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Biomarkers

Heart-Type Fatty Acid-Binding Protein Predicts Long-Term Mortality and Re-Infarction in Consecutive Patients With Suspected Acute Coronary Syndrome Who Are Troponin-Negative

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Objectives The purpose of this study was to establish the prognostic value of measuring heart fatty acid-binding protein (H-FABP) in patients with suspected acute coronary syndrome (ACS) (in particular, low- to intermediate-risk patients), in addition to troponin measured with the latest third-generation troponin assay.

Background We have previously shown that H-FABP is a useful prognostic marker in patients with proven ACS.

Methods Patients (n = 1,080) consecutively admitted to the hospital with suspected ACS were recruited over 46 weeks. Siemens Advia Ultra-TnI (Siemens Healthcare Diagnostics, Newbury, United Kingdom) and Randox Evidence H-FABP (Randox Laboratories, Ltd., Co., Antrim, United Kingdom) were analyzed on samples collected 12 to 24 h from symptom onset. After exclusion of patients with ST-segment elevation and new left bundle branch block, 955 patients were included in the analysis.

Results The primary outcome measure of death or readmission with myocardial infarction after a minimum follow-up period of 12 months (median 18 months) occurred in 96 of 955 patients (10.1%). The H-FABP concentration was an independent predictor of death or myocardial infarction, after multivariate adjustment. Patients with H-FABP concentrations >6.48 μg/l had significantly increased risk of adverse events (adjusted hazard ratio: 2.62, 95% confidence interval: 1.30 to 5.28, p = 0.007). Among troponin-negative patients (which constituted 79.2% of the cohort), the aforementioned cutoff of 6.48 μg/l identified patients at very high risk for adverse outcomes independent of patient age and serum creatinine.

Conclusions We have demonstrated that the prognostic value of elevated H-FABP is additive to troponin in low- and intermediate-risk patients with suspected ACS. Other studies suggest that our observations reflect the value of H-FABP as a marker of myocardial ischemia, even in the absence of frank necrosis. (J Am Coll Cardiol 2010; 55:2590–8) © 2010 by the American College of Cardiology Foundation

Heart-type fatty acid-binding protein (H-FABP) is a low-molecular-weight cytoplasmic protein that is involved in the intracellular uptake and buffering of free fatty acids in the myocardium (1). We have recently demonstrated that H-FABP predicts long-term mortality in a study of 1,448 patients with acute coronary syndrome (ACS) and that this prediction was independent of the GRACE (Global Registry of Acute Coronary Events) clinical risk factors, troponin and high-sensitivity C-reactive protein (2). In particular, H-FABP was able to identify those troponin-negative patients with unstable angina who were at high risk of subsequent death. This observation has been independently supported by O'Donoghue et al. (3) in their study of 2,287 ACS patients recruited in the Thrombolysis In Myocardial Infarction-16 trial. However, both these studies were performed on selected patients with independently confirmed ACS. Therefore, they offer predictive information on mortality for patients with ACS but cannot be used to provide diagnostic

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information, because they did not include consecutive series with chest pain; there were not significant numbers of either "troponin-negative" or low- to intermediate-risk patients (4). This group of apparently low-risk patients is likely to benefit the most from accurate risk stratification.

Over the past few years, troponin assays have improved, and newer assays can now achieve the analytical performance spelled out in the Joint European Society of Cardiology/American College of Cardiology consensus document on redefining myocardial infarction (MI) published in 2000—which recommended a coefficient of variation (CV) of <10% at the 99th percentile value of a reference healthy population (5). There is emerging evidence that these improvements increase the early diagnostic and prognostic value of the newer troponin assays (6–8). This raises the question of whether there is still additional value in measuring novel markers such as H-FABP in conjunction with the newer troponin assays (9). To address this, we designed and conducted the FAB (Heart-Type Fatty Acid Binding Protein in Suspected ACS) study, which recruited a consecutive series of patients who presented to hospital with suspected ACS. Our primary aim was the evaluation of the complementary value of H-FABP, particularly for those patients found to be negative for troponin measured with a third-generation troponin assay (Advia Centaur TnI-Ultra, Siemens Healthcare Diagnostics, Newbury, United Kingdom).

Methods

Study design and patient selection. The study was a prospective observational study of patients with suspected ACS presenting consecutively to a large teaching hospital in Leeds, United Kingdom. All individuals ages 18 years or above with chest pain of possible or definite cardiac etiology were deemed eligible for recruitment, irrespective of electrocardiographic (ECG) changes. Individuals presenting without chest pain but with other symptoms suggestive of ACS (dyspnea, diaphoresis, back pain, and so forth) were also included, if considered to be compatible with an atypical presentation of ACS by the assessing physician in the emergency department. We excluded patients: 1) who were unwilling or unable to provide informed consent; 2) from whom precisely timed additional blood samples could not be obtained; and 3) who were admitted with an identified noncardiac cause of chest pain on presentation (such as pneumonia or pulmonary embolism). When in doubt, the final judgment regarding appropriateness of inclusion into the study was made by the research team (2 specialist cardiology nurses and 1 cardiology research physician) at the time of obtaining written consent.

