Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 50, No. 21, 2007
ISSN 0735-1097/07/$32.00
doi:10.1016/j.jacc.2007.08.021

Heart-Type Fatty Acid-Binding Protein Predicts Long-Term Mortality After Acute Coronary Syndrome and Identifies High-Risk Patients Across the Range of Troponin Values

Niamh Kilcullen, MRCPI,* Karthik Viswanathan, MRCP,* Rajiv Das, MRCP,* Christine Morrell,* Amanda Farrin, MSc,† Julian H. Barth, MD, FRCP, FRCPath,‡ Alistair S. Hall, PhD, FRCP,* for the EMMACE-2 Investigators

Leeds, United Kingdom

Objectives Our aim was to determine if a high-performance assay for heart-type fatty acid-binding protein (H-FABP) has a role in predicting all-cause mortality after acute coronary syndrome (ACS).

Background Heart-type fatty acid-binding protein is released into the circulation following myocardial ischemia and necrosis and therefore may be of value to physicians when caring for patients admitted to hospital with a clinical diagnosis of ACS.

Methods This was a prospective observational study with a follow-up of 12 months. The H-FABP was measured 12 to 24 h after onset of symptoms in 1,448 patients admitted to hospital with ACS. The main outcome measure was all-cause mortality 1 year after index hospital admission. Multivariable analyses were conducted using the well validated GRACE (Global Registry of Acute Coronary Events) variables together with troponin I and highly sensitive C-reactive protein (hs-CRP).

Results After 12 months of follow-up, 296 patients had died. Multivariable analysis demonstrated that H-FABP quartiles were strongly predictive of outcome: Q1 hazard ratio (HR) 1.0; Q2 HR 2.32 (95% confidence interval [CI] 1.25 to 4.30; $p = 0.007$); Q3 HR 3.17 (95% CI 1.73 to 5.82; $p < 0.001$); Q4 HR 4.88 (95% CI 2.67 to 8.93; $p < 0.001$). The crude all-cause 1-year mortality for unstable angina patients with H-FABP <5.8 μg/l was 2.1% compared with 22.9% for patients above this cutoff. The adjusted all-cause mortality HR in this group was 11.35 (95% CI 2.00 to 64.34; $p = 0.006$).

Conclusions Heart-type fatty acid-binding protein predicts long-term mortality after ACS and identifies high-risk patients in a manner that is additive to the GRACE clinical risk factors, troponin, and hs-CRP, possibly as a result of identifying the occurrence of myocardial ischemia with or without necrosis. (J Am Coll Cardiol 2007;50:2061–7)

Heart-type fatty acid-binding protein (H-FABP) is a low-molecular-weight protein involved in the intracellular uptake and buffering of free fatty acids in the myocardium (1). It was first noted to be a marker of myocardial infarction (MI) in 1988 (2). Because it is rapidly released from the cytosol into the circulation after myocardial ischemia and necrosis (3), H-FABP has been shown to be a sensitive early marker of MI (4,5). Despite this fact, initial studies performed using nonspecific polyclonal antibody assays were disappointing. Consequently, there has been relatively little attention given to H-FABP as an early marker of myocardial necrosis/ischemia. However, O'Donoghue et al. (6) have recently reported on the prognostic value of H-FABP in a subset

See page 2068

From the *Coronary Artery Disease Clinical Research Network Group, Leeds Institute for Genetic, Health & Therapeutics, and †Clinical Trials Research Unit, University of Leeds, Leeds, United Kingdom; and the ‡Department of Clinical Biochemistry, General Infirmary at Leeds, Leeds, United Kingdom. Prof. Hall and Dr. Barth have received support in the form of free reagents from Beckman Coulter and Dainippon Pharmaceutical and research grants from AstraZeneca and Sanofi-Aventis in support of the EMMACE-2 study. Rajiv Das and Niamh Kilcullen held British Heart Foundation Junior Research Fellowships. Karthik Viswanathan is supported by grants from Pfizer and Medtronic. The structure of the EMMACE-2 Study Group is provided as an Online Appendix.

Manuscript received November 6, 2006; revised manuscript received July 23, 2007, accepted August 21, 2007.

Abbreviations and Acronyms

ACS = acute coronary syndrome
CV = coefficient of variation
ECG = electrocardiogram
H-FABP = heart-type fatty acid-binding protein
hs-CRP = highly sensitive C-reactive protein
MI = myocardial infarction
TnI = troponin I

of 2,287 participants (22%) of the TIMI (Thrombolysis In Myocardial Infarction)-16 trial demonstrating a clear association with a composite end point of death, MI, or heart failure at 10 months. This was present in all subsets of patients (i.e., based on age, gender, renal function, and so on) (6). We report on the clinical performance of a sensitive H-FABP assay to further clarify its role in predicting adverse prognosis after acute coronary syndrome (ACS) when used in combination with a routine measurement (12 to 24 h after onset of symptoms) of cardiac troponin.

