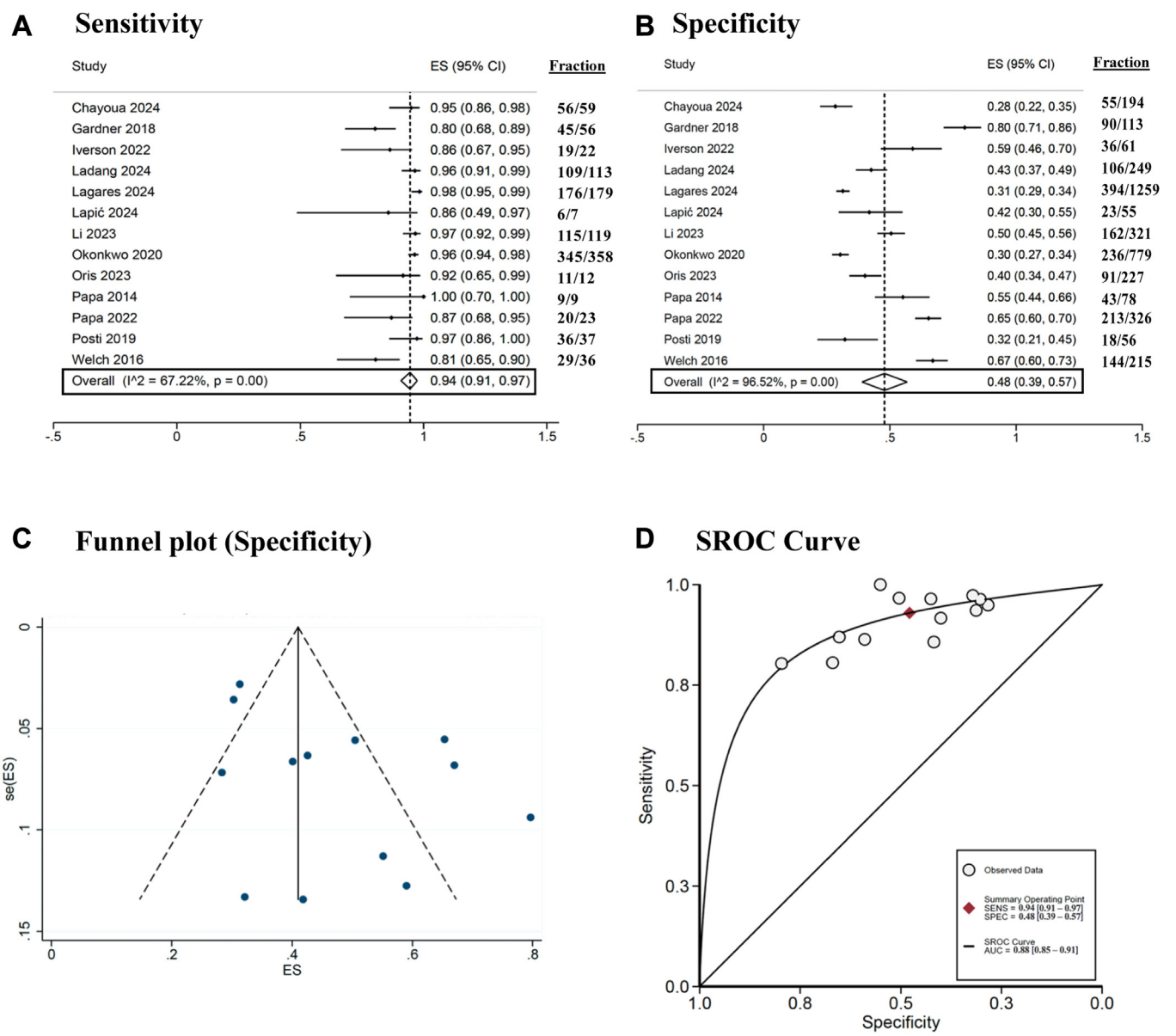

Figure 3. Diagnostic performance of GFAP for CCT scans findings. *A,* Forest plot showing the overall pooled sensitivity of GFAP for the exclusion of intracranial injury (n=13 studies). *B,* Forest plot showing the overall pooled specificity of GFAP. *C,* Funnel plot screening the distribution of the 13 studies for specificity. *D,* AUC of GFAP assessed by the SROC curve. *AUC,* area under the curve; *CI,* confidence interval; *GFAP,* glial fibrillary acidic protein, *SROC,* summary receiver operating characteristic.

from 29% to 31%. Consequently, a theoretical reduction of 31% in the number of unnecessary CCT scans is expected.