Between May 15, 2006, and April 1, 2007, we enrolled 1,080 consenting patients with suspected ACS, who all provided informed consent for participation and long-term follow-up. Consent was obtained at the earliest opportunity by the research team together with support from doctors and nursing staff working in the emergency department, as specified in the study protocol. All study subjects were managed in accordance with existing patient care pathways in the hospital, which require all patients with suspected ACS to be admitted either under the care of a cardiologist (if the patient was deemed intermediate or high clinical risk) or in the Clinical Decisions Unit run by the emergency department (if the patient was deemed low-risk) for a period of observation. All individuals enrolled into the study had additional serum and plasma samples taken for the purpose of research at the same time as the clinical blood sample taken for the "routine" troponin testing—at least 12 h from symptom onset. Only the "routine" troponin test sample was analyzed in real time, and the result was made available to the attending physician for further clinical management. Consequently, participation in the study did not influence the routine care and immediate/long-term management. Patients recruited into the study also had an earlier serum sample collected at the time of first contact with the emergency department whenever logistically possible. All additional research samples collected were centrifuged and stored in a −70°C freezer within 4 h of venipuncture and later analyzed as a single batch.

Abbreviations and Acronyms

ACS = acute coronary syndrome
CI = confidence interval
CV = coefficient of variation
ECG = electrocardiogram
H-FABP = heart-type fatty acid-binding protein
HR = hazard ratio
MI = myocardial infarction
TnI = troponin I

Most research blood samples collected were timed to coincide with "routine" venepuncture, carried out by clinical and support staff, to ensure little additional inconvenience to the patients. The study design was intentionally pragmatic, so as to be readily generalizable to other patients with suspected ACS as well as to maximize the chances of voluntary enrolment into the study to ensure that the final cohort was truly representative of unselected patients with suspected ACS. Demographic and relevant clinical data were collected at the time of consent from patient medical records and patient interview.

All patients were followed up for the occurrence or death and/or MI over a minimum period of 12 months. The date of censorship of follow-up data was April 1, 2008 (median follow-up 18 months). Survival status was obtained through the U.K. Office of National Statistics. We have ensured no loss to follow-up on mortality data from the Office for National Statistics by obtaining data from at least 4 months after the date of censorship to allow for lag time from death to documentation of death. We set out to obtain data on re-admission to hospital with MI from 2 sources—directly from the patients via patient follow-up questionnaires and from the hospital electronic records system. The first methodology yielded responses from only 68% of patients, with many patients expressing ambiguity about the diagnosis of

MI. By contrast, the information obtained from the hospital electronic records system was robust and reproducible. This was successfully validated on a random sample of patients with patient hospital records and patient follow-up questionnaires. Therefore, we have used this methodology for identification of adverse events of readmission with MI in all our analyses. Because our hospital is the sole provider of emergency care for patients with chest pain in the city of Leeds and the sole provider of primary angioplasty for the entire West Yorkshire region, we expect to have identified the vast majority of recurrent events. A small number of silent events and any occurring outside the West Yorkshire region might have been missed. This would not be expected to produce any bias to our results.

Laboratory analyses. Troponin was measured with the Advia Centaur system (Siemens Healthcare Diagnostics). The assay for measuring troponin in routine clinical practice at our recruiting hospital was upgraded partway through the study (September 12, 2006), from Advia Troponin I to Advia TnI-Ultra. However, the troponin value used in routine clinical practice for classifying patients as having had an acute MI was effectively unchanged throughout the study period. For the period when Advia Troponin I was used, the cutoff value was set at 0.18 μg/l, which represented the lowest concentration at which the CV was 10%. When the Advia TnI-Ultra was used, the cutoff value used was 0.14 μg/l, which corresponded to 0.18 μg/l for the Advia Troponin I assay (in-house evaluation). For the purpose of this study and to ensure that all study patients were categorized in accordance with the new universal definition of MI (10), all stored serum samples were analyzed as a single batch at the end of the study, with the highly sensitive Advia TnI-Ultra assay. We determined the cutoff value corresponding to the 99th percentile value of healthy adults for this assay as 0.05 μg/l locally (with a reference population of 299 healthy adults) and have used this throughout this report. We also observed that the single batch analysis showed considerable precision with an intra-assay CV of 10% at a concentration <0.01 μg /l for Advia TnI-Ultra.

The H-FABP was measured with the Biochip array technology on the fully automated Evidence system (Randox Laboratories, Ltd., Co., Antrim, United Kingdom) with the Cardiac Biochip. This biochip uses a high-precision, chemiluminescent immunometric assay for measuring H-FABP with 2 mouse monoclonal antibodies. We assessed the inter-assay CV to be 5% at a concentration of 5.8 μg/l (99th percentile values for subjects with estimated glomerular filtration rate >60 ml/min) (11).

