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ORIGINAL ARTICLE

The utility of heart-type free fatty-acid binding-protein (HFABP) levels for differentiating acute ischemic stroke from stroke mimics

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Abstract

Background Heart-type fatty acid-binding protein (HFABP) is found in the myocardium, brain, and some organs and is rapidly released from damaged cells into the circulation in case of ischemia.

Aims We aimed to determine the diagnostic utility of HFABP levels in patients suggesting acute ischemic stroke (AIS).

Methods This study was a prospective, single-center, observational diagnostic accuracy study with a nested cohort design. The estimated sample size was 126 patients, with a 1:1 case and control ratio. We included all consecutive patients with a lateralizing symptom (motor or sensory) or finding suggesting AIS (139 patients) who presented to ED within 24 h of their symptom onset and collected plasma at admission to the ED. After further evaluations, 111 patients (79.8%) were diagnosed with AIS and 28 with other neurological diseases (stroke-mimics).

Findings In our study, the median HFABP levels of the cases and controls were 2.6 µg/ml and 2.2 µg/ml, respectively, without any statistically significant difference ($p = 0.120$). The diagnostic accuracy of HFABP for AIS was also insignificant at 0.60 (95% CI 0.51–0.68; $p = 0.119$).

Discussion Plasma HFABP level is not a marker that can differentiate AIS from other neurological pathologies in patients presenting to the ED, with findings suggesting AIS.

Keywords The heart-type fatty acid-binding protein · Stroke · Diagnosis

Introduction

Stroke ranks as the second-leading cause of death globally after coronary artery disease. It is the foremost cause of disability and loss of workforce. Each year, approximately 17 million individuals worldwide experience a stroke [1]. According to the Heart Disease and Stroke Statistics 2021 update report, the mortality rate due to stroke among US adults aged 35–64 has risen from 14.7 per 100,000 in 2010 to 15.4 per 100,000 in 2016 [2]. In the USA alone, an estimated 800,000 people encounter a new or recurrent stroke annually; around 610,000 are first-time incidents, while 185,000 are recurrent attacks [2]. Notably, 10% of all strokes occur in individuals aged 18–50 [3].

Stroke is broadly categorized based on its pathophysiology into ischemic (IS, accounting for 87%) and hemorrhagic (ICH, accounting for 10%) types [2]. IS transpires due to an occlusion in a cerebral artery, leading to ischemia and a subsequent loss of brain function. Thrombolytics are the primary therapeutic approach for qualifying patients presenting

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with acute ischemic stroke (AIS), provided they can be administered within 4.5 h of symptom onset [4]. Mechanical thrombectomy is a secondary treatment option for AIS resulting from a significant artery occlusion in the anterior circulation, applicable up to 24 h post-symptom onset at specialized stroke centers [4]. Without intervention, or in cases of treatment failure, a progressive increase in mortality and morbidity is anticipated annually.

Prompt and precise differential diagnosis of AIS is pivotal for patients arriving at the emergency department (ED). Nonetheless, several conditions can emulate stroke symptoms, making thrombolytics potentially detrimental for such patients. Conditions mimicking stroke encompass demyelinating diseases (e.g., multiple sclerosis), tumors, abscesses, other infections, metabolic imbalances (ranging from hyper- to hypoglycemia, hyper- to hypocalcemia, and hyper- to hyponatremia), postictal motor deficits, trauma, and migraines. Conventional physical examinations and routine tests in the ED might not suffice in pinpointing the exact cause. Consequently, there's a growing emphasis on identifying neurochemical markers that can aid in AIS diagnosis, gauge the extent of brain damage, forecast outcomes, or ascertain treatment efficacy. To date, the employed biochemical markers comprise Neuron Specific Enolase (NSE), Protein S-100B, Glial Fiber Acidic Protein (GFAP), and heart-type fatty acid-binding protein (HFABP) [5].

