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Suspected acute coronary syndrome in the emergency room: Limited added value of heart type fatty acid binding protein point of care or ELISA tests: The FAME-ER (Fatty Acid binding protein in Myocardial infarction Evaluation in the Emergency Room) study

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Abstract

Background: Timely recognition of acute coronary syndrome remains a challenge as many biomarkers, including troponin, remain negative in the first hours following the onset of chest pain. We assessed the diagnostic accuracy of heart-type fatty acid binding protein (H-FABP), a cardiac biomarker with potential value immediately post symptom onset.

Methods and results: Prospective monocentre diagnostic accuracy study of H-FABP bedside point of care (CardioDetect®) and ELISA tests in acute coronary syndrome suspected patients presenting within 24 hours of symptom onset to the emergency department, in addition to clinical findings, electrocardiography and the currently recommended biomarker high sensitivity troponin-T (hs-cTnT). The final diagnosis of acute coronary syndrome was adjudicated by two independent cardiologists, blinded to H-FABP results. Acute coronary syndrome was diagnosed in 149 (32.9%) of 453 unselected patients with suspected acute coronary syndrome (56% men, mean age 62.6 years). Negative predictive values were similar for H-FABP point of care and ELISA tests (79% *vs*. 78% respectively), but inferior to initial hs-cTnT (negative predictive value 86%). The addition of H-FABP point of care results to hs-cTnT increased the negative predictive value to 89%. In a multivariable logistic regression model, H-FABP point of care and ELISA tests yielded relevant diagnostic information in addition to clinical findings and ECG (likelihood ratio test $p<0.001$) and increased area under the receiver operating characteristics curve (AUC; 0.82 *vs*. 0.84 and 0.84). This added value attenuated, however, after inclusion of hs-cTnT in the diagnostic model (AUC 0.88).

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Conclusions: In patients suspected of acute coronary syndrome presenting to the emergency department, H-FABP testing improves diagnostic accuracy in addition to clinical findings and electrocardiography. H-FABP, however, has no additional diagnostic value when hs-cTnT measurements are also available.

Keywords
Acute coronary syndrome (ACS), biomarker, heart-type fatty acid binding protein (H-FABP), point-of care test, high sensitivity troponin

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Introduction

Early diagnosis of acute coronary syndrome (ACS) is essential in patients presenting to the emergency department (ED) with chest pain, because timely interventions improve patient prognosis. Furthermore, ruling out ACS as early as possible reduces healthcare costs (e.g. diagnostic procedures, hospital admissions) and patient burden. Biomarkers are part of routine clinical assessment in suspected ACS, together with signs and symptoms, risk factor stratification and electrocardiography (ECG).[1] The diagnostic accuracy of cardiac troponins for acute myocardial infarction (AMI) has improved considerably since the introduction of high sensitivity cardiac troponin (hs-cTn) assays.[2] However, the negative predictive value (NPV) of a single hs-cTn measurement at the time of presentation is not sufficient to safely rule out ACS, especially in those presenting early, within 3–6 h after chest-pain onset. This results in serial measurements of high-sensitivity troponin-T (hs-cTnT) being routinely performed.[3] Further identification of low risk patients and multimarker strategies, using additional early diagnostic (but often less heart-specific) biomarkers such as heart-type fatty acid binding protein (H-FABP) and copeptin, have been proposed as an alternative to optimize both rapid rule-out and rapid rule-in for ACS, including patients with unstable angina who remain troponin-negative by definition.

H-FABP, a small (15 kDa) protein which transports intracellular long-chain fatty acids, has shown to be a promising early diagnostic marker of ACS in addition to cardiac troponin I or T.[4–8] H-FABP is released into the circulation within 2 h after the onset of ischaemia and peaks at 3–6 h, returning to normal values within 24–36 h.[9] Both rapid qualitative point of care tests with potential value in preclinical settings (e.g. CardioDetect®) and quantitative ELISA assays are available to determine H-FABP.[10,11] To date, most of the evidence supporting the (added) value of H-FABP in early recognition of ACS has been derived from studies involving conventional cardiac troponins instead of the currently recommended high sensitivity troponins. Of the five studies[12–16] that did assess the diagnostic value of H-FABP in addition to hs-cTnT, improved diagnostic sensitivity for AMI or NSTEMI was observed in only one.[14] Nonetheless, all of the studies had important limitations. Firstly, patients with unstable angina were excluded from the main analyses, thereby discounting a substantial subset of patients at risk of developing AMI and sudden cardiac death. In addition, none of the studies performed a multivariable analysis (including for example, symptoms and ECG).

