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RESEARCH ARTICLE

TBICheck: A Rapid Test to Rule-Out CT scans in Mild Traumatic Brain Injury Patients

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ABSTRACT

Introduction: The TBICheck™ Rapid test is an immunochromatographic rapid test capable of assisting in the triage of patients with mild Traumatic Brain Injury suspected of brain lesions. It quantitatively determines heart-type Fatty Acid Binding Protein (H-FABP) levels in whole blood, serum, or plasma. The aim of the present study was to evaluate its technical performance and test it in two different cohorts of mTBI patients as a potential diagnostic tool for detecting brain lesions in patients with mTBI.

Material and methods: Description of the assay: Linearity and low limit of quantification of TBICheck™ lateral flow assay were determined using serial dilution of standardized samples. Results were read using the TBICheck™ Reader, a mobile photometric immunoassay analyzer based on reflectance measurements to capture the optical density. Obtained results were compared to classical ELISA assays, Meso Scale Diagnostics. Patient cohorts: Two different cohorts of adult mTBI patients were included: a retrospective one including 82 patients and a prospective one including 65 patients. Values of H-FABP area under the curve, specificity, sensitivity were calculated.

Results: The H-FABP dose response fitted a linear regression within the range of 0.5-25 ng/mL. LLOQ in blood was 0.5 ng/mL. High Spearman correlation was found (ρ=0.933, $p<0.001$) when MSD ELISA and TBICheck™ concentrations were compared.

In the retrospective cohort, when the clinical sensitivity was set at 100%, a specificity value of 32.9% was obtained. In the prospective cohort, the SP value raised to 66.1% with 100% SE, meaning that 6 out of 10 patients might be discharged on the basis of their serum H-FABP concentration at hospital admission.

Conclusions: The quantification of H-FABP by using the TBICheck™ Rapid test on adult mTBI patients may allow to rule out the need of a CT-scan reducing the radiation exposure and avoiding the long waiting times in emergency units. It may lead to savings in hospital resources and assists medical doctors to provide the most appropriate treatment to the patients.

1. Introduction

Traumatic Brain Injury (TBI) is a major global public health concern, and is among the primary causes of morbidity and mortality worldwide [1] . According to population-based studies, every year around 50-60 million people experience new cases of TBI, with at least 3.5 million cases reported in the US and 2.5 million in Europe [2,3]. Around 60-95% of the cases are mild TBI, which is defined as a history of loss of consciousness, amnesia, or disorientation, with Glasgow Coma Scale (GCS) from 13 to 15 [4].

Computed tomography (CT) scans are widely used for the diagnosis of mild traumatic brain injury due to their ability to rapidly and accurately detect intracerebral haemorrhages [5]. However, despite being utilized in about 80% of those patients, over 90% of CT scans do not reveal any clinically significant brain injury [6,7]. It is essential for healthcare providers to carefully consider the risks and benefits of CT scans and limit their usage to cases where they are truly necessary, to minimize the associated drawbacks [8]. The high volume of usage has contributed to increased healthcare costs, placing a strain on healthcare budgets. Secondly, there are mounting concerns about the potential risks of radiation exposure from unnecessary CT scans, which can lead to adverse health effects. Additionally, the excessive use of CT scanning exacerbates the existing problems of emergency department overcrowding, where patients may have to wait for hours before receiving necessary medical attention [9,10]. This can have serious consequences, especially for critically ill patients who require prompt treatment [11].

To address the limitations of CT scans for TBI diagnosis, point-of-care testing (POCT) has emerged as a promising alternative approach that utilizes biomarkers to diagnose TBI [12-14]. Among the most studied biomarkers are glial fibrillary acidic protein (GFAP) and S100 calcium binding protein B (S100B), which have been shown to be promising indicators of brain damage [15-19]. Another promising biomarker that has gained attention in recent years is heart type Fatty Acid Binding Protein (H-FABP). As an intracellular vascular and brain fatty-acid transporter, H-FABP has been postulated as a reliable indicator of brain injury, with previous studies showing elevated levels in patients with mTBI and correlations with the presence and severity of brain damage [20-24]

In this context, we have developed a lateral flow POCT device that measures H-FABP levels in patients with suspected mTBI postulating that it could offer several advantages over traditional laboratory-based tests, such as ease-of-use, testing by non-medical personnel, and rapid results, which makes it particularly useful in situations where quick and accurate assessments are needed (emergency rooms, sports fields, or in military settings) [25,26].