Fatty acid-binding proteins (FABP) are cytoplasmic proteins with a molecular weight of 14–15 kilodaltons. They are prominently expressed in tissues with active lipid metabolism, such as the heart and liver, and cell types specialized for lipid storage, trafficking, and signaling, including adipocytes and macrophages [6]. Twelve distinct FABPs have been identified, each named based on the tissue where they are most abundantly expressed [7]. HFABP, also known as FABP3 or heart-type FABP, is predominantly expressed in cardiomyocytes and skeletal muscles. However, it is also present in various other tissues, including the brain, testis, kidneys, and adrenal glands, albeit in reduced concentrations [8]. Clinically, HFABP is a biomarker for cardiac injuries and heart failure, especially in diagnosing myocardial infarction (MI). Recent research has proposed that increased levels of HFABP might be associated with conditions such as mild brain injuries, Creutzfeldt-Jakob disease, Alzheimer's disease, and, notably, stroke [9–15]. Elevated HFABP levels have been observed in patients with transient ischemic attacks (TIA), mild stroke, and cardiovascular diseases [9, 10, 15].

Based on the recent literature, HFABP is a potential biomarker for predicting stroke. Therefore, we hypothesized that HFABP levels might be significantly elevated in patients with stroke who present to the ED with a lateralizing symptom compared to those with other neurological conditions that mimic stroke.

Methods

We conducted a prospective, single-center, observational diagnostic accuracy study with a nested-cohort design in the Emergency Department (ED) of a University Hospital with an annual load of 500,000 patients. We included all consecutive patients who presented to our ED within 24 h of symptom onset between May 2018 and December 2019. The local ethics committee approved the study and obtained the consent of all patients. This report has been compiled following the STARD guideline [16].

Patient population, inclusion, and exclusion criteria

Inclusion criteria:

All non-pregnant patients aged 18 years and older were eligible for the study if they met either of the following criteria upon presentation to the ED:

(a) Complaint of loss of strength or sensation in their extremities, which had begun within the past 24 h (symptoms could be ongoing or have subsided).
(b) Presence of lateralizing motor findings upon physical examination.

Exclusion criteria:

Patients were excluded from the study during the assessment and follow-up if they met the following criteria, indicating they did not have an ischemic stroke or a stroke mimic. However, their serum samples were retained until the study's conclusion for potential further analysis:

(a) Patients found to have a cerebral hemorrhage, intracranial mass, or evident traumatic pathologies on head CT or MRI.
(b) Patients diagnosed with Acute Coronary Syndrome (ACS) per AHA/ESC guidelines.
(c) Patients with a carboxyhemoglobin (COHb) level > 5 d/L, as determined through blood gas analysis, and whose symptoms subsided following treatment and monitoring.
(d) Patients with fingertip blood sugar levels < 50 gr/dL or those whose symptoms abated post-dextrose infusion, indicating pure hypoglycemia.
(e) Patients known or calculated to have a GFR < 15 ml/min/1.73 m^2 or definitively diagnosed with end-stage renal disease (ESRD), such as those on continuous dialysis or transplant recipients.

(f) Patients known or identified as undergoing active treatment for malignancy or those diagnosed with oncological/hematological malignancy during the ED evaluation.
(g) Patients with a medical history or presentation consistent with intoxication and whose symptoms fully resolved post-administration of an antidote such as flumazenil, naloxone, atropine, physostigmine, etc.
(h) Patients diagnosed with an acute orthopedic extremity pathology, spinal injury, or a lesion of the distal nerve motor root corresponding to the side exhibiting lateralizing symptoms.
(i) Patients who could not be diagnosed with stroke because no reference standard diagnostic tests were conducted.
(j) Patients diagnosed, following consultation with specialists and neurologists, with other metabolic, surgical, neurological, or psychiatric conditions other than stroke that could account for their symptoms.
(k) Patients who opted to withdraw their consent from the study.

Reference standard: diagnosis of stroke

In our study, a combination of test results was employed to validate stroke diagnosis. Notably, during the diagnostic phase of stroke, all personnel involved in the patient's management and those conducting radiological evaluations remained blinded to the HFABP values.

For patients who underwent imaging: Initial CT, CTA, and MRI reports were assessed by a designated assistant researcher from the Radiology Department. A specialist in Radiology then reviewed these images a second time to either confirm the stroke diagnosis or rule it out.