Therefore, the aim of this study was to determine the diagnostic value of both a H-FABP bedside point of care (POC) test and H-FABP ELISA for the (early) detection of ACS among patients who present to the ED with chest pain, both as a single marker and in conjunction with hs-cTnT along with readily available information from clinical examination and ECGs.

Methods

Setting and study population

The FAME-ER (Fatty Acid binding protein in Myocardial infarction Evaluation in the Emergency Room) study is a single centre, prospective diagnostic study of patients presenting to the ED of the Meander Medical Centre, Amersfoort, the Netherlands, within 24 h of the onset of chest pain suggestive of ACS. Patients were enrolled between May 2007 and November 2007. Clear cut ST-segment elevation myocardial infarction (STEMI) was the only exclusion criterion, as there is no diagnostic uncertainty in these cases. The majority of these patients underwent emergency percutaneous coronary intervention (PCI) in one of the affiliated hospitals (University Medical Centre Utrecht and St Antonius Hospital Nieuwegein). The Meander Medical Centre is a large regional teaching hospital, providing healthcare for a population of 300,000 patients. The study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Routine clinical assessment

All patients underwent routine clinical assessment by the attending physician (either a cardiologist or ED physician), including obtaining a medical history, performing a physical

examination, serial 12 lead ECGs and chest radiography. Directly upon presentation, four drops of capillary whole blood were drawn in order to perform the rapid H-FABP POC test and venous blood was drawn to determine cardiac troponin I, among other routine laboratory parameters. An additional 5ml tube of blood was drawn to determine H-FABP ELISA, the remainder being stored for future analyses (including hs-cTnT). The plasma component was frozen and stored at –80°C until sample analysis. Patients were diagnosed and treated according to ESC guidelines at time of presentation to the ED. Patients with positive POC test results who otherwise would have been discharged home from the ED were admitted to the hospital for further review.

Measurement of H-FABP

H-FABP was measured quantitatively by sensitive non-competitive sandwich ELISA (Department of Genetics & Cell Biology, Maastricht University, The Netherlands), using a cut-off value of 7ng/ml, identical to the POC. The assay coefficient of variation (CV) was <10%. The H-FABP POC test was determined by a rapid chromatographic lateral flow test CardioDetect® (threshold value 7ng/ml, Rennesens GmbH, Berlin) designed for qualitative detection. Once the four drops of blood were placed onto the test funnel, this was temporarily stored in a blinded box and read after 20 min by the attending nurse. Positive results showed two red–purple signal lines, 'control' and 'result'; negative results showed only one line, 'control'. Tests that only showed the 'result' line or no lines were invalid. In the case of invalid test results (n=28) a second test was performed. The cardiologist/ED physician remained blinded to the test result until the patient was discharged from the ED.

Measurement of troponin I

Troponin I (cTnI) levels were measured by sandwich chemoluminescence immunoassay (Synchron Lxi 725 integrated clinical chemistry, Beckman Coulter). The lower detection limit for cTnI was 0.01μg/l, with a 99th percentile cut-off level for positivity of ⩾0.04μg/l and the CV <10% at 0.06μg/ml.

Measurement of hs-cTnT

hs-cTnT levels were determined post hoc by the Elecsys troponin T high-sensitive assay fourth generation (Roche Diagnostics). The lower detection limit was 3pg/ml, with a 99th-percentile cut-off point of ⩾14pg/ml and the CV <10% at 13pg/ml.

Outcome

The primary outcome was ACS (i.e. STEMI, non-STEMI (NSTEMI) and unstable angina), determined according to the universal definition of myocardial infarction.[1] The diagnosis of NSTEMI was made when a rise and/or fall in cardiac troponin with at least one value above the 99th percentile was observed in a clinical setting consistent with myocardial ischaemia. Unstable angina was diagnosed on the basis of signs and symptoms, accompanied by dynamic ECG changes, evidence of ischaemia on functional testing or new coronary angiographic changes. The final diagnosis was adjudicated by an outcome panel consisting of two cardiologists (from a pool of four) and a resident in cardiology, based on all available clinical information as well as baseline hs-cTnT, (serial) cTnI measurements, (serial) ECG findings, coronary angiography, echocardiography, cardiac exercise test and hospital discharge letters. The outcome panel was blinded to the H-FABP results.