Statistical analyses. Data are presented as mean ± SD unless otherwise stated, and all statistical analyses were performed with SPSS software (version 16, SPSS, Inc., Chicago, Illinois). Groups of patients were compared with the chi-square test for categorical variables and analysis of variance for continuous variables. Event-free survival curves were generated as univariable Kaplan-Meier estimates. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated by constructing first a univariable and subsequently a multivariable Cox proportional hazards regression model, with death or MI as the dependent variable. For all tests, $p \leq 0.05$ was considered to be statistically significant.

Results

Cohort description and long-term outcomes. The FAB study identified 1,080 patients admitted to hospital with suspected ACS, all of whom had troponin I (TnI) measured >12 h from symptom onset. Approximately one-half of all individuals admitted with suspected ACS during the recruitment period were included in the study. Figure 1 shows the enrolment of patients into the study. We excluded 97 patients presenting with ST-segment elevation or new left bundle branch block on their ECG and 28 patients in whom H-FABP results were unavailable. The remaining 955 patients were included in the analyses. The mean age of patients included was 60 ± 15 years; 577 were male (60.5%) and 378 were female (39.5%). Advia TnI-Ultra (Siemens Healthcare Diagnostics) had a non-Gaussian distribution with a median concentration of 0.02 μg/l (interquartile range 0.01 to 0.04 μg/l). We classified 199 of 955 (20.8%) patients as having non–ST-segment elevation MI, with Advia TnI-Ultra ≥0.05 μg/l (as per the Universal definition of MI). Heart-type fatty acid-binding protein was measured in all 955 patients. We observed a non-Gaussian distribution, with a median concentration of 2.42 μg/l (interquartile range 1.57 to 3.95 μg/l). Table 1 provides a summary of the baseline characteristics across the cohort and further stratified according to H-FABP concentration.

As of April 1, 2008 (median follow-up 18 months), 59 of 955 (6.2%) had died, and the primary outcome measure of death or readmission with MI had occurred in 96 of 955 (10.1%). Table 2 shows the occurrence of death and death or MI—hereafter referred to as "events"—across the study cohort, which has been split into 4 groups on the basis of an equal number of "events." There is a gradient of increasing risk with increasing concentrations of troponin across the 4 groups. It is also notable that the frequency of inpatient angiography and revascularization in Groups 1 and 2 (in whom all patients were deemed troponin "negative") is significantly lower than in Groups 3 and 4 (where the vast majority of patients were deemed troponin "positive"). In contrast, the event rate observed increased progressively across all groups.

Prognostic value of H-FABP across the entire cohort. Table 3 shows the occurrence of "events" (death or MI) across the cohort stratified by H-FABP results. For this analysis, the entire cohort was split into 4 groups on the basis of equal number of events as follows: Group 1: H-FABP <3.26 μg/l, Group 2: 3.27 to 6.48 μg/l, Group 3: 6.49 to 12.77 μg/l, Group 4: >12.77 μg/l. Patients were