The objective of this study is to evaluate the efficacy of our TBICheck™ device in triaging patients with mTBI, and to compare its performance with traditional methods of TBI diagnosis. For this purpose, we used two different cohorts of patients, a retrospective one that will be used as a discovery cohort and a prospective one (BIOTRABIS) that will be used as a validation cohort.

3 Methods

3.1 POINT OF CARE TEST DEVELOPMENT

The TBICheck™ Rapid Test is an immunochromatographic test for the quantitative determination of heart-type Fatty Acid Binding Protein (H-FABP) in whole blood, serum or plasma (ABCDx, Switzerland). After the addition of 60 µL of sample into the well of the test, H-FABP binds to a colloidal gold-labeled antibody on the conjugate release pad. The resulting complex flows over a nitrocellulose membrane where a specific capture reagent is precoated and a red line appears at the test zone. Unreacted colloidal gold-labeled antibodies in the sample are captured at the control zone. The TBICheck™ Rapid Test must be read in 15 minutes after the addition of the sample and in combination with the TBICheck™ Reader (ABCDx, Switzerland): a mobile photometric immunoassay analyzer for in vitro diagnostic (IVD) colorimetric tests based on reflectance measurements to capture the optical density.

The concentration of H-FABP in the sample is proportional to the signal intensity in the test zone and can be measured by the TBICheck™ reader with a pre-set calibration curve. The information for the calibration curve is stored in the specific TBICheck™ RFID card supplied with the TBICheck™ Rapid Test.

Linearity test and limit of quantification determination

Linearity: Hytest (Hytest LTD, Turku, Finland) H-FABP recombinant was reconstituted in 2 mL of distilled water, obtaining a stock solution of 0.25 mg/mL. 3 µL of stock solution were mixed with 27 µL of free serum in order to dilute 10 times the stock solution. 3 µL of diluted stock solution (0.025 mg/mL) were again diluted 10 times in order to obtain a solution of 2500 ng/mL.

From this 2500 ng/mL solution, we started with the preparation of the different standard (STD) samples. For this purpose, we mixed 20 µL of 2500

ng/mL with 480 µL of free serum, obtaining the STD of 100 ng/mL. From this 100 ng/mL STD, serial dilutions were performed to obtain the STD of 25, 10, 5, 2.5 and 1.25 ng/mL.

Low limit of quantification (LLOQ): The analytical sensitivity was calculated from the mean plus two standard deviations of twenty replicated analyses of H-FABP free serum (Hytest).

3.2 STUDY POPULATION

3.2.1 Retrospective cohort: inclusion and exclusion criteria

The present study included a total of 82 patients recruited in three different European cohorts: Geneva (Switzerland), Barcelona (Spain) and Seville (Spain). All included patients or their legal representatives gave a written informed consent prior to inclusion. To participate, patients needed to fulfil several inclusion criteria: diagnosis of mTBI with a GCS score of 15; presence of at least one clinical symptom (loss of consciousness, amnesia, vomiting or nausea, headache or equilibrium disorder); CT scan performed within 24 h of the trauma (where the presence of epidural haemorrhage, subdural haemorrhage, subarachnoid haemorrhage, intracerebral haemorrhage, contusion with haemorrhage, cerebral oedema or skull fracture was classified as CT-positive); blood sample collected at admission; and age above 14 years old. Exclusion criteria were: pregnancy; GCS score below 15 at admission to hospital; absence of clinical symptoms; no head CT scan; and no signed informed consent form.

Samples that could not complete with the whole verification process due to equipment or human factors were excluded from the study. Similarly, samples with incomplete detection data or those with abnormal value when doing the error analysis were not included in the study.