Methods

Patient recruitment. Over a 6-month period (April 28, 2003 to October 28, 2003) the EMMACE (Evaluation of Methods and Management of Acute Coronary Events)-2 study identified and validated 2,499 consecutive consenting patients with ACS from 11 adjacent hospitals in West Yorkshire, United Kingdom (7). Of these, 1,448 had sufficient blood sample remaining after routine local analyses for the measurement of H-FABP and troponin I (TnI) centrally. Patients were potentially eligible if they were admitted to hospital either through the emergency department or directly to the ward with an admission diagnosis of suspected ACS. The judgement regarding appropriateness of inclusion was made by a specialist cardiology research nurse in conjunction with a cardiology research registrar, taking into account the views and opinions of the attending medical team. This was based first on the clinical context and second on the results of cardiac biomarkers. Specifically, patients were included in the study if they fulfilled a revised European Society of Cardiology/American College of Cardiology definition (8) of myocardial infarction: raised troponin value above the 10% coefficient of variation (CV) taken 12 to 24 h after the onset of symptoms accompanied by at least 1 of the following: 1) ischemic symptoms; 2) development of pathologic Q waves on the electrocardiogram (ECG); 3) ECG changes indicative of ischemia; 4) delivery of primary coronary angioplasty; and 5) compatible postmortem findings. We also included some unstable angina patients who were TnI negative (measured centrally) on the basis of clinical and ECG diagnosis. In these patients, symptoms were sufficient to warrant acute admission to hospital and diagnosis was based on an appropriate clinical history (chest pain believed to be indicative of myocardial ischemic origin), ECG findings (ST-segment depression, T-wave changes, bundle branch block, other abnormalities), and the results of locally used troponin assays. Patients may also have been considered to be troponin positive on a local assay but later reclassified as negative based on central measurement using the Beckman Coulter Accu TnI assay (High Wycombe, Buckinghamshire, United Kingdom). Patients were excluded if they refused consent or if they were judged not to have a diagnosis of ACS. Our intention was to include a wide range of consenting ACS patients into the study. The study was approved by the Multi Research Ethics Committee and the Local Research Ethics Committees for each of the participating hospitals. All patients were followed up long term for survival which was tracked through the Office of National Statistics (ONS).

Blood sampling and assays. The serum or plasma sample obtained from patients 12 to 24 h after the onset of symptoms, taken for routine diagnostic purposes, was saved for later measurement of TnI and H-FABP. Of the 1,448 samples analyzed, there were 1,180 (82%) serum and 268 (18%) plasma samples. Previous work showed that H-FABP concentrations were 13% higher in serum samples compared with plasma (N. Kilcullen, unpublished data, 2004). Serum samples were also obtained from 96 (48 male, 48 female) healthy controls to assess the reference limits for H-FABP in our laboratory. The mean age of controls was 45 years (SD 9.5 years; median age 44 years; range 27 to 69 years). All samples were centrifuged and frozen at −70°C within 6 h of venipuncture. Sample stability tests showed no significant change in H-FABP concentrations after 12 months' storage (N. Kilcullen, unpublished data, 2004). The H-FABP was measured using a 2-step direct sandwich enzyme-linked immunosorbent assay using 2 distinct mouse antihuman H-FABP monoclonal antibodies (Dainippon Pharmaceutical Co., Osaka, Japan). A cutoff value of 5.8 μg/l was used to define abnormality based on the 99th percentile for the 96 healthy controls. The intra-assay CV for H-FABP was 3.4% at 6 μg/l and 6.9% at 12 μg/l. The interassay CV for H-FABP was 6.3% at 6 μg/l and 8.3% at 12 μg/l. The mean minimum detectable concentration of H-FABP is 1.25 μg/l (manufacturer's data). Patients with H-FABP concentrations ≤5.8 μg/l were subsequently defined as being H-FABP—and those with values of >5.8 μg/l were defined as being H-FABP+.

Patients were included in the EMMACE-2 cohort based predominantly, though not exclusively, on initial troponin measurements performed in each hospital using a variety of different assays. However, residual blood samples were then brought to a central laboratory for repeat measurement using a single high-performance assay. Biochemical analysis of TnI was performed using the Accu TnI assay (Beckman Coulter). The interassay CV for TnI was 10% at 0.06 μg/l. Potential decision points for TnI are 0.04 μg/l (99th percentile value [9]) and 0.06 μg/l (10% CV value [10]). Given the implied imprecision of the assay below values of 0.06 μg/l, and in line with local practice and most current thinking, we defined patients as being TnI− when measured concentrations were ≤0.06 μg/l and as

TnI+ when >0.06 μg/l. This assay was selected for use in the present study because it had a much better performance profile than most of the other assays that were in routine use (11).