Statistical methods

We followed the Standards for Reporting of Diagnostic Accuracy (STARD) checklist.[17] Statistical analyses were performed using SPSS 20.0 along with Fix Pack 1, and R-2.15.0 using the RMS package. To evaluate the diagnostic characteristics of H-FABP ELISA (dichotomized at ⩾7ng/ml), H-FABP POC test, hs-cTnT (dichotomized at ⩾14pg/l) and their combinations, we constructed 2 × 2 tables with ACS as the outcome. The positive predictive value (PPV), NPV, sensitivity and specificity were calculated. The discriminative value of H-FABP and hs-cTnT was determined by the area under the receiver operating characteristics (ROC) curve (AUC) or c-statistic indicating the probability that two patients (one with and one without ACS) are classified correctly. Odds ratios (based on univariable logistic regression analysis) of dichotomized biomarkers and candidate predictors of ACS (e.g. age, sex, history, cardiovascular risk factors, ECG) were estimated. The latter predictors were selected based on available medical literature and clinical experience. Multiple imputation techniques were applied in the case of missing values.[18]

All predictors were included in a stepwise multivariable logistic regression analysis mimicking the chronological availability of the diagnostic elements in daily practice at the ED. The first steps in the model were to estimate the diagnostic value of 1) readily available patient characteristics and history and 2) the addition of ECG to patient characteristics and history. Subsequently the diagnostic value of the H-FABP POC and ELISA (dichotomized) test in adjunct to 3) patient characteristics, history and ECG, and 4) patient characteristics, history, ECG and initial hs-cTnT (dichotomized) were estimated. The linear predictor of the preceding model was used as an offset variable for the subsequent model, thereby forcing the regression coefficient to be exactly 1. Restricted cubic splines were used to test whether continuous variables had a linear association with the outcome. Likelihood ratio (LR) tests, (changes in) the AUCs, and continuous net reclassification improvement (NRI)[19]