3.2.2 BIOTRABIS prospective cohort: inclusion and exclusion criteria

The present study included a total of 65 patients recruited in different Spanish centers: Barcelona's Hospital Universitari Vall d'Hebron, Seville's Virgen del Rocío University Hospital, Madrid´s La Paz University Hospital, Barcelona´s German Trias y Pujol University Hospital, Barcelona´s Clinic University Hospital, Alcazar de San Juan´s General Hospital la Mancha Centro. All included patients or their legal representatives gave a written informed consent prior to inclusion. To participate, patients needed to fulfil BIOTRABIS inclusion criteria : adults more than 18 years old having a traumatic brain injury episode in less than 24 hours, GCS 14-15 and showing at least one of the next symptoms: loss of consciousness in the first 20 minutes after the episode, post traumatic amnesia in the first 30 minutes after the episode, persistent headache, dizziness and nausea, vertigo, confusion and disorientation.

Exclusion criteria were rejection of the participation in the study, age lower than 18 years old, previous traumatic brain injury in less than 1 month before the episode, epilepsy, schizophrenic and patients showing alcohol or drugs intoxication.

3.2.3 Ethics committee

Geneva's Human Research Ethics Committee (CER: 12–194 / NAC 12–074); Barcelona's Hospital Universitari Vall d'Hebron Ethics Committee (PR_AG_195–2012); and Seville's Virgen del Rocío University Hospital Institutional Review Board (2012PI/120), Madrid´s La Paz University Hospital (PI-4003), Barcelona´s German Trias y Pujol University Hospital (PI-20-064), Barcelona´s Clinic University Hospital (HCB-2020-0117) and Alcazar de San Juan´s General Hospital la Mancha Centro (134-C) ethics committees approved the study.

The trial registry for the prospective study: https://classic.clinicaltrials.gov/ct2/show/NCT04641767

3.3 SAMPLE COLLECTION, STORAGE AND TRANSPORTATION

For the retrospective study, upon hospital arrival, each patient had a serum (Seville and Barcelona) or plasma (Geneva) sample withdrawn, centrifuged, aliquoted and stored at -80°C until analysis. Frozen samples were completely thawed, mixed well and allowed to reach room temperature before testing. HFABP values were retrospectively measured using two different methods: ELISA kit and TBICheck POCT.
In the prospective cohort HFABP measurement was performed in whole blood samples using the TBICheck POCT.

3.4 SAMPLE MEASUREMENT

ELISA

Different ELISA kits were used to measure H-FABP concentration. In Geneva, HK402 kit from Hycult (Hycult Biotech, Uden, The Netherlands) with a limit of quantification (LOQ) ranging between 102–25000 pg/mL was selected. However, for patients recruited in Sevilla and Barcelona H-FABP was measured using a K151HTD kit from Meso Scale (Meso Scale Diagnostics, Rockville, MD, USA) LOQ 137–100000 pg/mL.

ELISA H-FABP measurements obtained from the retrospective study using three different cohorts,

were merged as a single cohort. Due to the high heterogeneity in the sample type (serum and plasma) as well as in the assays used to measure H-FABP concentrations, the biomarker results were normalized using the median concentration of negative CT patients as corrector factor.

TBICheck rapid test:

In the retrospective study 60 µL of sample (plasma or serum) were added to the sample well. One drop of the dilution buffer was added afterwards. In the prospective study, 120 µL of whole blood were added to the sample well. The cassette was inserted into the TBICheck™ reader. It was activated and the RFID card was placed over it. The result was automatically measured 15 minutes after addition of the sample and displayed on the TBICheck™ Reader screen.

3.5 STATISTICAL ANALYSIS

Patients were dichotomised into CT-positive and CT-negative groups for statistical analyses. According to Kolmogorov-Smirnov test ($p < 0.001$) H-FABP values were non-parametrically distributed and so, non-parametric Mann-Whitney U test, Fisher's exact test and the chi-square test were applied to test the differences between the two CT groups. Continuous data correlation was evaluated using the Spearman test.