Statistical analyses. Statistical analyses were performed using SPSS software (version 11.1, SPSS Inc., Chicago, Illinois). Data are presented as median (range) unless otherwise stated. Groups were compared using the chi-squared test for categorical variables and analysis of variance for continuous variables. Survival curves were generated by means of Kaplan-Meier estimates (univariable). Hazard ratios (HR) and 95% confidence intervals (CI) were also calculated by constructing a univariable or multivariable logistic regression model. To explore the extent to which H-FABP produces prognostic information that is statistically independent, we performed logistic regression analyses using the nine variables from the GRACE (Global Registry of Acute Coronary Events) prediction model (12): age, previous history of heart failure, previous MI, heart rate on admission, systolic blood pressure on admission, ST-segment depression on ECG, creatinine on admission, elevated cardiac markers, and inpatient percutaneous coronary intervention plus highly sensitive C-reactive protein (hs-CRP). We used TnI as a continuous variable rather than as the categoric variable described in the GRACE model. For all tests, p values of <0.05 were considered to be statistically significant. We also explored the extent to which the selected H-FABP clinical cutoff value produced prognostic information that was independent of standard clinical predictors of risk as well as being complementary to the clinical cutoff values for TnI. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Cohort description according to H-FABP quartile. The EMMACE-2 study identified and validated 2,499 patients with ACS. The 12-month mortality was identical for patients included and excluded from this study. Furthermore, a range of other descriptive factors were identical in those included and excluded from this study (data not shown). The median age of patients included was 72.5 (SD 13) years; 885 were male (61%) and 563 were female (39%). The majority of patients had non–ST-segment elevation myocardial infarction (62.9%; n = 911) compared with ST-segment elevation myocardial infarction (24.8%; n = 359) and unstable angina (12.3%; n = 178). Table 1 provides a summary of the cohort characteristics based on quartiles for H-FABP.

Table 1 Baseline Factors According to H-FABP Quartile

	H-FABP Q1: ≤6.4 μg/l (n = 360)	H-FABP Q2: 6.4–12.4 μg/l (n = 364)	H-FABP Q3: 12.4–36.2 μg/l (n = 362)	H-FABP Q4: >36.2 μg/l (n = 362)
Median age (yrs)*	65.0 (SD 12.5)	73.7 (SD 11.7)	75.5 (SD 12.1)	74.2 (SD 13.2)
Gender (male)	61.7% (222)	55.5% (202)	63.8% (231)	63.5% (230)
Unstable angina	59.0% (105)	31.5% (56)	9.6% (17)	0% (0)
NSTEMI	24.8% (226)	27.0% (246)	26.3% (240)	21.8% (199)
STEMI/BBBMI	8.1% (29)	17.3% (62)	29.2% (105)	45.4% (163)
Heart failure*	1.7% (6)	8.2% (30)	13.3% (48)	7.5% (27)
Previous MI*	27.7% (100)	28.0% (102)	30.7% (111)	21.8% (79)
Prior PCI/CABG	17.8% (64)	13.7% (50)	9.9% (36)	5.5% (20)
Diabetes*	13.1% (47)	20.1% (73)	18.0% (65)	16.3% (59)
Smoking	29.2% (105)	23.4% (85)	21.8% (79)	30.1% (109)
Median heart rate (beats/min)*	75 (SD 22.2)	80 (SD 24.5)	84 (SD 25.3)	80 (SD 22.5)
Median SBP (mm Hg)*	142 (SD 28.0)	145 (SD 28.9)	144 (SD 31.1)	138 (SD 29.7)
ST-segment depression on ECG	15.8% (57)	19.5% (71)	24.6% (89)	27.9% (101)
Median creatinine (mg/dl)*	1.01 (SD 0.24)	1.12 (SD 0.33)	1.70 (SD 1.28)	1.11 (SD 1.24)
Troponin*†				
Negative (<0.06 μg/l)	35.3% (127)	18.1% (66)	6.4% (23)	0.3% (1)
Q1 (0.07–0.47 μg/l)	31.7% (114)	26.6% (97)	19.3% (70)	6.4% (23)
Q2 (0.48–2.39 μg/l)	25.0% (90)	29.4% (107)	21.3% (77)	8.0% (29)
Q3 (2.40–12.68 μg/l)	5.8% (21)	19.2% (70)	30.7% (111)	27.9% (101)
Q4 (>12.68 μg/l)	1.7% (6)	5.8% (21)	21.5% (78)	55.5% (201)
Inpatient PCI*	12.8% (46)	7.7% (28)	4.1% (15)	5.0% (18)
Inpatient CABG	3.6% (13)	2.5% (9)	3.3% (12)	1.1% (4)
Median hs-CRP‡ (μg/l)	0.47 (SD 2.67)	0.75 (SD 4.87)	1.62 (SD 6.73)	4.28 (SD 6.91)

*Factor in GRACE risk model. †Troponin measured in central laboratory. ‡hs-CRP = highly sensitive C-reactive protein, measured in central laboratory.

BBBMI = bundle branch block myocardial infarction; CABG = coronary artery bypass surgery; ECG = electrocardiogram; GRACE = Global Registry of Acute Coronary Events; H-FABP = heart-type fatty acid-binding protein; MI = myocardial infarction; NSTEMI = non–ST-segment elevation myocardial infarction; PCI = percutaneous coronary intervention; Q = quartile; SBP = systolic blood pressure; STEMI = ST-segment elevation myocardial infarction.