For patients who did not undergo imaging but were admitted to our institution (internal follow-up patients): The stroke diagnosis was based on several criteria:

- Consultations with the Neurology department.
- Review of hospital admission records.
- Analysis of ICU discharge summaries and death notification notes. Diagnostic criteria included consistent or escalating loss of strength suggestive of a stroke, ruling out alternative diagnoses through pertinent diagnostic tests to determine stroke as the most likely cause, or explicit documentation confirming the stroke diagnosis.
- In some cases, if imaging was conducted at any point during the patient's hospital stay and indicated a stroke, this confirmed the diagnosis.

For patients who did not undergo imaging and were transferred to an external facility (external follow-up patients): Stroke diagnosis was established like the approach for internal follow-up patients:

- Neurology consultations, examination of hospital admission records, ICU discharge summaries, and death notification notes were instrumental. The criteria for diagnosis matched those applied to the internal follow-up cohort: a continuous or intensifying loss of strength indicative of a stroke, eliminating other potential diagnoses, and identifying stroke as the primary cause or direct documentation of the stroke diagnosis.
- As with the internal follow-up patients, the stroke diagnosis could also be corroborated if any imaging performed at the external facility during the patient's stay indicated its occurrence.

Patient flow and management

All patients who presented to our ED exhibiting the aforementioned key indicators and symptoms of stroke were assessed, treated, and diagnosed following the prevailing AHA stroke guidelines. In cases where the diagnosis remained inconclusive or there was ambiguity about the presence of an ischemic stroke, a specialized committee was entrusted with making the final decision. This committee consisted of three faculty members, operated autonomously from the primary research team, and was kept uninformed about the H-FABP levels of the patients.

For those patients who were transferred without undergoing imaging or were admitted either within our facility or to an external one, the validation or refutation of stroke evidence rested on examining medical records combined with the committee's discernment. If a patient passed away before their hospital records could be thoroughly examined, before a discharge summary was provided, or before a comprehensive evaluation for stroke, they were excluded from the study. However, their data were considered in the study's sensitivity analysis.

Data and sample collection, sample storage, and ELISA analysis

The data for our study was derived from routine observational records amassed by the emergency medicine residents and specialists staffing the ED during both day and night shifts. During the standard blood collection at admission for diagnostic work-up, we obtained a 5 ml blood sample, which we then stored in a gel-free dry tube designed for plasma extraction. This sample was refrigerated in the ED for the duration of the respective shift. At the end of each shift, we executed a centrifugation process, separating the

plasma at 3000 rpm for 15 min. Subsequently, the plasma was transferred to an Eppendorf tube, labeled, and stored in a refrigerator set at −20 °C. These samples underwent a weekly transfer to a storage unit maintained at −80 °C, where they remained until the analysis. Notably, the nursing staff responsible for blood collection operated blind to any clinical or radiological data concerning the patients.

To determine the plasma concentration of HFABP, we utilized a direct, non-competitive sandwich-type enzyme-linked immunosorbent assay (ELISA). This was conducted using monoclonal antibodies sourced from the commercially accessible ELISA kits HK402, provided by HyCult Biotechnology, located in Uden, the Netherlands. We adhered strictly to the manufacturer's protocol during this procedure. These ELISA kits were procured through a grant bestowed by our university's scientific research and project center. It is crucial to note that the biochemistry team entrusted with the HFABP measurements had no access to, and thus remained blind to, the patient's clinical and radiological particulars.

Index test: plasma HFABP level

Based on a previous study by Pelsers et al., the 95th percentile upper reference limit (URL) of the HFABP was reported to be 6 µg/ml [9]. In a recent study by Vera et al., published in 2021, the 97.5th-percentile URL of the HFABP was found to be 7.4 ng/ml (95% CI: 6.3–9.2), while the 99th-percentile URL was 12.1 ng/ml (8.6–14.9) in a sample population of 355 people (with 170 males and 185 females) consisted of individuals aged 18–74 years, in the USA [17]. We, therefore, used both cut-off values and categorized levels above as high and below as low during secondary analysis.