Statistical analyses were done using IBM SPSS software, version 20.0 (SPSS Inc., Chicago, IL, USA). The proteins' diagnostic performances were tested using receiver operating characteristics (ROC) curves with TIBCO Spotfire S+® version 8.2 software (TIBCO software Inc., Palo Alto, CA, USA). For each protein, the thresholds were selected at the best cut-off for a sensitivity of 100%.

Receiver operating characteristic (ROC) curves were calculated for H-FABP. To avoid a false negative result, this is, to not misdiagnose a patient presenting a brain lesion, sensitivity (SE) value was restricted to 100%. Afterwards, specificity (SP) value was evaluated. The pROC package for S+ (version 8.1., TIBCO Software Inc.) was used to calculate the values of areas under the partial area under the curve (AUC), specificity (SP), sensitivity (SE), and 95%confidence intervals (95% CI).

Results

3.6 DEMOGRAPHIC CHARACTERISTICS

The retrospective part of this study included 82 mTBI patients. A total of 12 patients were classified as CT+. Most of them were men and significantly older than those patients with CT-. The most common clinical symptoms were loss of consciousness, amnesia and headache. Demographics on individual cohorts were comparable with the global demographics table (Table 2).

Table 1. Test result of whole blood and plasma samples from healthy patients

Sample ID	Whole blood concentration (ng/mL)	Plasma concentration (ng/mL)	CV%
1	3.50	3.28	4.60
2	2.93	2.95	0.51
3	2.60	2.15	13.29
4	1.15	1.60	23.18
5	2.56	3.66	24.99
6	1.62	1.61	0.65
7	2.37	3.04	17.67
8	0.76	1.07	23.59
9	1.32	1.48	8.25
10	2.46	3.35	21.64
11	3.59	2.77	18.39
12	0.86	1.82	50.45
13	1.68	1.87	7.70
14	1.05	1.89	40.69
15	8.21	8.06	1.32
16	2.36	3.09	18.90
17	1.46	1.40	2.94
18	11.08	10.78	1.93
19	2.69	4.52	36.02
20	0.86	1.07	15.44

Table 2. Demographic characteristics of the retrospective cohort

	BARCELONA		SEVILLA		GENEVA		3 COHORTS		
	CT- (n=7)	CT+ (n=2)	CT- (n=31)	CT+ (n=4)	CT- (n=32)	CT+ (n=6)	CT- (n=70)	CT+ (n=12)	*p* value
Age, median (IQR)	31 (27-34)	59.5 (57-62)	34.5 (21.8-48.5)	72 (31.3-83.5)	41 (27-56.5)	74 (24.5-81)	34.5 (26.75-53.25)	71 (25-75)	0.046[a]
Gender (F), n (%)	5 (71.4%)	0 (0%)	10 (32.3%)	1 (25%)	10 (31.3%)	2 (33.3%)	25 (35.7%)	3 (25%)	ns[b]
Headache, n (%)	1 (14.3%)	1 (50%)	22 (70.9%)	3 (75%)	10 (31.3%)	1 (16.7%	33 (47.1%)	5 (41.7%)	ns[b]
Vomiting, n (%)	0 (0%)	0 (0%)	6 (18.8%)	1 (25%)	3 (9.4%)	1 (16.7%	9 (12.9%)	2 (16.7%)	ns[b]
Loss of Conciousness, n (%)	6 (85.7%)	1 (50%)	24 (77.4%)	4 (100%)	29 (90.6%)	5 (83.3%)	59 (84.3%)	10 (83.3%)	ns[b]
Amnesia, n (%)	-	-	19 (61.3%)	4 (100%)	22 (68.8%)	4 (66.6%)	47 (67.1%)	8 (66.7%)	ns[b]
Time from accident to blood (min), n (%)	245 (121-324	175 (155-195)	210 (131.3-273.8)	210 (73.75-297.5)	297 (171.3-416.3)	135 (82.5-221.3)	225 (135-320)	157.5 (97.5-223.8)	ns[a]
H-FABP result (ng/mL), median (IQR)	2.62 (1.99-5.58)	4.4 (3.5-5.3)	2.55 (1.27-4.1)	4.6 (2.1-12.4)	2.8 (1.8-3.6)	4.95 (2.2-7.5)	2.62 (1.62-3.8)	4.95 (2.9-6.3)	<0.001[a]