JACC Vol. 50, No. 21, 2007
November 20, 2007:2061–7

Cardiac markers. Heart-type fatty acid-binding protein was measured in 1,448 samples and was observed to have a nongaussian distribution with a median concentration of 12.2 μg/l (range 1.5 to 944.4 μg/l). Three hundred five patients had H-FABP concentrations ≤5.8 μg/l, and 1,143 had concentrations >5.8 μg/l. Troponin I had a nongaussian distribution with a median concentration of 1.35 μg/l (range 0.01 to 101 μg/l). Two hundred seventeen patients had TnI concentrations ≤0.06 μg/l and 1,210 patients had concentrations >0.06 μg/l.

Prognostic value of H-FABP. Figure 1 shows Kaplan-Meier all-cause mortality curves according to H-FABP quartiles. Table 2 summarizes the findings of the univariable and multivariable analyses that we performed to assess the magnitude of risk of 1-year all-cause mortality that was associated with TnI and H-FABP quartiles. Next, we assessed the independence of each biomarker from other clinical risk factors in the GRACE model inclusive of hs-CRP. The adjusted HRs for H-FABP quartiles were: Q1 1.0, Q2 2.32 (95% CI 1.25 to 4.30; p = 0.007), Q3 3.17 (95% CI 1.73 to 5.82; p < 0.001), and Q4 4.88 (95% CI 2.67 to 8.93; p < 0.001). We then included TnI in the multivariable adjustment model to assess independence of H-FABP prognostic information from troponin. These sensitivity analyses were performed in 3 separate ways, including: 1) elevated TnI (>0.06 μg/l) as an additional categoric variable: H-FABP Q1 HR 1.0, Q2 HR 2.44 (95% CI HR 1.29 to 4.60; p = 0.006), Q3 HR 3.26 (95% CI 1.72 to 6.18; p < 0.001), Q4 HR 4.77 (95% CI 2.50 to 9.12; p < 0.001); 2) quartiles of elevated TnI relative to negative troponin (<0.06 μg/l) as an additional categoric variable: H-FABP Q1 HR 1.0, Q2 HR 2.52 (95% CI 1.33 to 4.77;

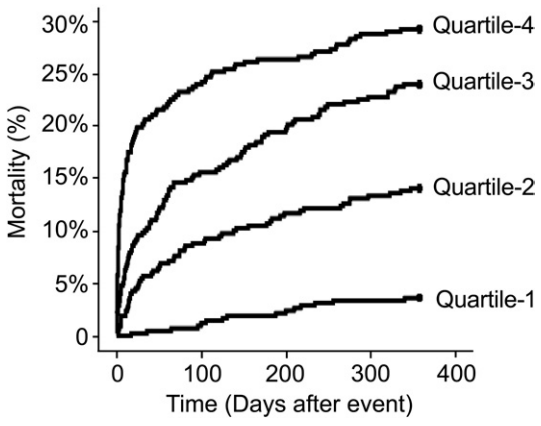

Figure 1 Kaplan-Meier All-Cause Mortality Curves According to H-FABP Quartiles

Kaplan-Meier all-cause mortality curves according to heart-type fatty acid-binding protein (H-FABP) quartiles (Q). After adjusting for all GRACE risk factors, highly sensitive C-reactive protein (hs-CRP), and troponin I as a continuous variable the following hazard ratios were observed: Q1 1.0, Q2 2.58 (95% confidence interval [CI] 1.37 to 4.85; p = 0.003), Q3 3.77 (95% CI 2.01 to 7.07; p < 0.001), and Q4 6.59 (95% CI 3.40 to 12.74; p < 0.001).