Sample size calculation

Our objective was to identify the optimal threshold level and evaluate the diagnostic efficacy of HFABP by utilizing the Area Under the Curve (AUC) of the Receiver Operating Curve (ROC) analysis. Based on our calculations, to achieve an AUC of at least 0.65, which would be statistically distinct from the non-significant AUC of 0.5—with a type 1 error rate of 5% and type 2 error rate of 20% (equivalent to 80% power)—a minimum of 57 patients would be required in each group. Factoring in an additional 10% for potential attrition or unexpected exclusions, we determined the need to recruit at least 126 patients for our study.

Statistical analysis

We checked the fit of continuous variables with the normal distribution using the Shapiro–Wilks test, histogram, or Q–Q plots. Then, we reported continuous variables with their mean, standard deviation, 95% confidence interval, or median and interquartile range. We summarized categorical variables with their counts and frequencies. *T*-tests or ANalyses Of Variance (ANOVA) were used to compare the means, and Mann–Whitney *U* or Kruskal–Wallis tests were used to compare the medians of continuous variables between groups. We reported the mean difference and the confidence intervals (CIs) for those comparisons. Contingency tables and chi-square tests were used to compare the frequencies between the levels of categorical variables.

The reference variable (outcome, reference test) in this diagnostic accuracy study was the presence of stroke, a composite binary outcome variable. We used ROC curve analysis to find the AUC of HFABP level in diagnosing the presence of stroke. Then, we calculated Youden J Index to determine the optimum threshold value. We reported the sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios of the HFABP at this threshold, with 95% CIs. Previous studies reported those diagnostic accuracy metrics using the threshold value of 6 µg/ml. Therefore, we also calculated and reported those diagnostic utility metrics for this previously reported level.

The accepted type 1 error rate was 5% in this study. All analyses and sample size estimation were performed with MedCalc Statistical Software version 19.2.6 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org; 2020) and jamovi version 2.0 (The jamovi project, https://www.jamovi.org; 2021).

Results

We screened 201 patients for participation, of which 190 were eligible for study inclusion, but 51 were excluded. This resulted in a final study cohort of 139 patients. Of this group, 111 (79.8%) received a stroke diagnosis. The patient flow chart is depicted in Fig. 1.

Demographically, 66 (47.5%) were male. When examining medical histories, 21 (18.9%) had Atrial Fibrillation (AF), 44 (31.7%) were diagnosed with Diabetes Mellitus (DM), 28 (20.1%) had Coronary Artery Disease (CAD), and 66 (47.5%) had Hypertension (HT). The median NIHSS score across the cohort was 3 points (IQR 5, as shown in Table 1). Notably, most admission characteristics exhibited slight variance between groups, with the exception of age, history of AF, and the NIHSS score (refer to Table 1). A detailed breakdown of the etiological classification of stroke patients is presented in Table 2.

The median HFABP levels for patients diagnosed with stroke versus those without were 2.6 µg/ml and 2.2 µg/ml, respectively. This difference was not statistically significant ($p = 0.120$, as shown in Table 3). Our analysis determined that HFABP was not a reliable marker for identifying stroke, yielding an AUC of 0.60 (95% CI: 0.51–0.68;