[a]: Mann-Whitney U test, [b]: Chi square test

65 patients were included in the prospective part of the present study. Even if no significant differences were found, median age of CT+ patients were slightly higher that the age of CT- patients (Table 3). Similarly, as in the previous patients' demographic characteristics the average time to have the CT from the accident time was 200 min for the CT+ patients and 250 min for the CT- patients. Most of the patients had a good recovery at 6 months and did not present sequels in most of the cases.

Table 3. Demographic characteristics of the BIOTRABIS prospective cohort

BIOTRABIS			
	Normal (N=59)	Pathologic (N=6)	p value
Age, median (IQR)	71 (51 - 80)	81.000 (71.3 - 85.5)	0.234[a]
Gender (F), n (%)	34 (57.6%)	3 (50.0%)	0.719[b]
Neurological pathology, n (%)	1 (4.3%)	1 (33.3%)	0.076[b]
Tobacco, n (%)	3 (5.2%)	1 (16.7%)	0.268[b]
Time from accident to blood c.(min), n (%)	195 (120 - 287.5)	730 (446.2 - 1043.7)	0.003[b]
H-FABP result(OD), median (IQR)	8.805 (4.88 - 16.49)	13.6 (11.7 - 16.7)	0.112[a]
GOSe, , n (%)			0.934[b]
Death	3 (8.3%)	0 (0.0%)	
Work to a lower performance level	1 (2.8%)	0 (0.0%)	
Moderate disability, works at his post with some adjustments	2 (5.6%)	0 (0.0%)	
Good recovery, with lower deficits	9 (25.0%)	1 (25.0%)	
Good recovery without sequel	21 (58.3%)	3 (75.0%)	
N-Miss	23	2	

3.7 TECHNICAL VERIFICATION OF THE TBICHECKTM RAPID TEST

In order to assess the efficacy of our device, we first evaluate whether the TBICheck™ Rapid Test, used in combination with the TBICheck™ Reader, had a good correlation and equivalence when comparing with Meso Scale Human FABP3 reference kit.

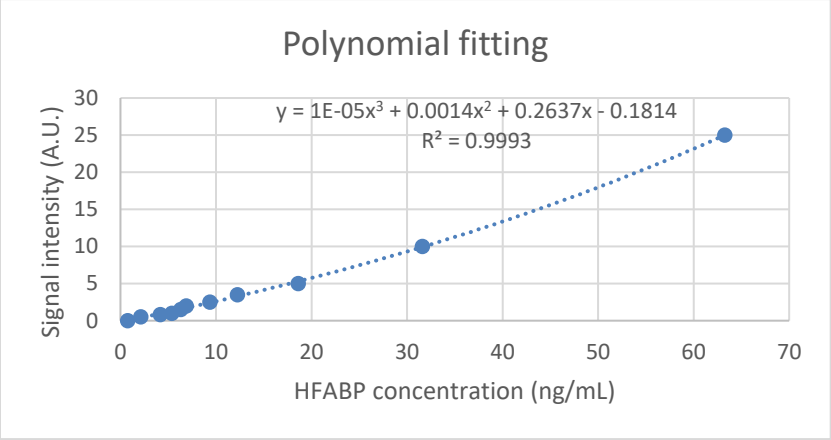
3.7.1 Analytical sensitivity

The limit of detection was calculated from the mean plus two standard deviations of twenty replicated analyses of H-FABP free serum (Hytest) and was found to be less than 0.5 ng/mL.

3.7.2 Linearity

Linearity study was performed using samples of already known H-FABP concentration and ranging from 0.5-25 ng/mL. Optical densities obtained with the cube reader and expressed as a function of H-FABP concentration were set by following polynomial formulation. The measuring range was 0.5-25 ng/mL (Figure 1).