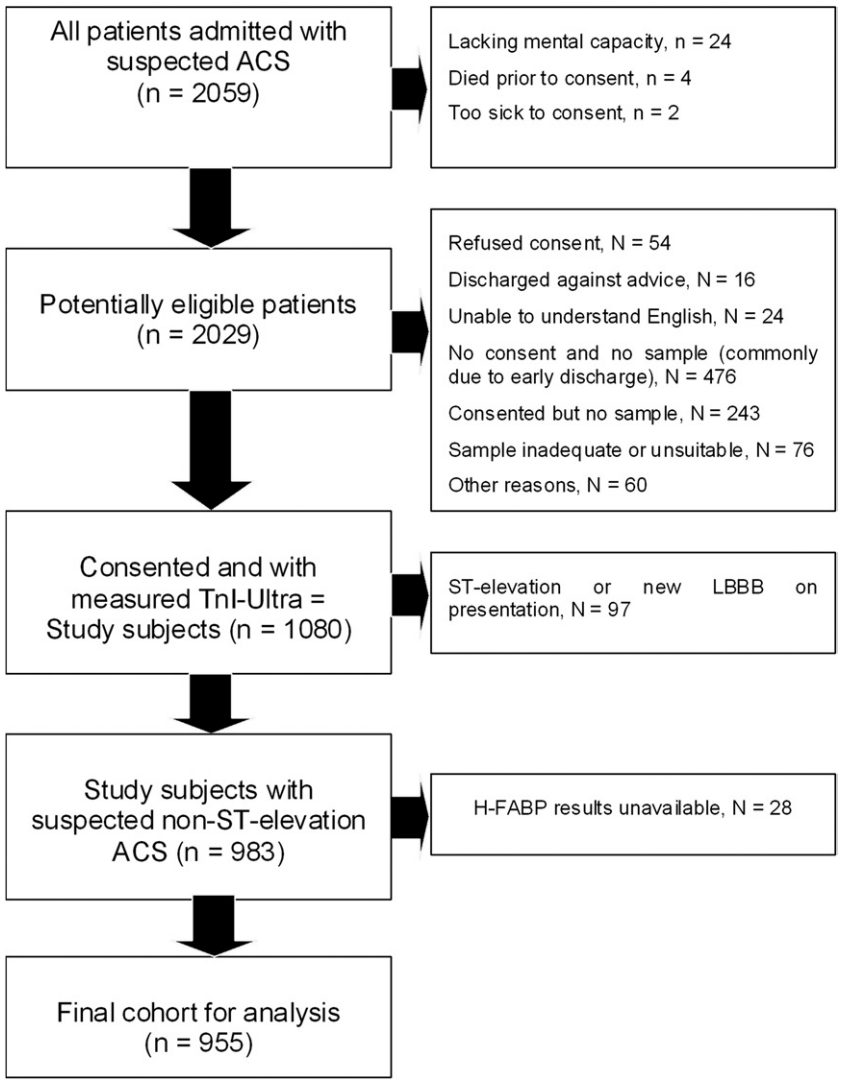

Figure 1 **Enrollment of Patients Into the Study**

Flowchart shows patient enrolment into the study. TnI-Ultra (Siemens Healthcare Diagnostics, Newbury, United Kingdom).
ACS = acute coronary syndrome; H-FABP = heart-type fatty acid-binding protein; LBBB = left bundle branch block.

further classified as MI (troponin-positive) or Not MI (troponin-negative) in accordance with the new Universal Definition of MI (10), with the cutoff value of troponin corresponding to the 99th percentile value for healthy adults (i.e., 0.05 μg/l). There is a gradient of increasing risk across increasing concentrations of H-FABP ($p < 0.001$), irrespective of the associated troponin result. Unadjusted HRs for patients in Groups 3 and 4 according to H-FABP concentration (as shown in Table 4) were 15.67 (95% CI: 8.16 to 30.07) and 20.37 (95% CI: 10.38 to 40.00), respectively (both $p < 0.001$), compared with Group 1. Figure 2 shows the corresponding Kaplan-Meier event-free survival curves for the 4 groups according to H-FABP concentration. Table 4 summarizes the findings of the univariate analyses that were performed to assess the magnitude of risk (death or MI at median follow-up of 18 months) associated with various baseline risk factors that were statistically significant across the 4 groups of H-FABP (Table 1). All factors that were significantly associated with risk on univariate analysis were then included in a Cox proportional hazards regression model, and the results of this multivariable analysis are shown in Table 4. The results show that age, previous MI, admission heart rate, and H-FABP concentration remained statistically significant as independent predictors of long-term risk. Patients with H-FABP concentrations in Group 3 (6.49 to 12.77 μg/l) had a significantly increased risk of adverse events with an adjusted HR of 2.62 (95% CI: 1.30 to 5.28, $p = 0.007$), with Group 4 (>12.77 μg/l) showing a statistically nonsignificant increase in risk with an adjusted HR of 1.54 (95%

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Table 1 Baseline Characteristics According to H-FABP Groups by Equal Number of Events

	Entire Cohort (Events = 96 of n = 955)	H-FABP Group 1 ≤3.26 μg/l (Events = 24 of n = 635)	H-FABP Group 2 3.27–6.48 μg/l (Events = 24 of n = 203)	H-FABP Group 3 6.49–12.77 μg/l (Events = 24 of n = 63)	H-FABP Group 4 >12.78 μg/l (Events = 24 of n = 54)	p Value
Age, yrs (SD)	60.01 (15.00)	55.44 (13.99)	67.37 (12.22)	72.03 (11.26)	71.93 (14.71)	<0.001
Male sex	60.5%	59.9%	58.6%	68.3%	64.8%	0.50 (NS)
Diabetes	15.1%	11.3%	23.3%	22.6%	19.6%	<0.001
Smoking	24.4%	25.8%	20.2%	21.7%	26.9%	0.40 (NS)
Hypertension	61.2%	40.7%	67.0%	71.0%	61.2%	<0.001
Family history of CAD	55.0%	57.4%	51.5%	42.0%	53.2%	0.13 (NS)
Previous MI	30.5%	22.4%	40.3%	65.1%	47.2%	<0.001
Prior PCI/CABG	23.3%	19.8%	30.7%	33.9%	24.5%	0.003
Previous heart failure	5.1%	2.2%	8.1%	10.9%	23.4%	<0.001
ST-segment depression on ECG	10.6%	4.9%	15.3%	23.8%	44.4%	<0.001
Creatinine, mg/dl (SD)	1.14 (0.30)	1.06 (0.16)	1.22 (0.27)	1.36 (0.30)	1.54 (0.71)	<0.001
Systolic BP, mm Hg (SD)	138.01 (25.41)	136.29 (22.67)	140.98 (27.47)	133.92 (29.13)	152.45 (36.89)	<0.001
Heart rate, beats/min (SD)	77.59 (20.39)	75.05 (17.15)	80.98 (23.47)	82.63 (29.05)	88.74 (24.49)	<0.001
Troponin-negative <0.05 μg/l	79.2%	90.2%	72.9%	50.8%	5.6%	<0.001

BP = blood pressure; CABG = coronary artery bypass grafting; CAD = coronary artery disease; ECG = electrocardiogram; H-FABP = heart-fatty acid-binding protein; MI = myocardial infarction; PCI = percutaneous coronary intervention; Q = quartile.