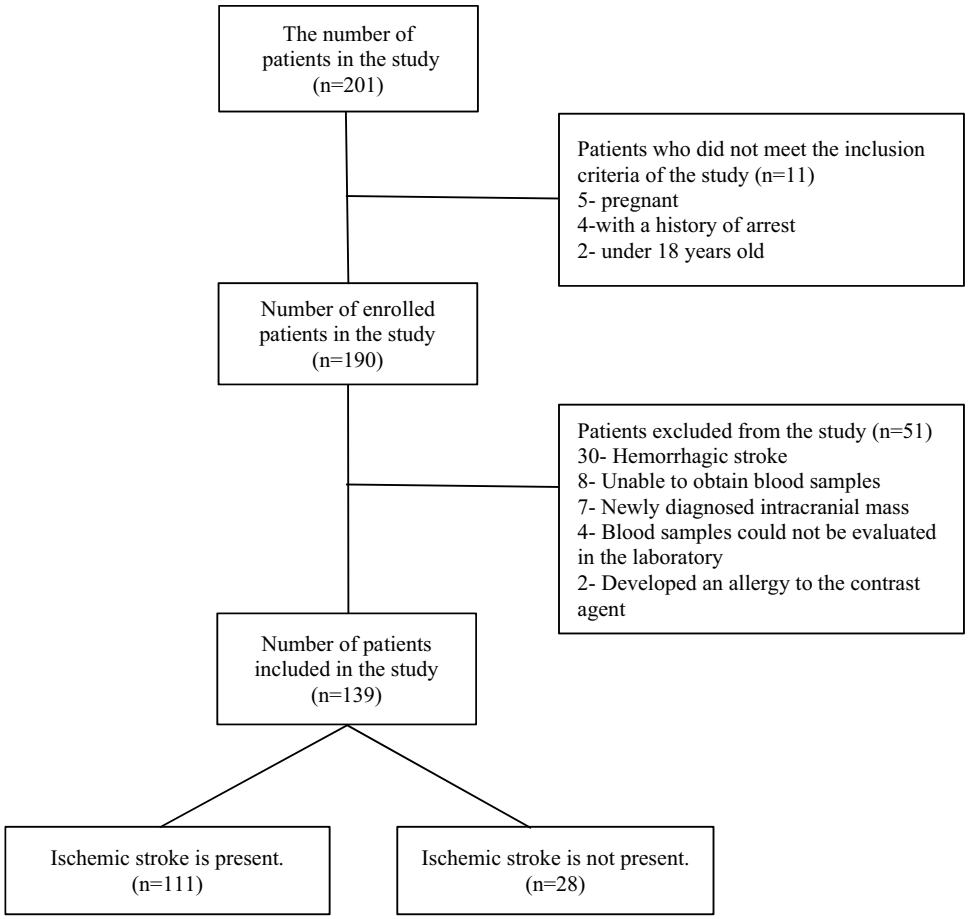

Fig. 1 The patient flow chart

Table 1 Comparison of the study population and groups in terms of demographic characteristics and values at the time of application

	All patients (n = 139)	Stroke (n = 111)	Stroke mimics (n = 28)	*p*
Age (years), median (IQR)	69.0 (25.5)	70.0 (22.0)	56.0 (30.5)	**0.001**
Sex, male, n (%)	66 (47.5)	55 (49.5)	11 (39.3)	0.339
AF, n (%)	21 (15.1)	21 (18.9)	0 (0.0)	**0.008**
DM, n (%)	44 (31.7)	36 (32.4)	8 (28.6)	0.822
HT, n (%)	66 (47.5)	55 (49.5)	11 (39.3)	0.399
CAD, n (%)	28 (20.1)	26 (23.4)	2 (7.1)	0.066
PICE, n (%)	33 (23.7)	30 (27.0)	3 (10.7)	0.084
NIHSS, median (IQR)	3 (5)	4 (6)	2 (2)	**< 0.001**
SBP (mmHg), median (IQR)	140.0 (33.5)	140.0 (32.5)	130.5 (33.0)	0.109
DBP (mmHg), median (IQR)	80.0 (19.0)	81.0 (18.5)	79.0 (22.5)	0.902
MAP (mmHg), median (IQR)	100.7 (20.7)	101.3 (20.0)	97.2 (17.5)	0.395
Symptom duration, n (%)				0.160
0–4 h	63 (45.3)	46 (41.4)	17 (60.7)	
> 4–12 h	48 (34.5)	42 (37.8)	6(21.4)	
> 12–24 h	28 (20.1)	23 (20.7)	5 (17.9)	

IQR interquartile range, *AF* atrial fibrillation, *DM* diabetes mellitus, *HT* hypertension, *CAD* coronary artery disease, *PICE* past ischemic cerebrovascular event

Medians were compared with the Mann–Whitney *U* test; frequencies were compared with Fisher's Exact test if needed. *p* values smaller than 5% were accepted as statistically significant and marked with bold font

Table 2 TOAST classification of stroke patients

TOAST classification	n (%)
Large artery atherosclerosis	40 (36.0)
Cardioembolic stroke	19 (17.1)
Small artery disease	47 (42.3)
Other determined etiology	4 (3.6)
Undetermined etiology	1 (0.9)

$p = 0.119$; see Fig. 2). Youden J index was highest at an HFABP level of 2.7 µg/ml. The sensitivity, specificity, PPV, NPV, + LR, and −LR were 48.7%, 75%, 88.5%, 26.9%, 1.946, and 0.685, respectively, at this threshold (Table 4). Additionally, we evaluated the clinical utility metrics using threshold values that had been proposed in earlier studies. However, these metrics presented lower clinical utility in comparison (Table 4).