Table 2 Hazard Ratios for 1-Year All-Cause Mortality

	Hazard Ratio (95% CI)	p Value
(A) Univariable		
Age (yrs)	1.08 (1.07–1.10)	<0.001
History of heart failure	2.82 (1.88–4.22)	<0.001
Previous MI	1.24 (0.94–1.64)	0.14 (NS)
Heart rate (per beat/min)	1.02 (1.01–1.02)	<0.001
SBP (per mm Hg)	0.99 (0.99–0.99)	<0.001
ST-segment depression on ECG	1.81 (1.36–2.41)	<0.001
Creatinine (per mg/dl)	1.82 (1.47–2.26)	<0.001
Inpatient PCI	0.10 (0.03–0.33)	<0.001
hs-CRP (per μg/l)	1.12 (1.09–1.14)	<0.001
TnI negative (<0.06 μg/l)	1.00	
Q1 (0.07–0.47 μg/l)	2.21 (1.32–3.69)	0.003
Q2 (0.48–2.39 μg/l)	2.08 (1.24–3.49)	0.005
Q3 (2.40–12.68 μg/l)	2.53 (1.53–4.21)	<0.001
Q4 (>12.68 μg/l)	2.74 (1.65–4.54)	<0.001
H-FABP		
Q1 (<6.38 μg/l)	1.00	
Q2 (6.38–12.39 μg/l)	4.45 (2.47–8.00)	<0.001
Q3 (12.39–36.2 μg/l)	8.78 (4.99–15.46)	<0.001
Q4 (>36.2 μg/l)	11.69 (6.67–20.49)	<0.001
(B) TnI multivariable		
Age (per yr)	1.07 (1.06–1.09)	<0.001
History of heart failure	1.55 (0.96–250)	0.073 (NS)
Previous MI	1.11 (0.79–1.55)	0.55 (NS)
Heart rate (per beat/min)	1.007 (1.001–1.013)	0.028
SBP (per mm Hg)	0.995 (0.990–1.000)	0.039
ST-segment depression on ECG	1.37 (0.98–1.93)	0.069
Creatinine (mg/dl)	1.36 (1.12–1.66)	0.002
Inpatient PCI	0.37 (0.11–1.23)	0.11 (NS)
hs-CRP (per μg/l)	1.08 (1.06–1.11)	<0.001
TnI		
Negative (<0.06 μg/l)	1.00	
Q1 (0.07–0.47 μg/l)	1.56 (0.88–2.76)	0.13 (NS)
Q2 (0.48–2.39 μg/l)	1.66 (0.94–2.95)	0.081 (NS)
Q3 (2.40–12.68 μg/l)	1.91 (1.09–3.37)	0.025
Q4 (>12.68 μg/l)	2.38 (1.35–4.19)	0.003
(C) H-FABP multivariable		
Age (per yr)	1.06 (1.05–1.08)	<0.001
History of heart failure	1.45 (0.90–2.33)	0.12 (NS)
Previous MI	1.15 (0.82–1.60)	0.43 (NS)
Heart rate (per beat/min)	1.008 (1.002–1.014)	0.013
SBP (per mm Hg)	0.995 (0.990–1.000)	0.045
ST-segment depression on ECG	1.36 (0.97–1.91)	0.076 (NS)
Creatinine (per mg/dl)	1.20 (0.99–1.44)	0.061 (NS)
Inpatient PCI	0.40 (0.12–1.33)	0.14 (NS)
hs-CRP (per μg/l)	1.08 (1.05–1.10)	<0.001
H-FABP		
Q1 (<6.38 μg/l)	1.0	
Q2 (6.38–12.39 μg/l)	2.32 (1.25–4.30)	0.007
Q3 (12.39–36.2 μg/l)	3.17 (1.73–5.82)	<0.001
Q4 (>36.2 μg/l)	4.88 (2.67–8.93)	<0.001

(A) Unadjusted for other factors; (B) TnI quartiles adjusted for the GRACE risk factors plus hs-CRP; (C) H-FABP quartiles adjusted for the GRACE risk factors plus hs-CRP with TnI as a continuous variable.

CI = confidence interval; hs-CRP = highly sensitive C-reactive protein; TnI = troponin I; other abbreviations as in Table 1.

Table 3 TnI and H-FABP Concentration Quartiles

	TnI Negative (<0.06 μg/l)	TnI Q1 (0.07–0.47 μg/l)	TnI Q2 (0.48–2.39 μg/l)	TnI Q3 (2.40–12.68 μg/l)	TnI Q4 (>12.68 μg/l)
(A) H-FABP mortality					
Q1 (<6.38 μg/l)	3.1% (4)	5.3% (6)	4.4% (4)	0% (0)	0% (0)
Q2 (6.38–12.39 μg/l)	18.2% (12)	17.5% (17)	18.7% (20)	11.4% (8)	9.5% (2)
Q3 (12.39–36.2 μg/l)	30.4% (7)	41.4% (29)	32.5% (25)	24.3% (27)	15.4% (12)
Q4 (>36.2 μg/l)	0% (0)	47.8% (11)	37.9% (11)	34.6% (35)	30.3% (61)
All quartiles	10.6% (23)	20.7% (63)	19.8% (60)	23.1% (70)	24.5% (75)
(B) H-FABP adjusted HR (95% CI)					
Q1 (<6.38 μg/l)	1.00	1.00	1.00	1.00	1.00
Q2 (6.38–12.39 μg/l)	6.50 (1.53–27.71) p = 0.011	2.09 (0.72–6.08) p = 0.18 (NS)	2.99 (0.94–9.57) p = 0.065 (NS)	—*	—*
Q3 (12.39–36.2 μg/l)	5.79 (1.08–31.12) p = 0.041	4.93 (1.73–14.03) p = 0.003	4.30 (1.31–14.17) p = 0.016	—*	—*
Q4 (>36.2 μg/l)	—*	8.26 (2.19–31.09) p = 0.002	6.03 (1.58–23.01) p = 0.009	—*	—*

(A) All-cause mortality and (B) adjusted hazard ratio modeled using the significant GRACE risk factors plus hs-CRP and TnI as an additional continuous variable. *Because there were no deaths in the group (H-FABP Q4 and TnI Negative), it was not possible to calculate the hazard ratios (HRs).