For GFAP alone, 3 studies were excluded based on funnel plot analysis.[40,41,44] These studies reported higher Spe (80%, 67%, and 65%), but lower sensitivities. These differences are likely due to the different cut-off values used for GFAP. Our results for GFAP are comparable with those of Amoo et al,[14] who reported a Se of 93% and a Spe of 36%.

For UCH-L1 alone, no previous meta-analysis has evaluated its performance in the context of mTBI. Indeed, few studies provide robust data. Based on the funnel plot analysis, 5 studies were excluded, due to heterogeneity in Spe values, which differed by up to 60%.[37,40,42,43,48] Therefore, the studies with the highest and the lowest Spe were excluded.

The performance of the combination of GFAP and UCH-L1 is slightly similar to that of S100B. A meta-analysis performed in 2010 reported a Se of 96% and a Spe of 30% for a S100B threshold of 0.10 µg/L.[18] Given the similar diagnostic performance, particularly in terms of specificity, the relevance of using this combination of 2 biomarkers lies in the fact that their concentrations are not affected by skin

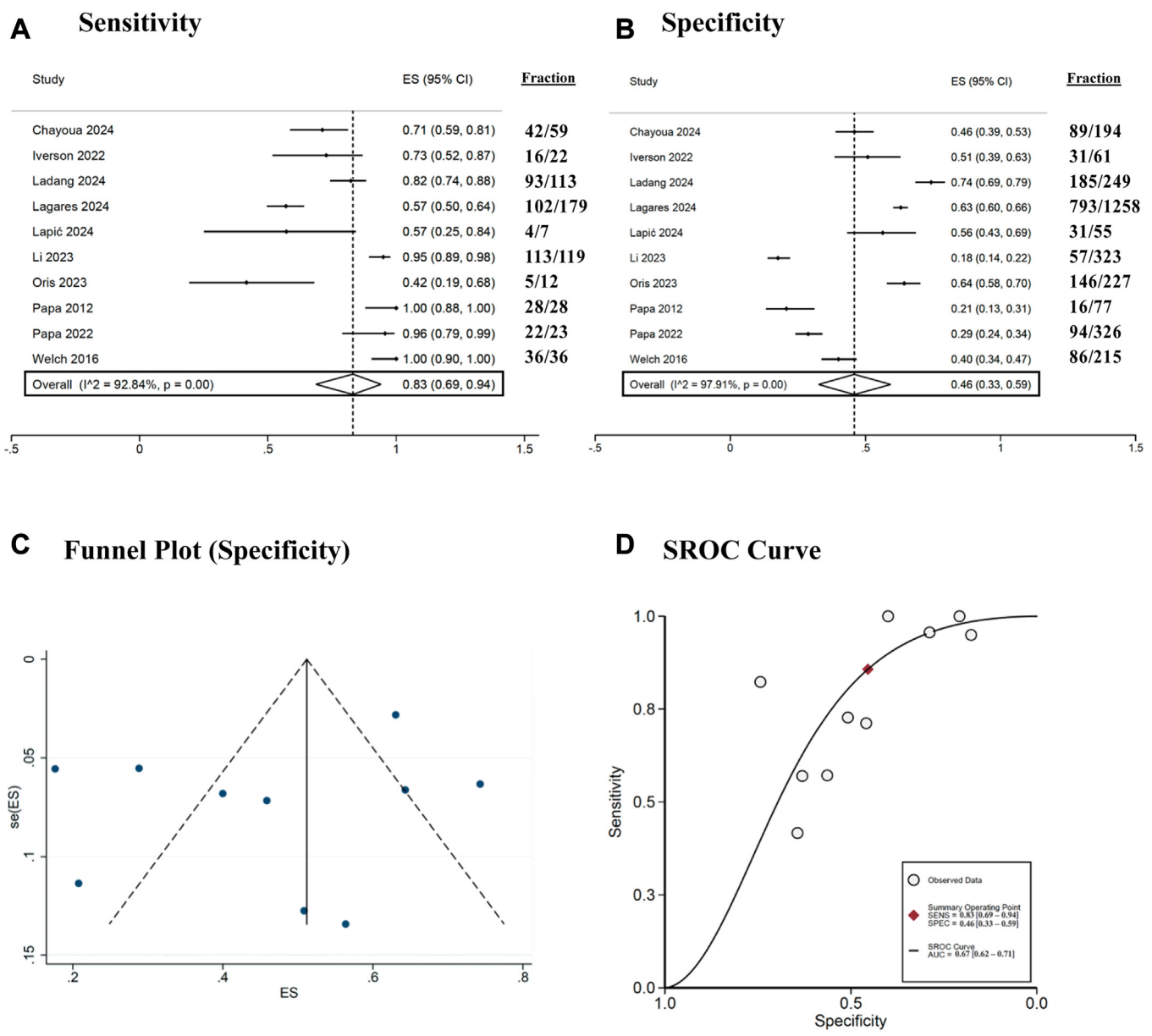

Figure 4. Diagnostic performance of UCH-L1 for CCT scans findings. A, Forest plot showing the overall pooled sensitivity of UCH-L1 for the exclusion of intracranial injury (n=10 studies). B, Forest plot showing the overall pooled