Additionally, we explored the utility of HFABP based on the Upper Reference Limits (URLs) indicated in prior studies, specifically at levels of 6, 7.4, and 12.1 µg/ml. Using the 6 µg/ml threshold, a mere 17.1% of stroke patients versus 10.7% of those with stroke mimics tested positive for HFABP. This difference was not statistically significant (Table 3). The utility metrics at other mentioned thresholds similarly demonstrated limited value.

Table 3 Comparison of plasma H-FABP levels, which is the index test of our study, according to the groups

	All patients (n = 139)	Stroke (n = 111)	Stroke mimics (n = 28)	*p*
HFABP (µg/ml), median (IQR)	2.5 (2.8)	2.6 (3.3)	2.2 (1.3)	0.121
HFABP ≥ 2.7 µg/ml, n (%)	61 (43.9)	54 (48.6)	7 (25.0)	**0.032**
HFABP ≥ 6 µg/ml, n (%)	22 (15.8)	19 (17.1)	3 (10.7)	0.566
HFABP ≥ 7.4 µg/ml, n (%)	11 (7.9)	8 (7.2)	3 (10.7)	0.694
HFABP > = 12.1 µg/ml, n (%)	3 (2.2)	2 (1.8)	1 (3.6)	0.494

Medians were compared with the Mann–Whitney *U* test; frequencies were compared with Fisher's Exact test if needed. *p* values smaller than 5% were accepted as statistically significant and marked with bold font

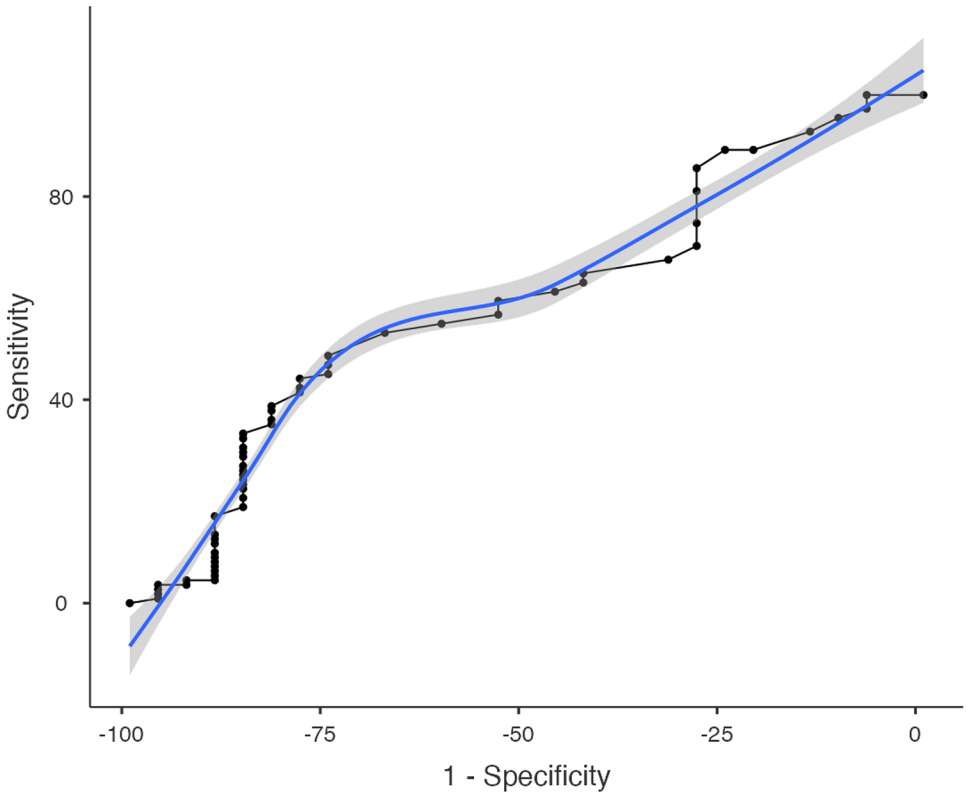

Fig. 2 ROC curve showing the accuracy of HFABP level for stroke

Gray cloud marks the standard error of the ROC curve.