Abbreviations as in Tables 1 and 2.

p = 0.005), Q3 HR 3.53 (95% CI 1.82 to 6.82; p <0.001), Q4 HR 5.54 (95% CI 2.69 to 11.38; p <0.001); and 3) TnI as an additional continuous variable: H-FABP Q1 HR 1.0, Q2 HR 2.58 (95% CI 1.37 to 4.85; p = 0.003), Q3 HR 3.77 (95% CI 2.01 to 7.07; p < 0.001), Q4 HR 6.59 (95% CI 3.40 to 12.74; p < 0.001). Table 3 extends this analysis to assess the magnitude of risk associated with H-FABP quartiles once the cohort had been stratified by TnI quartile. In all 4 TnI quartiles H-FABP provided concentration-related additional information regarding clinical risk. Assessment of TnI quartile 4 was made more difficult by the absence of any deaths in the H-FABP reference group (Q1), although this in itself provides strong support for the complementary information carried by a low concentration of H-FABP.

Next we described the mortality and the adjusted multivariable prognostic value of the H-FABP clinical cutoff across a range of acute coronary syndrome subtypes defined by ECG in conjunction with troponin (Table 4). This demonstrated the additive value of H-FABP, particularly for ACS subtypes such as unstable angina, traditionally considered to be associated with a low long-term risk. The crude all-cause 1-year mortality for unstable angina patients with H-FABP <5.8 μg/l was 2.1% compared with 22.9% for patients above this cutoff. The adjusted all-cause mortality HR in this group was 11.35 (95% CI 2.00 to 64.34; p = 0.006). Figure 2A depicts Kaplan-Meier mortality curves for patients in each of the 4 subgroups based on the results of TnI and H-FABP measurement, i.e., TnI+/H-FABP+, TnI−/H-FABP+, TnI+/H-FABP−, and TnI−/H-FABP−. Figure 2B depicts the corresponding Cox regression curves after adjustment for other covariables. As shown, measurement of H-FABP discriminated between high- and low-risk patients with ACS, both before and after adjustment for other covariables. The occurrence of a negative test result for both TnI and H-FABP was associated with zero mortality (no deaths) before 6 months.

Table 4 All-Cause Mortality According to H-FABP Diagnostic "Cutoff" With Hazard Ratio Adjusted Using GRACE Risk Factors Plus hs-CRP With TnI as an Additional Continuous Variable

	Unstable Angina	NSTEMI	STEMI/BBBMI
H-FABP (≤5.8μg/l)	2.1% (2)	4.8% (9)	0% (0)
H-FABP (>5.8μg/l)	22.9% (19)	26.1% (189)	23.0% (77)
Adjusted HR	11.35	3.11	—*
95% CI	2.00–64.34	1.45–6.70	—*
p Value	0.006	0.004	—*

Unstable angina is defined as TnI ≤0.06 μg/l and no ST-segment elevation or new BBB on admission ECG. NSTEMI is defined as TnI >0.06 μg/l and no ST-segment elevation or new BBB on ECG. STEMI/BBBMI is defined as ST-segment elevation or new bundle branch block on admission ECG with supporting clinical and/or biomarker data). *Because there were no deaths in the reference group (H-FABP <5.8 μg/l) it was not possible to calculate the HR.

Abbreviations as in Tables 1, 2, and 3.

Discussion

The present data indicate that measurement of H-FABP 12 to 24 h after the onset of symptoms provides information that is additive to that provided by the GRACE risk factors, including as well TnI and hs-CRP as continuous variables. This reinforces the conclusions of a recent investigation regarding measurement of H-FABP in conjunction with troponin T within 6 h of the onset of chest pain (13) and a more recent report of samples taken later after a mean of 41 h (6). These studies used different diagnostic cutoffs of 9.8 and 8 μg/l, respectively, owing to the performance of the 2 assays. Our decision to use a value of 5.8 μg/l as a basis for discriminating between patients was based on an assessment of the 99th percentile for healthy caucasian Europeans. It is apparent that H-FABP is able to distinguish between low- and high-risk patients across a wide range of TnI values (Table 3) and at all parts of the ACS spectrum (Table 4).