specificity of UCH-L1. C. Funnel plot screening the distribution of the 10 studies for specificity. D. AUC of UCH-L1 assessed by the SROC curve. *AUC,* arear under the curve; *GFAP,* glial fibrillary acidic protein; *SROC,* summary receiver operating characteristic.

pigmentation, facilitating their use in routine practice.[20,22] Moreover, the sampling window can be extended up to 12 hours post-mTBI, whereas, for S100B, the window is 3 hours in France and 6 hours according to Scandinavian recommendations.[24,58] As with S100B, age-specific GFAP and UCH-L1 thresholds in adults, particularly in the elderly, would help improve specificity.[12,48,59-61]

Regarding methodological assessment, no studies included in the meta-analysis were found to have a high risk of bias according to the QUADAS-2 tool criteria.

This meta-analysis in adults should be complemented by a multicenter randomized interventional trial to assess the number of CCT scans avoided by the use of GFAP and UCH-L1 in current practice.

In summary, the combined use of GFAP and UCH-L1 demonstrates a sensitivity of 100% in ruling out intracranial injury in adults following mTBI and has the potential to reduce the number of unnecessary CCT scans by 31%, making it a superior option compared with GFAP alone, and UCH-L1 alone. Although specific clinical guidelines could not be established from this meta-analysis,