Table 4 Clinical utility of plasma H-FABP levels

	Accuracy, %	Sensitivity, % (95% CIs)	Specificity, % (95% CIs)	+LR	−LR
HFABP ≥ 2.7 µg/ml	54.0 (45.3–62.4)	48.7 (39.0–58.3)	75.0 (55.1–89.3)	1.946 (0.996–3.801)	0.685 (0.517–0.906)
HFABP ≥ 6 µg/ml	31.7 (24.0–40.1)	17.1 (10.6 – 25.4)	89.3 (71.8–97.7)	1.598 (0.508–5.020)	0.928 (0.796–1.082)
HFABP ≥ 7.4 µg/ml	23.7 (16.9–31.7)	7.2 (3.2–13.7)	89.3 (71.8–97.7)	0.673 (0.191–2.373)	1.039 (0.905–1.194)
HFABP ≥ 12.1 µg/ml	20.9 (14.4–28.6)	1.8 (0.2–6.4)	96.4 (81.7–99.9)	0.505 (0.047–5.367)	1.018 (0.944–1.098)

Discussion

In our study, we ascertained that the discriminative capability of HFABP in distinguishing stroke from its mimics within the Emergency Department (ED) setting is not optimal. Though there was a statistically significant difference of 23.6% between stroke and stroke-mimic cases at the HFABP threshold level of 2.7 µg/ml, this level is still deemed within the normal limits according to prior research. This suggests that minor yet significant variations in HFABP levels might aid in stroke diagnosis, especially when combined with other biomarkers.

Lagerstedt et al. discerned HFABP's potential in differentiating CT-negative from CT-positive mild brain injury patients, especially when used with other biomarkers [14]. They notably identified H-FABP as the standout protein, achieving an impeccable sensitivity of 100% and a specificity of 32%. This specificity jumped to 46% with the introduction of GFAP to HFABP, suggesting its significant diagnostic potential.

Previous studies offer varying results on HFABP levels. For instance, Pelsers et al. deduced a threshold value of plasma HFABP as 6 mg/ml using CSF fluid samples from stroke patients and plasma from healthy volunteers [9]. Contrarily, Wunderlich et al. found HFABP levels surpassing the threshold suggested by Pelzer et al. [18]. Furthermore, Zimmerman-Ivol et al. reported an AUC of 0.531 for the diagnostic value of H-FABP in stroke, suggesting a sensitivity of 68% and a remarkable specificity of 100% [19]. Other studies, such as those by So-Young Park et al., revealed varying AUC, sensitivity, and specificity values, all of which inform our understanding of HFABP's diagnostic potential [20].

Our study's AUC stood at 0.60, noticeably lower than several preceding studies. A key distinction in our approach was the choice of our study population and control group. Unlike previous studies that used healthy individuals as controls, we leaned on real-life conditions by choosing individuals with stroke-like complaints. Our control group had various diagnoses, from migraine with aura to facial paralysis. In our control group (n = 28), 5 patients were diagnosed with migraine headaches with aura, 7 had epilepsy, 4 had non-convulsive status, and 12 had facial paralysis. Healthy individuals can introduce selection bias, which might exaggerate differences between cases and controls. Our methodology likely reflects a more accurate estimate of HFABP's diagnostic utility in routine clinical settings. The only study with a similar control group was So-Young Park et al.'s first study, which included patients admitted to the hospital with other neurological complaints and found similar utility metrics to our study [20]. We think that our findings represent a more accurate estimate of the utility of this marker in standard daily practice compared to previous literature.