CI: 0.55 to 4.32, p = 0.41). Analyses with receiver-operator curves comparing the value of H-FABP and TnI in predicting long-term adverse events showed H-FABP to be comparable to TnI (area under the curve for H-FABP = 0.79 [95% CI: 0.74 to 0.84] vs. TnI = 0.77 [95% CI: 0.72 to 0.82]), as shown in Figure 3.

Prognostic value of H-FABP in the TnI-negative subgroup. In the troponin-negative subgroup (n = 756), there were 40 major adverse events (death or MI) during the follow-up period. Table 3 shows the event rate across each of the 4 groups on the basis of H-FABP concentrations as described in the earlier section. Table 5 shows the unadjusted HRs for troponin-negative patients, after stratifying patients into the same 4 groups. This shows increasing risk in Groups 2, 3, and 4 as compared with Group 1 with a very significantly increased event rate in patients with H-FABP concentrations above 6.48 μg/l (Groups 3 and 4)—unadjusted HR in Group 3: 11.20 (95% CI: 4.95 to 25.36), p < 0.001. Table 5 shows the adjusted HRs after adjustment for age and serum creatinine in a Cox proportional hazards regression model. This confirms the additional prognostic value of H-FABP >6.48 μg/l (adjusted HR: 3.12, 95% CI: 1.11 to 8.76, p = 0.03), independent of age and serum creatinine.

Discussion

Despite enormous research interest in cardiac biomarkers in recent years, very few have established themselves unequivocally in routine clinical practice. Cardiac troponin remains the cornerstone in the risk stratification of patients with suspected ACS. One of the important criteria for a biomarker is to be able to inform clinical decision-making and thus influence patient management (12). Heart-type fatty acid-binding protein has emerged as an independent prognostic marker for patients with ACS in at least 2 large studies (2,3). The FAB study was specifically designed to complement our earlier study on H-FABP (2) and extend the applicability of the results to contemporary clinical practice by including low-risk subjects. Our study population (excluding those presenting with ST-segment elevation or new left bundle branch block) included 79.2% of subjects with TnI below the 99th percentile, which is consistent with several routine laboratory audits. In this first report resulting from the FAB study, we have confirmed our previous finding for patients with proven ACS—that H-FABP identifies high-risk patients who are troponin-negative.

Since the publication of the New Definition of MI in 2000 (5), there have been few studies reporting long-term major adverse outcomes (death, MI) for a large consecutive

Table 2 Management and Long-Term Outcomes on the Basis of Troponin Results

	Entire Cohort (n = 955)	TnI Group 1 0.00–0.02 μg/l (Events = 24 of n = 641)	TnI Group 2 0.03–0.08 μg/l (Events = 24 of n = 146)	TnI Group 3 0.09–3.04 μg/l (Events = 24 of n = 99)	TnI Group 4 >3.04 μg/l (Events = 24 of n = 69)	p Value
Inpatient angiogram	15.4% (147)	6.1% (39)	8.2% (12)	49.5% (49)	68.1% (47)	<0.001
Inpatient PCI/CABG	8.1% (77)	1.4% (9)	2.1% (3)	33.3% (33)	46.4% (32)	<0.001
Death	6.2% (59)	2.7% (17)	9.6% (14)	12.1% (12)	23.2% (16)	<0.001
Death or MI	10.1% (96)	3.7% (24)	16.4% (24)	24.2% (24)	34.8% (24)	<0.001

TnI = Troponin I; other abbreviations as in Table 1.

Table 3 Number of Major Adverse Events Across the 4 Subgroups on the Basis of H-FABP Concentrations and Stratified by Troponin Results

H-FABP Concentration	"Not MI" According to Universal Definition (Troponin <0.05 μg/l)	"MI" According to Universal Definition (Troponin ≥0.05 μg/l)	Total
Group 1 = 0.15–3.26 μg/l (events = 24 of n = 635)	16/573 (2.8%)	8/62 (12.9%)	24/635 (3.8%)
Group 2 = 3.27–6.48 μg/l (events = 24 of n = 203)	14/148 (9.5%)	10/55 (18.2%)	24/203 (11.8%)
Group 3 = 6.49–12.77 μg/l (events = 24 of n = 63)	9/32 (28.1%)	15/31 (48.4%)	24/63 (38.1%)
Group 4 = 12.78–151.00 μg/l (events = 24 of n = 54)	1/3 (33.3%)	23/51 (45.1%)	24/54 (44.4%)
Entire cohort (N = 955)	40/756 (5.3%)	56/199 (28.1%)	96/955 (10.1%)
p value	<0.001	<0.001	<0.001

Major adverse events are death or MI after a median follow-up period of 18 months.
Abbreviations as in Table 2.

population of patients presenting with chest pain/suspected ACS (13–15). In keeping with these published studies, the majority of our subjects were at low-to-intermediate risk.