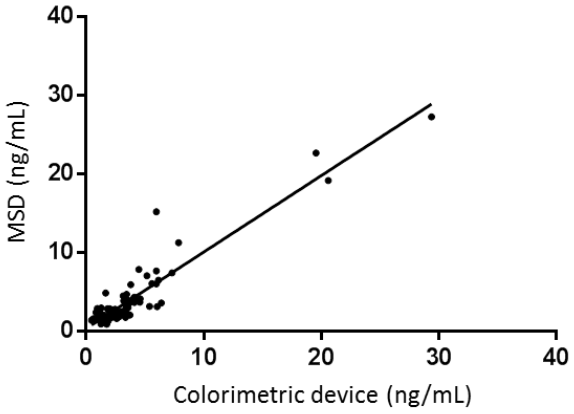
Figure 1.



3.7.3 Comparison between reference MSD H-FABP 3 kit and TBICheck™ Rapid Test

H-FABP concentration was measured in plasma samples of 82 mTBI subjects. Pearson correlation or Pearson's r was used to evaluate the correlation between concentrations obtained using Meso Scale ELISA kit and those obtained using the TBICheck™ Rapid Test. With an alpha of 0.05 the Pearson correlation coefficient was 0.933 ($p<0.0001$). The results showed a positive and significant correlation between the two methods (Figure 2).

Figure 2. Comparison of the concentrations measured with the TBICheck™ Rapid Test and with the ELISA kit from Meso Scale.



3.7.4 Whole blood and plasma specimens measured by TBICheck™ Rapid Test

The aim of TBICheck™ Rapid Test is to eliminate the need for separation of plasma and to be able to use it at the patient's side in a healthcare setting, therefore analyses should be performed in blood samples.

A total of 20 freshly collected heparinized whole blood specimens were collected and their corresponding plasma samples from healthy individuals from the Transfusion Center of the Geneva University Hospital (HUG) were prepared. The specimens were included testing whole blood and plasma at the same time by the TBICheck™ Rapid Test.

Similar results were found between H-FABP concentrations measured in plasma and whole blood; 17 out of 20 samples presented CV values below 25%. Correlations between the two variables revealed a Spearman's Rho correlation of 0.848 ($p<0.0001$) (Supplementary Figure 1).

3.8 VALIDATION OF H-FABP TEST AS TBI BIOMARKER USING TBICHECK™

3.8.1 Retrospective cohort: comparison between the use of the rapid test in combination with the colorimetric device and the CT-scan

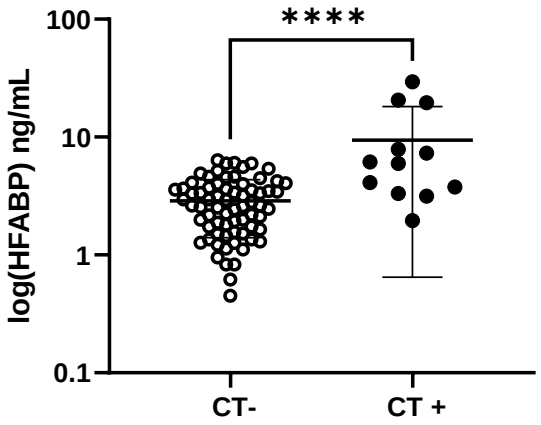
In order to evaluate if the combination of the TBICheck™ Rapid Test and the TBICheck™ reader could be used to detect the presence of a brain lesion in a retrospective cohort of mTBI patients, the H-FABP concentration was measured in 82 mTBI patients who got a CT-scan. The results were classified between patients in which the CT-scan has evidenced the presence of a brain lesion (CT+) and patients for whom the CT-scan have not reported a brain lesion (CT-). The statistical parameters of the two groups are reported in Figure 2 and proved that there is a statistically significant difference among the 2 groups. Setting the clinical sensitivity at 100%, meaning that no false negatives were present, we obtained a specificity of 32.9% for a cut-off value of 1.9 ng/mL.