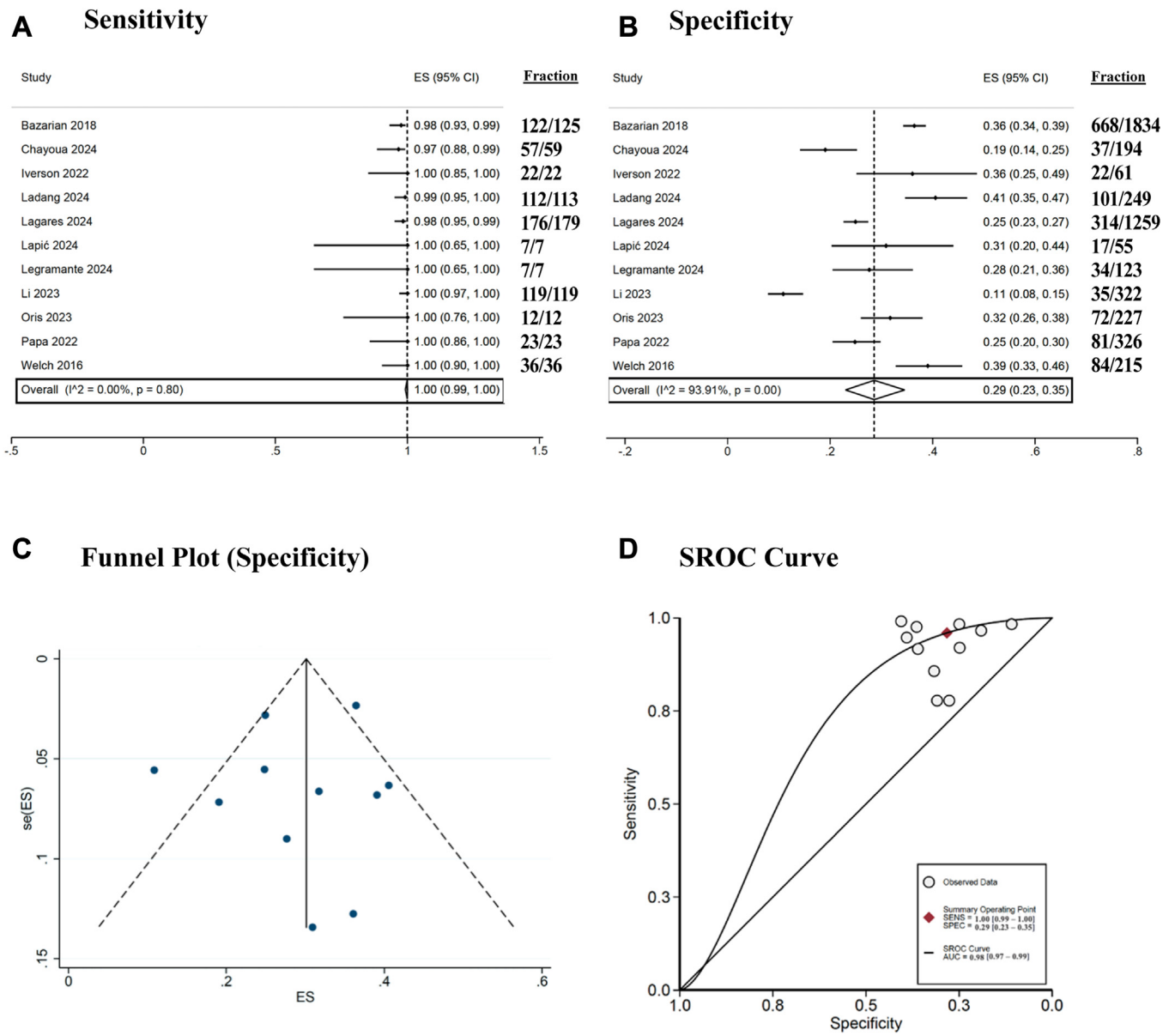

Figure 5. Diagnostic performance of GFAP and UCH-L1 for CCT scans findings. A. Forest plot showing the overall pooled sensitivity of GFAP and UCH-L1 in combination for the exclusion of intracranial injury (n = 11 studies). B. Forest plot showing the overall pooled specificity of GFAP and UCH-L1 in combination. C. Funnel plot screening the distribution of the 11 studies for specificity. D. AUC of GFAP and UCH-L1 in combination assessed by the SROC curve. *AUC,* arear under the curve; *CI,* confidence interval; *GFAP,* glial fibrillary acidic protein; *SROC,* summary receiver operating characteristic.

the findings support further evaluation and potential integration of GFAP and UCH-L1 assays into routine diagnostic protocols. Nevertheless, we still recommend following the guidelines of scientific societies and collecting samples from the patient within 12 hours of mTBI using a commercially available kit for routine use.

The authors would like to thank Nathalie Pinol, MA, from the University Library of the Faculty of Medicine in Clermont-Ferrand, for her help in constructing the bibliographic search equations.

Supervising editor: Clifton Callaway, MD, PhD. Specific detailed information about possible conflicts of interest for individual editors is available at https://www.annemergmed.com/editors.

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Author contributions: DB and VS designed the study, AP searched databases, and AP, SK, and CO collated all data and assessed studies for bias. AP, DB, and BP analyzed all data. All authors interpreted the results and contributed to the manuscript. DB takes responsibility for the entire paper.

Data sharing statement: The authors confirm that this systematic review adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for reporting. All data pertinent to the study are included in the article or provided as supplementary information. Raw data used in this study are available from the corresponding author on reasonable request. Additional materials related to the study can also be obtained by directly contacting the authors.

All authors attest to meeting the four ICMJE.org authorship criteria: (1) Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND (2) Drafting the work or revising it critically for important intellectual content; AND (3) Final approval of the version to be published; AND (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding and support: By *Annals'* policy, all authors are required to disclose any and all commercial, financial, and other relationships in any way related to the subject of this article as per ICMJE conflict of interest guidelines (see www.icmje.org). The authors have stated that no such relationships exist. The authors report this article did not receive any outside funding or support.

Publication dates: Received for publication September 18, 2024. Revisions received February 4, 2025, and March 13, 2025. Accepted for publication March 18, 2025.

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