Table 4 HRs for Death or MI After Median Follow-Up Period of 18 Months

Univariate*	HR (95% CI)	p Value
Age	1.11 (1.08–1.12)	<0.001
Diabetes	2.43 (1.48–3.99)	<0.001
Hypertension	1.95 (1.25–3.05)	0.003
Previous PCI/CABG	1.64 (1.03–2.61)	0.043
Previous heart failure	5.62 (2.89–10.96)	<0.001
Previous MI	4.60 (2.94–7.18)	<0.001
Heart rate	1.024 (1.015–1.033)	<0.001
Systolic BP	1.005 (0.97–1.014)	0.21
ST-segment depression on ECG	6.48 (3.98–10.55)	<0.001
Creatinine	1.025 (1.018–1.033)	<0.001
TnI 0.00–0.02 μg/l	1.00	<0.001
TnI 0.03–0.08 μg/l	5.06(2.78–9.20)	<0.001
TnI 0.09–3.04 μg/l	8.22 (4.55–15.2)	<0.001
TnI >3.04 μg/l	13.71 (7.22–26.05)	<0.001
H-FABP 0.15–3.26 μg/l	1.00	<0.001
H-FABP 3.27–6.48 μg/l	3.41 (1.89–6.16)	<0.001
H-FABP 6.49–12.77 μg/l	15.67 (8.16–30.07)	<0.001
H-FABP 12.78–151.0 μg/l	20.37 (10.38–40.00)	<0.001
Multivariable†		
Age	1.06 (1.03–1.08)	<0.001
Diabetes	1.67 (0.97–2.87)	0.062
Hypertension	1.06 (0.65–1.73)	0.81
Prior heart failure	1.29 (0.65–2.56)	0.47
Prior MI	1.79 (1.08–2.98)	0.025
Heart rate	1.01 (1.00–1.02)	0.004
ST-segment depression on ECG	1.73 (0.98–3.05)	0.59
Creatinine	1.00 (0.99–1.01)	0.48
TnI 0.00–0.02 μg/l	1.00	0.10
TnI 0.03–0.08 μg/l	2.15 (1.11–4.17)	0.024
TnI 0.09–3.04 μg/l	2.09 (1.01–4.36)	0.048
TnI >3.04 μg/l	2.41 (0.95–6.09)	0.063
H-FABP 0.15–3.26 μg/l	1.00	0.003
H-FABP 3.27–6.48 μg/l	0.78 (0.39–1.55)	0.48
H-FABP 6.49–12.77 μg/l	2.62 (1.30–5.28)	0.007
H-FABP 12.78–151.0 μg/l	1.54 (0.55–4.32)	0.41

*Unadjusted univariate analysis; †multivariate analysis including all factors noted to be significant on univariate analysis (except previous PCI/CABG in view of borderline significance only on univariate analysis).
CI = confidence interval; HR = hazard ratio; other abbreviations as in Table 2.

Consequently, we note significantly lower occurrence of death and MI compared with previously published studies on H-FABP that largely included high-risk ACS patients. The event rate in our study was slightly lower than the consecutive chest pain population studies mentioned earlier. This is consistent with global improvement in care of patients with ACS secondary to the increasing use of evidence-based pharmacological therapy (aspirin, clopidogrel, statin, beta-blocker, ACE inhibitor) and coronary intervention.

Recently, McCann et al. (16) reported on the prognostic value of H-FABP among other markers in 664 patients presenting to coronary care unit with ischemic-type chest pain recruited over 3 years. The authors showed that H-FABP and N-terminal pro–B-type natriuretic peptide had independent prognostic value in addition to troponin. Our study confirms the long-term prognostic value of H-FABP demonstrated in this and earlier studies (2,3) in a

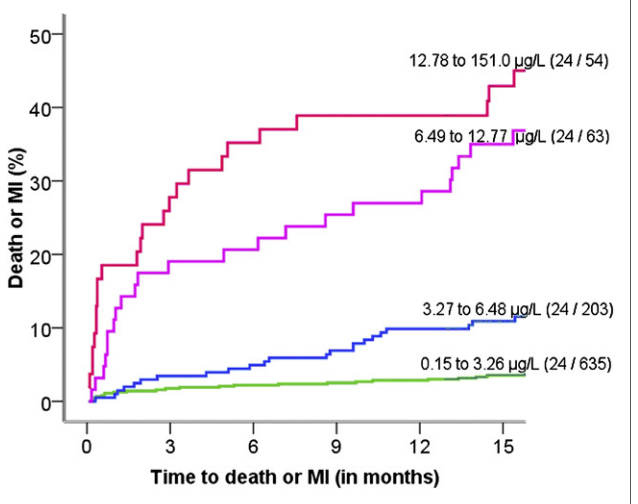

Figure 2 Kaplan Meier Event-Free Survival Curves for Death or MI in the 4 Groups of Patients on the Basis of H-FABP Concentrations

Each **curve** represents a subgroup of patients defined by the measured concentration of heart-type fatty acid-binding protein (H-FABP). Group 1: H-FABP <3.26 μg/l, Group 2: 3.27 to 6.48 μg/l, Group 3: 6.49 to 12.77 μg/l, Group 4: >12.77 μg/l. For hazard ratios for each of the groups, see Table 4. MI = myocardial infarction.