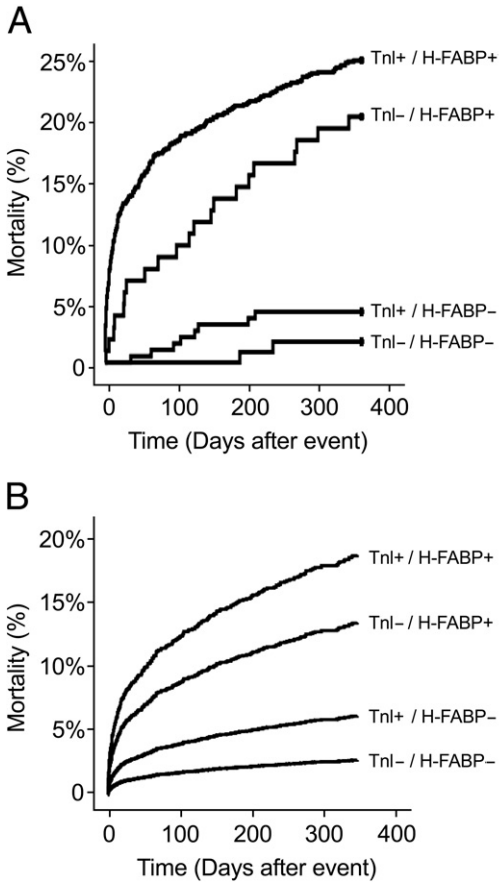

Figure 2 **All-Cause Mortality by TnI and H-FABP Clinical Cutoff Values**

(A) Kaplan-Meier mortality curves for patients with either TnI ≤ 0.06 μg/l (unstable angina) or TnI >0.06 μg/l (MI inclusive of STEMI/BBBMI) according to the H-FABP cutoff value of 5.8 μg/l. One-year univariable hazard ratios for each group relative to TnI−/H-FABP− patients are: TnI+/H-FABP− 2.38 (95% CI 0.48 to 11.65; p = 0.29), TnI−/H-FABP+ 4.93 (95% CI 1.09 to 22.30; p = 0.038), and TnI+/H-FABP+ 7.11 (1.71 to 29.64; p = 0.007). **(B)** Cox regression mortality curves for patients with either TnI ≤ 0.06 μg/l (unstable angina) or TnI >0.06 μg/l (MI inclusive of STEMI/BBBMI) according to the H-FABP cutoff value of 5.8 μg/l. One-year multivariable (GRACE risk factors plus hs-CRP) hazard ratios for each group relative to TnI−/H-FABP− patients are: TnI+/H-FABP− 2.31 (95% CI 0.49 to 10.90; p = 0.29), TnI−/H-FABP+ 5.21 (95% CI 1.21 to 22.47; p = 0.027), and TnI+/H-FABP+ 7.41 (95% CI 1.83 to 30.00; p = 0.005). BBBMI = bundle branch block myocardial infarction; MI = myocardial infarction; STEMI = ST-segment elevation myocardial infarction; TnI = troponin I; other abbreviations as in Figure 1.

Despite the use of H-FABP in earlier studies, it has not gained widespread use. This is likely owing to the relatively poor analytic sensitivity of early assays, the relatively small clinical studies, and the absence of long-term clinical outcome data. We have shown that H-FABP is useful as a marker in conjunction with TnI. Heart-type fatty acid-binding protein not only offers independent prognostic information but also, more importantly, confirms the finding of O'Donoghue et al. (6) that H-FABP identifies high-risk patients who are considered to be troponin negative. The importance of the present study is that it was done on a population-based cohort of patients diagnosed with ACS (mostly MI) in whom an important factor involved in selection was the presence of sufficient redundant serum after routine analysis. The patients were of an age range which is older than seen in clinical trials and consequently also had a higher mortality. Both of these factors enhance the statistical power of our assessment of H-FABP. Our samples were taken at 12 to 24 h after symptom onset, whereas the O'Donoghue study used subjects from the OPUS (Orbofiban in Patients with Unstable Coronary Syndromes)–TIMI-16 study who were younger than the present population, and their samples were taken even later, at a mean time of 41 h after the onset of symptoms.

For patients who present with symptoms and ECG changes of ischemic heart disease and who are diagnosed clinically as having ACS, detectable troponin is considered to be a marker of myocyte necrosis. However, the cutoff value used to distinguish between probable normality and possible abnormality has been subject to limited assay precision and consequently remains poorly defined. Some have recommended use of the value at which the assay has a CV (a measure of precision) of 10% (10), whereas others have recommended use of the 99th percentile (3 standard deviations above the mean) based on a population of normal individuals. For the TnI assay that we have used within the context of a central laboratory, the 99th percentile is reported to be 0.04 μg/l (9). Despite being one of the most precise assays available at the time, the CV at 0.04 μg/l remained high at 14%. The implications of this would be that patients may be incorrectly stratified and treated. Fortunately, more precise troponin assays have now started to emerge. Nevertheless, Table 3 indicates that across the full range of troponin concentrations, H-FABP measurement provides prognostic information that is independent of other clinical risk factors and additive to the information denoted by troponin.

In contrast with other prognostic markers, such as hs-CRP and B-type natriuretic peptide, H-FABP provides direct evidence not only of acute myocyte necrosis, but also of myocardial ischemia (14,15). Meng et al. (14) demonstrated that in rats the H-FABP concentration in peripheral blood was 4× the baseline after just 15 min induced myocardial ischemia. Also, 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. 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 because of its small molecular size, because both of these features permit early leakage through the porous membranes of ischemic myocardial cells.

Study limitations. Limitations of the present investigation include the fact that we studied a high-risk cohort of patients with confirmed ACS and that we studied a single blood sample taken at 12 to 24 h after symptom onset.

However, further studies are currently being conducted using multiple measurements in unselected patients presenting to the emergency room with undifferentiated chest pain. Other limitations in our conclusions may relate to the method of risk adjustment that we used. We opted to include factors that form part of the well validated GRACE risk model to permit the reasons for factor selection to be fully transparent. However, because this model uses troponin elevation as a categoric variable, we also assessed the effects of using troponin as a continuous variable and adjusted as well for baseline hs-CRP measurement. Another weakness, although also possibly a strength, of this study was the low rate of in-hospital percutaneous coronary intervention (<13%). This reflects provincial United Kingdom practice in 2003 and may inadvertently have permitted a clearer assessment of the natural history of H-FABP changes in the context of ACS. The fact that 87.7% of the patients studied had an elevated TnI (MI) and just 12.3% had troponin-negative unstable angina should also be noted when interpreting these data.