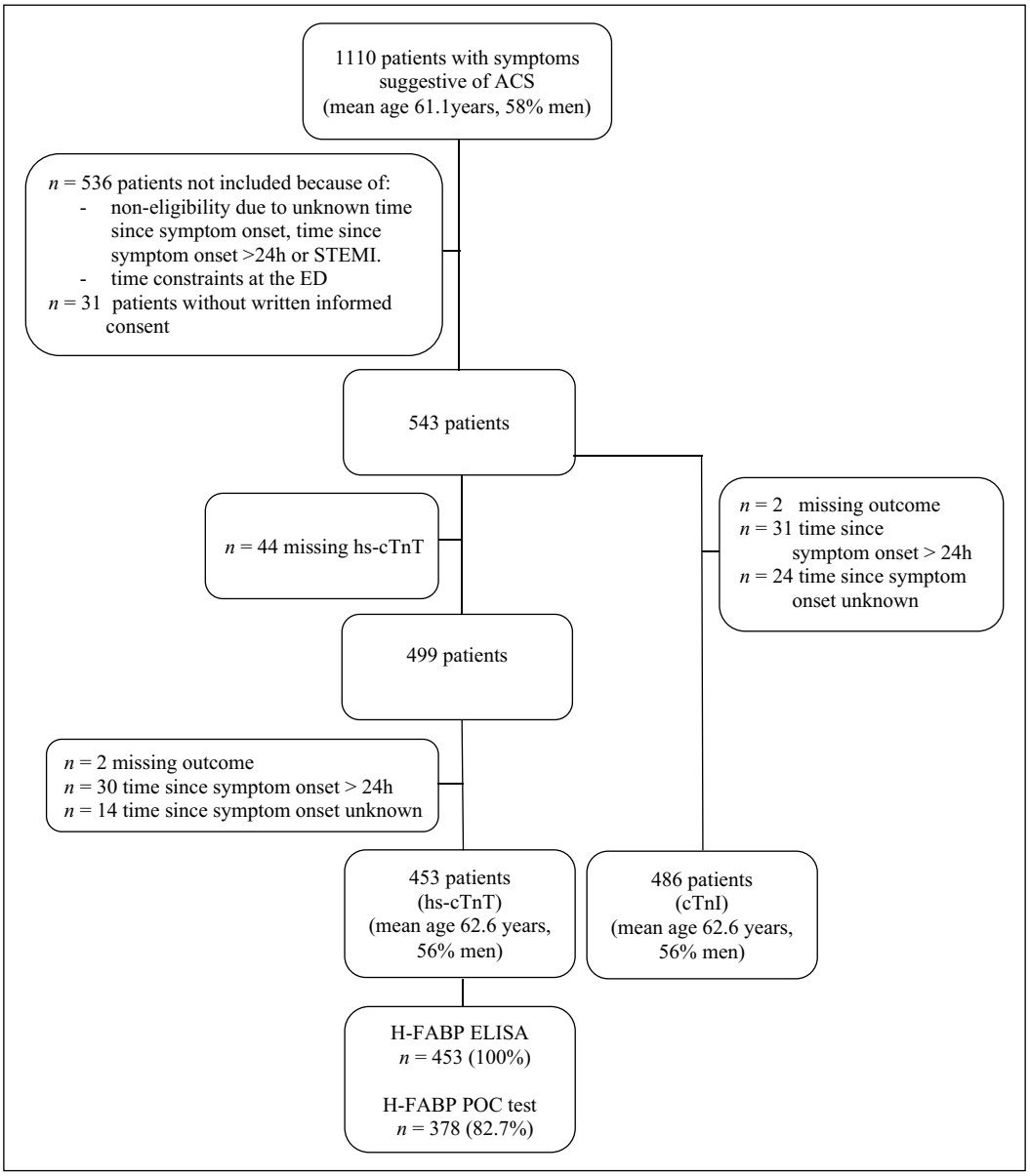

Figure 1. Flowchart of n=1110 patients presenting to the emergency department with suspected ACS in the FAME-ER study.
ACS: acute coronary syndrome; STEMI: ST-segment elevation myocardial infarction; ED: emergency department; hs-cTnT: high sensitivity troponin-T; cTnI: troponin I; H-FABP: heart-type fatty acid binding protein; POC: point of care

were used to quantify the added value of diagnostic items. Bootstrapping techniques were used as a validation method to adjust the estimated associations between tests and the outcome ACS for over-optimism.[20] Pre-specified subgroup analyses were performed to assess whether the diagnostic accuracy differed according to time since symptom onset (<3 h, <6 h).

Results

Patient characteristics

A total of 543 patients with suspected ACS were evaluated (Figure 1), of which two patients were excluded due to missing outcomes and 44 patients were excluded due to missing baseline high sensitivity troponin values. Of the remaining 497 patients, 44 were excluded as the time from symptom onset to presentation was unknown (*n*=14) or time since symptom onset > 24 h (*n*=30). In total, 453 patients were included in the analysis. In 87 patients no H-FABP POC test results were available (either because there was no time to perform the test or because the control line of the POC test did not turn red). In another 28 patients the H-FABP POC test did show an evident red control line, but the result line was uninterpretable.

Baseline characteristics of the 453 patients are shown in Table 1. As adjudicated by the panel, the final diagnosis of ACS applied to 149 (32.9%) of the patients, 13 of whom

Table 1. Baseline characteristics.

Characteristics	*N*	All patients	Patients within 6 h of symptom onset
Age, years	453	62.6 ± 14.5	62.0 ± 15.0
Male	453	253 (56%)	161 (54%)
Duration of symptoms, median (IQR)	430	3.0 (1.8–6.8)	2.5 (1.4–3.3)
Hypertension	447	193 (43%)	121 (41%)
Hypercholesterolaemia	447	148 (33%)	103 (35%)
Diabetes mellitus	447	72 (16%)	46 (16%)
Current smoker	444	114 (25%)	78 (26%)
Former smoker	444	111 (25%)	78 (26%)
Family history of CVD	442	181 (41%)	117 (40%)
BMI, mean kg/m²	320	27.0 ± 4.7	26.6 ± 4.5
Previous MI	446	96 (22%)	64 (22%)
Previous PCI	447	97 (22%)	69 (23%)
Previous CABG	446	45 (10%)	31 (11%)
Any MI, PCI or CABG	450	150 (33%)	102 (34%)
Previous CVA	447	7 (2%)	4 (1%)
Previous TIA	447	22 (5%)	11 (4%)
Heart failure	448	24 (5%)	17 (6%)
Peripheral arterial disease	447	25 (6%)	17 (6%)
Current aspirin use	440	187 (43%)	125 (43%)
Current clopidogrel use	436	50 (12%)	35 (12%)
Current coumarin use	436	47 (11%)	29 (10%)
Current ß-blocker use	437	171 (39%)	114 (39%)
Current statin use	439	176 (40%)	124 (43%)