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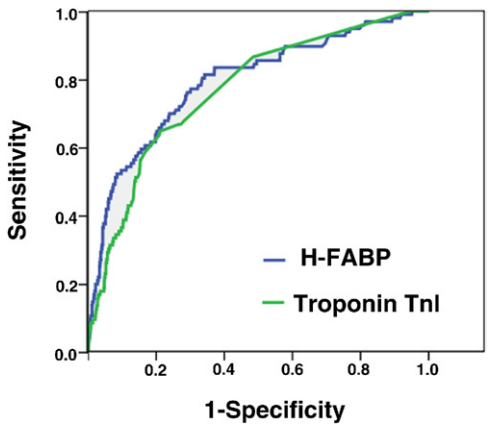

Figure 3 Receiver-Operator Curves for H-FABP and TnI-Ultra in the Prediction of Death or MI

Receiver-operator curves for H-FABP and troponin I (TnI) in prediction of death or MI after median of 18 months follow-up. Area under the curve for H-FABP = 0.79 (95% confidence interval 0.74 to 0.84). Area under the curve for TnI = 0.77 (95% confidence interval: 0.72 to 0.82). Abbreviations as in Figures 1 and 2.

larger and unselected consecutive cohort of suspected ACS patients. We have shown that the risk of death or recurrent MI increases with increasing concentrations of H-FABP with a significant increase in patients with H-FABP >6.48 μg/l, and this is independent of other established clinical risk factors, including troponin. One of the particular strengths of studying an unselected cohort of consecutive patients with suspected ACS (as in our study) is that the results can be extrapolated more readily to contemporary clinical practice. Therefore, we believe that this takes us 1 step closer toward establishing the clinical utility of routine measurement of H-FABP in suspected ACS.

The ideal cutoff value for defining troponin positivity in suspected ACS has been long identified as the 99th percentile value in a population of healthy adults, and this has been reinforced in the more recently published Universal Definition of Myocardial Infarction (10). However, the fact that no commercially available assays were able to achieve the standard of a CV <10% at the 99th percentile value until recently has remained a major limitation (17). Therefore, it was likely that before 2008 some patients were misclassified as low-risk, thus overestimating the magnitude of benefit of biomarkers studied in conjunction with troponin. In this study, we measured troponin with the ultra-sensitive TnI-Ultra (Siemens Healthcare Diagnostics) assay, a third-generation troponin assay that satisfies the requirements stated in the Universal Definition of MI (10). It is notable that the independent prognostic value of H-FABP demonstrated in patients with suspected ACS is not negated by the use of the newer ultra-sensitive troponin assays.

We have demonstrated, in particular, that the long-term prognostic value of H-FABP in troponin-negative patients is independent of age and serum creatinine, both of which have been shown to influence H-FABP concentrations in apparently healthy subjects (11). Our study suggests that troponin-negative patients with H-FABP >6.48 μg/l represent a very-high-risk group of patients, and we suggest that further investigations such as coronary angiography and appropriate pharmacotherapy are warranted in this small subgroup of patients (35 of 756, 4.6% of all troponin-negative patients in our study).

In a recent publication by our group, we have defined the 99th percentile values for H-FABP measured with 2 commercially available assays in a population of primary and secondary care outpatients (11). The 99th percentile values for subjects with estimated glomerular filtration rate >60 ml/min for the Evidence Investigator H-FABP assay used in this study were 5.3 and 5.8 μg/l in female and male subjects, respectively. In our present study, with this cutoff value to define patients as "elevated H-FABP," these patients had significantly increased event rate. The unadjusted HRs for death or MI for those with "elevated H-FABP" as defined by this cut-off value were 3.70 (95% CI: 1.82 to 7.56, $p < 0.0001$) in troponin-positive patients and 6.57 (95% CI: 3.05 to 14.11, $p < 0.0001$) among troponin-negative patients.

We acknowledge a few limitations of this study. It must be noted that 53.2% of eligible patients admitted during the period of recruitment were enrolled into the main study. This was largely because, although patients with suspected ACS present 24 h/day and 7 days/week, participation in a study of this nature requires informed consent and precisely timed sample collection within a few hours of hospital admission—making the study logistically quite challenging. The exclusion of patients from the study (Fig. 1) was random, and we believe that this was unlikely to introduce any systematic bias to our results. We also note the limitations inherent to any statistical modeling and multivariate adjustment. Although we only included the most important clinical variables likely to cause confounding during statistical modeling, we recognize the potential for over-fitting of the statistical model. However, we note that in usual clinical care it is rare for diagnostic tests to be used in any other than the unadjusted form.