Conclusions

Our results indicate that raised concentrations of H-FABP are strongly predictive of mortality after ACS. Furthermore, H-FABP identifies patients who are high risk across the full range of TnI concentrations. This might be explained by a possible role for H-FABP in denoting the presence of myocardial ischemia either in the presence or the absence of myocardial necrosis denoted by detecting raised levels of TnI. The occurrence of a negative test result for both TnI and H-FABP was associated with zero mortality (no deaths) before 6 months. This appears to represent a particularly worthwhile clinical outcome, especially because it was observed in patients admitted into hospital for suspected ACS. Such an observation would give confidence to physicians assessing unselected suspected ACS patients in the emergency room, particularly if reproduced using much earlier blood samples. In this regard, we believe that measurement of H-FABP may have a great deal to offer in routine clinical care and therefore deserves further research.

Reprint requests and correspondence: Dr. Niamh Kilcullen, C-NET Group, Clinical Cardiology, G Floor Jubilee Building, General Infirmary at Leeds, Leeds, LS1 3EX Yorkshire, United Kingdom. E-mail: niamhkilcullen@doctors.org.uk.

REFERENCES

1. Glatz JF, Luiken JJ, van Nieuwenhoven FA, Van der Vusse GJ. Molecular mechanism of cellular uptake and intracellular translocation of fatty acids. Prostaglandins Leukot Essent Fatty Acids 1997;57:3–9.
2. Glatz JF, Van Bilsen M, Paulussen RJ, Veerkamp JH, Van der Vusse GJ, Reneman RS. Release of fatty acid-binding protein from isolated rat heart subjected to ischaemia and reperfusion or to the calcium paradox. Biochim Biophys Acta 1988;96:1148–52.
3. Glatz JFC, van der Voort D, Hermens WT. Fatty acid-binding protein as the earliest available plasma marker of acute myocardial injury. J Clin Ligand Assay 2002;25:167–77.
4. Okamoto F, Sohmiya K, Ohkaru Y, et al. Human heart-type cytoplasmic fatty acid-binding protein (H-FABP) for the diagnosis of acute myocardial infarction. Clinical evaluation of H-FABP in comparison to myoglobin and creatine kinase isoenzyme MB. Clin Chem Lab Med 2000;38:231–8.
5. Glatz JFC, Van der Vusse GJ, Simoons ML, Kragten JA, van Dieijen-Visser MP, Hermens WT. Fatty acid-binding protein and the early detection of acute myocardial infarction. Clin Chim Acta 1998;272:87–92.
6. O'Donoghue M, de Lemos JA, Morrow DA, Murphy SA, Buros JL, Cannon CP, Sabatine MS. Prognostic utility of heart-type fatty acid binding protein in patients with acute coronary syndromes. Circulation 2006;114:550–7.
7. Das R, Kilcullen N, Morrell C, Robinson MB, Barth JH, Hall AS. The British Cardiac Society Working Group definition of myocardial infarction: implications for practice. Heart 2006;92:21–6.
8. Joint European Society of Cardiology/American College of Cardiology Committee for Redefinition of Myocardial Infarction. Myocardial infarction redefined: a consensus document of the Joint European Society of Cardiology Committee for Redefinition of Myocardial Infarction. Eur Heart J 2000;21:1502–13.
9. Uettwiller-Geiger D, Wu AHB, Apple FS, et al. Multicentre evaluation of an automated assay for troponin I. Clin Chem 2002;48:869–76.
10. Apple FS, Wu AHB, Jaffe AS. European Society of Cardiology and American College of Cardiology guidelines for redefinition of myocardial infarction: how to use existing assays clinically and for clinical trials. Am Heart J 2002;144:981–6.
11. Venge P, Lagerqvist B, Diderholm E, Lindahl B, Wallentin L, FRISC II study. Clinical performance of three cardiac troponin assays in patients with unstable coronary artery disease (a FRISC II substudy). Am J Cardiol 2002;89:1035–41.
12. Eagle KA, Lim MJ, Dabbous OH, et al., GRACE Investigators. A validated prediction model for all forms of acute coronary syndrome: estimating the risk of 6-month postdischarge death in an international registry. JAMA 2004;291:2727–33.
13. Ishii J, Ozaki Y, Lu J, et al. Prognostic value of serum concentration of heart-type fatty acid-binding protein relative to cardiac troponin T on admission in the early ours of acute coronary syndrome. Clin Chem 2005;51:1397–404.
14. Meng X, Ming M, Wang E. Heart fatty acid binding protein as a marker for postmortem detection of early myocardial damage. Forensic Sci Int 2006;160:11–6.
15. Ming M, Meng XZ. Immunohistochemical observation of heart-type fatty acid binding protein in early myocardial ischaemia. Fa Yi Xue Za Zhi 2004;20:15–7.

APPENDIX

For the structure of the EMMACE-2 Study Group, please see the online version of this article.