The utility of HFABP extends beyond stroke. HFABP has been posited as a marker for conditions like Creutzfeldt-Jakob disease, Alzheimer's disease, and even traumatic brain injuries [10, 11]. Recent studies further highlight its potential as a predictor for cardiovascular events [12]. Lyang et al., in particular, showcased HFABP's impressive predictive capacity for mortality in acute ischemic stroke patients, with a sensitivity of 80% and specificity of 74.4% [13]. Dolmans et al. found a statistically significant difference in HFABP levels when comparing patients with (19.7 ng/ml) and without (17.4 ng/ml) TIA/minor stroke, suggesting its wider applicability in the realm of neurological and cardiovascular conditions ($p = 0.05$; MWU) [15].

Limitations

First, our study was designed with the anticipation of achieving an AUC of 65%, deeming it statistically significant if 57 patients were enrolled in both the stroke and control groups. However, the 1:1 ratio was not attainable due to a higher prevalence of stroke cases encountered during our study period. We only managed to secure half of the required minimum sample for the control group. While this could affect findings, especially if we had discovered higher AUCs, the performance of HFABP was significantly below our expectations. Hence, we confidently conclude that HFABP lacks clinical predictive value, given our study's power.

We measured the HFABP levels once upon admission, and we could only collect a bare definition of the exact time of onset of stroke symptoms. The variability in time intervals between symptom onset and the blood draw could potentially affect the levels and, consequently, our findings. As inflammatory and hemostatic markers can elevate due to non-cerebral conditions like systemic infections or metabolic

diseases, future studies might also benefit from serial measurements, not once at admission. We also did not collect extensive background information on other medical conditions that could potentially influence HFABP levels.

We defined the presence of stroke as the reference variable. There may be inherent limitations and inaccuracies depending on how the presence of a stroke is confirmed (e.g., clinical diagnosis, imaging, or other criteria). We also focused only on patients presenting to the ED with specific symptoms. This could introduce selection bias, as there might be a different cohort of stroke patients who present with atypical or no symptoms, potentially affecting the generalizability of the study findings. However, those atypical presentations are probably not candidates for HFABP evaluation, and this selection bias has an effect towards null.

The median NIHSS score of our participants was on the lower side, suggesting that our sample likely had smaller cerebral ischemic regions compared to those with elevated NIHSS scores. This lower score could have introduced spectrum bias. Our findings indicating a generally low median HFABP level might be attributed to this bias. Consequently, HFABP could still be predictive when evaluating patients with more extensive ischemic zones.

The control group consisted of patients with other neurological complaints (e.g., migraine headache with aura, epilepsy, nonconvulsive status, facial paralysis), rather than a truly healthy cohort. This can introduce challenges in distinguishing true markers for stroke versus other neurological conditions.

The study focuses on threshold values of HFABP as determined by previous studies, and even when looking at optimal threshold values through the Youden J index, it may not capture the full range of clinical presentations or nuances in HFABP levels across different patient cohorts. We primarily focused on HFABP, but in practice, a combination of biomarkers might yield better diagnostic accuracy. The exclusion of other potential markers may have limited our study's comprehensiveness.

Conclusion

The plasma HFABP level did not demonstrate significant clinical utility in distinguishing strokes from stroke mimics in the Emergency Department (ED). By addressing the aforementioned limitations, future studies may yield more conclusive results.

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Author contributions E.U.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Roles/Writing—original draft, Writing—review & editing; H.A.: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Software, Supervision, Validation, Roles/Writing—original draft, Writing—review & editing; E.S., S.K.: Resources, Supervision, Validation, Writing—review & editing; M.E.S.: Investigation, Project administration, Resources, Supervision; O.O.: Conceptualization, Methodology, Project administration, Supervision, Validation, Writing—review & editing; A.D.: Conceptualization, Methodology, Project administration, Supervision, Validation, Writing—review & editing. All authors were involved with the organization/supervision of the article; contributed to the analysis of data, interpretation of the data, drafting/writing of the manuscript; and reviewed/approved the final version to be published. EU takes responsibility for the paper as a whole.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors report no conflict of interest.

Ethical approval The Ethics Committee approved this study of the Marmara University School of Medicine in September 2016 with the Approval No. 09.2016.297.

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