3.8.2 Prospective cohort: use of the rapid test in combination with the colorimetric device as TBI biomarker in a prospective cohort

In this prospective cohort results were classified in the same way as in the retrospective cohort: CT+ and CT- according to the result of the CT scan.

Differences in H-FABP concentrations are shown in Figure 3. CT+ patients present significative higher values of H-FABP than CT- patients. When the SE value was fixed to 100%, a cut-off value of 2.5 ng/mL gave a SP value of 66.1%, meaning that 66.1% of the patients may be discharged on the basis or their serum H-FABP concentration, excluding the risk of a brain trauma.

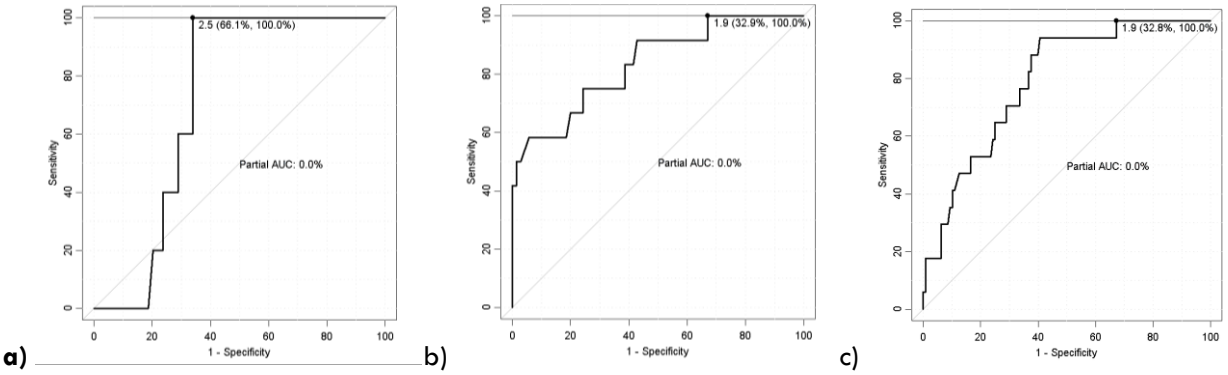
Figure 3. H-FABP concentrations measured with the TBICheck™ Rapid Test in mTBI patients: the long horizontal lines indicate the means. The top and bottom short horizontal lines illustrates the standard deviation. The differences in H-FABP concentrations between the groups are statistically significant ($P < 0.0001$).



3.8.3 Merged cohorts

Afterwards/secondly, both cohorts were normalized and merged to evaluate how the TBICheck™ Rapid Test could perform in a larger cohort of patients. A total of 147 patients were included in the analyses. 128 were classified as CT- and 19 were classified as CT+. Figure 4 shows that when the SE value was fixed at 100% a SP value of 32.8 was obtained.

Figure 4: ROC curves representing the capacity of HFABP to differentiate between CT+ and CT- patients. Predictive performance was investigated at 100% SE in the three different cohorts. a: prospective cohort, b: retrospective cohort, c: merged cohort



a) b) c)

4 Discussion

Early diagnosis of TBI has been linked to improved patient outcomes, leading to a pressing need for efficient and accurate diagnostic tools. The current gold standard in the field, the Glasgow Coma Scale (GCS) and imaging tests such as MRI and CT scans, have their limitations [27-29]. The subjective nature of the GCS and its inability to be administered to sedated or intubated patients are significant drawbacks. Meanwhile, imaging tests are often expensive and time-consuming, making them an obstacle to those seeking a simple and objective TBI diagnosis.

To address these challenges, our team has developed a lateral flow assay that quickly diagnoses mTBI through the detection of HFABP protein in blood.

Our research underscores the performance of TBICheck™ to serve as a pre-hospital diagnostic tool for ruling out patients without brain injury. HFABP measurement using the TBICheck Rapid Test in combination with the TBICheck™ Reader, was able to effectively distinguish mild traumatic brain injury patients with positive and negative CT scans. This was demonstrated in a retrospective cohort of 82 patients and subsequently validated in a prospective cohort of 65 patients.