Table 5 HRs for Death or MI Stratified by H-FABP Results Among Troponin-Negative Patients

Unadjusted	HR (95% CI)	p Value
H-FABP 0.15–3.26 μg/l	1.00	<0.001
H-FABP 3.27–6.48 μg/l	3.46 (1.69–7.10)	0.001
H-FABP 6.49–12.77 μg/l	11.20 (4.95–25.36)	<0.001
H-FABP 12.78–151.0 μg/l	16.64 (2.21–125.51)	0.006
Adjusted for Age and Serum Creatinine	**Adjusted HR (95% CI)**	
H-FABP 0.15–3.26 μg/l	1.00	0.01
H-FABP 3.27–6.48 μg/l	1.55 (0.72–3.36)	0.26
H-FABP 6.49–12.77 μg/l	3.12 (1.11–8.76)	0.03
H-FABP 12.78–151.0 μg/l	16.67 (2.19–127.06)	0.007

Abbreviations as in Tables 1 and 4.

We report here results for biomarkers measured at only 1 time-point (i.e., >12 h from symptom onset). This was our pre-stated objective and is in keeping with standard practice in our institution and indeed most other hospitals in the United Kingdom. Initial risk stratification of patients into high and low risk was done primarily on the basis of clinical and ECG parameters rather than admission biomarker assessment. The likelihood of a false negative troponin in our study is very low, because of the high precision of the Ultra-TnI (Siemens Healthcare Diagnostics) when run in a single batch. Patients recruited into the study also had an earlier serum sample collected at the time of first contact with the emergency department whenever logistically possible. Future analyses will assess the value of H-FABP (and other cardiac markers) measured at this earlier time point in the early diagnosis of MI in detail. Limited analyses of the 384 troponin-negative patients who had an admission serum sample collected (of the 756 troponin-negative patients in the study [i.e., 50%]) showed that the occurrence of death or MI among those with raised H-FABP at the time of admission (male >5.8 μg/dl, female >5.3 μg/dl) was 22.2% (6 of 27 patients) as compared with a much lower event rate of 5.3% in patients with normal H-FABP at admission (19 of 357 patients), which was statistically significant ($p = 0.001$) (HR: 5.08, 95% CI: 1.84 to 14.07, $p = 0.002$).

We also note the substantial influence of the troponin results in deciding further management, as illustrated by the very low rate of inpatient coronary angiography in troponin-negative patients (Table 2). This underlines the importance of using the new ultra-sensitive troponin assays and classifying all patients with troponin values >99th percentile value as potentially high risk. Importantly, many centers around the world have yet to make this change, and our data indicate that this would be improved further by the parallel measurement of H-FABP with a similarly high-precision, high-sensitivity assay. The multimarker cardiac biochip that we used in this study (Randox Laboratories, Ltd.) permits such parallel measurements to be easily made. Furthermore, the recent development of a point-of-care platform permits rapid delivery of results and repeat measurements. Further studies will assess the value of this system in the context of suspected ACS and also in the detection of myocardial ischemia and injury in other contexts, such as after PCI or surgical intervention.

The H-FABP seems to provide direct evidence of myocardial ischemia even when frank myocyte necrosis is absent (18,19). Meng et al. (18) demonstrated that in rats the H-FABP concentration in peripheral blood was 4 times the baseline after just 15 min of induced myocardial ischemia. Additionally, in human autopsy cases it was possible to demonstrate depletion of H-FABP in the myocardium of individuals dying suddenly after the onset of chest pain. These changes were present despite the absence of myocyte necrosis on electron microscopy (19). This caused those investigators to conclude that H-FABP is both a sensitive and an early marker of myocardial ischemia. Such a conclusion is intuitive, given that H-FABP is confined to the cytoplasm and also because of its small molecular size, because both these features permit early leakage through the porous membranes of ischemic myocardial cells. This might explain the strong correlation between H-FABP and ST-segment depression noted on ECG (Table 1). The presence of myocardial ischemia resulting in chest pain might result from coronary spasm in the presence of atheroma but in the absence of plaque erosion/rupture and thrombus formation. Such patients might be expected to have no detectable troponin and yet have an elevated H-FABP and, importantly, an increased risk of future major cardiovascular event.

Conclusions

We have demonstrated that the prognostic value of elevated H-FABP is additive to troponin in low- and intermediate-risk patients with suspected ACS. Importantly, this is true in spite of use of a very-high-sensitivity troponin assay that achieves the analytical performance prescribed in the Universal Definition of MI consensus document (10). We interpret this as supporting the role of H-FABP as a marker of myocardial ischemia, even in the absence of frank necrosis.

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