The examined POCT presented an analytical sensitivity of less than 0.5 ng/mL and a measuring range of 0.5-25 ng/mL. The linearity of the test was established using known concentrations of HFABP and the obtained results were compared between the reference Meso Scale HFABP-3 kit and the TBICheck™ Rapid Test. A positive and significant correlation with a Pearson correlation coefficient of 0.933 ($p<0.0001$) was found, highlighting that the TBICheck™ had a good analytical performance and high agreement with the reference laboratory method.

There are currently six other HFABP POCTs that have been commercialized and evaluated for diagnosing acute myocardial infarction [30-33]. These tests use finger-prick whole blood samples and provide results within 2 to 15 minutes, but the majority of them are qualitative, indicating a positive test with a colored strip on an individual cassette. The CardioDetect test is the only one that provides quantitative results, but its fixed threshold of 4 ng/mL is not sensitive enough for accurately diagnosing TBI patients [34,35]. This study is the first to highlight an H-FABP POCT that can provide quantitative results for accurately diagnosing traumatic brain injury patients, setting it apart from other tests in the field.

A previous clinical study established a cut-off level of 2.6 ng/mL for a positive test in the early diagnosis of mTBI patients using H-FABP [23]. The present study found a threshold of 1.9 ng/mL to achieve a clinical sensitivity of 100% and a specificity of 32.85% in the retrospective cohort, while a specificity of 66.1% was achieved in the prospective cohort with a cut-off value of 2.5 ng/mL.

It is worth noting that all the values reported in both the previous and present studies fall within the measuring range of the examined POCT, which technically validate its utility. However, the difficulty in establishing consistent threshold values across different studies and cohorts can significantly impact the diagnostic test's characteristics and the overall outcomes of the study.

The difference in accuracy and so consequently in threshold values may be explained, among other factors, by the fact that the samples were collected over a wide time range of 24 hours instead of 6 hours as previously reported. Additionally, the mean age of the prospective and retrospective cohorts was different, which is relevant since HFABP levels can vary with age and this may have affected the results.

The present study had several limitations that should be addressed. First of all, we were limited by the small sample size of both cohorts, particularly the prospective cohort. Further multicentric studies are needed to validate these findings.

It is worth noting that our study focused on the utility of the TBICheck™ in mild traumatic brain injury cases. However, future research could explore its applicability in moderate and severe traumatic brain injury scenarios.

While H-FABP has been evaluated as a standalone biomarker, research has shown that combining biomarkers, such as H-FABP and GFAP, can significantly improve diagnostic accuracy by incorporating proteins from various sources and pathways. A study examining diagnostic combinations of GFAP, H-FABP, S100B, and IL-10 found that a panel consisting of H-FABP and GFAP was highly effective, achieving 100% sensitivity and 46% specificity [36]. This has generated interest in developing a dual point-of-care test (DUO POCT) that incorporates both biomarkers.

The development of the TBICheck™ represents a significant advancement in the field of traumatic brain injury diagnosis. Its ability to provide rapid

and quantitative results sets it apart from other existing POCTs that are predominantly qualitative. The convenience and speed of the TBICheck™ make it a promising candidate for use in pre-hospital settings, emergency departments, and resource-limited healthcare settings. Its potential to exclude patients without brain injury effectively could lead to streamlined triage processes, ensuring that individuals with suspected TBI receive timely and appropriate care.

5 Conclusions

The TBICheck™ can be used to quickly assess H-FABP levels in a range of acute care settings where mTBI occurs, including emergency departments (EDs), forward-deployed military facilities, and low- and middle-income countries with limited access to laboratory and imaging resources. This test has the potential to reduce the need for unnecessary computed tomography scans resulting in potential benefit por patients and healthcare systems alike.

6 References

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SUPPLEMENTARY MATERIAL

Supplementary figure 1: blood plasma correlations of 20 ctr patients