Values are given as mean (±SD) proportion (%).
BMI: body mass index; CABG: coronary artery bypass grafting; CVA: cerebrovascular accident; CVD: cardiovascular disease; IQR: interquartile range; MI: myocardial infarction; PCI: percutaneous coronary intervention; TIA: transient ischaemic attack

(2.9%) were diagnosed as STEMI, 104 (23.0%) as NSTEMI and 32 (7.1%) as unstable angina. The non-ACS group consisted of 304 individuals with a final diagnosis of stable angina (*n*=45), rhythm disorders (*n*=12), heart failure (*n*=4), pericarditis (*n*=1), or non-cardiac conditions (*n*=245; mostly atypical chest pain, gastro-oesophageal reflux disease, cholecystolithiasis/cholecystitis, and myalgic chest pain). Median time from symptom onset to presentation to the ED was 3.0 h (interquartile range (IQR) 1.8–6.8), with no difference between ACS and non-ACS patients. Time from symptom onset to presentation was less than 3 h in 197 (43%) patients, and less than 6 h in 301 (70%) patients. In total, 246 (54%) patients were admitted to the hospital for further evaluation. Twenty-seven patients had a negative baseline cTnI, but a positive baseline hs-cTnT. These patients were diagnosed with the following: STEMI (*n*=1), NSTEMI (*n*=16), atrial fibrillation (*n*=3), pericarditis (*n*=1), non-cardiac conditions (*n*=6).

Univariable analysis

Strong predictors for the presence of ACS in suspected patients were ECG changes suggestive of acute STEMI or an ECG with ST-depression/(inverse)T-waves suggestive of ischaemia and all dichotomized individual cardiac biomarkers (Table 2). Furthermore, age, sex, history of hypertension or hypercholesterolaemia, heart failure, prior myocardial infarction (MI) or prior coronary event (MI, PCI or coronary artery bypass grafting (CABG)) were predictive for the diagnosis of ACS.

Single biomarkers: univariable analysis

Both H-FABP POC and ELISA tests yielded relevant diagnostic information on the presence of ACS (Table 3). The diagnostic performance of the qualitative H-FABP POC test was comparable to the dichotomized ELISA (⩾7ng/ml). The optimal ROC-derived cut-off value for H-FABP ELISA, as determined by the Youden Index, was 7.06ng/ml. H-FABP performed best in patients presenting 3–6 h after symptom onset. Amongst this subset of patients, the NPV of the H-FABP POC test alone was 90% (when unclear test results were considered positive), with a PPV of 49%. Diagnostic accuracy of the H-FABP tests reduced when time from symptom onset was greater than 6 h. The 59 patients with false positive H-FABP ELISA results were assigned the following panel diagnoses: eight (14%) stable angina, eight (14%) rhythm disorders, three (5%) heart failure, two (3%) cholecystolithiasis, two (3%) gastro-oesophageal reflux disease and 36 (62%) non-cardiac myalgic or

Table 2. Univariable analysis.

	Predictor	ACS	Non-ACS	Odds	95% CI
		n=149	*n*=304	ratio	
Risk factors	Age, years	69.0 ± 13.2	59.5 ± 14.1	1.05	1.04–1.07
	Male	95 (64%)	158 (52%)	1.6	1.1–2.4
	Hypertension	85 (57%)	112 (37%)	2.3	1.5–3.4
	Hypercholesterolaemia	62 (42%)	88 (29%)	1.7	1.2–2.6
	Diabetes mellitus	28 (19%)	44 (15%)	1.4	0.8–2.3
	Current smoker	37 (25%)	77 (25%)	1.0	0.6–1.5
	Former smoker	38 (26%)	80 (26%)	0.9	0.6–1.5
	Family history of CVD	67 (45%)	122 (40%)	1.2	0.8–1.7
History	Previous MI	43 (30%)	53 (18%)	2.0	1.2–3.1
	Previous PCI	35 (24%)	62 (21%)	1.2	0.8–2.0
	Previous CABG	20 (14%)	25 (8%)	1.8	0.9–3.3
	Any MI, PCI or CABG	64 (43%)	88 (29%)	1.8	1.2–2.8
	Heart failure	19 (12%)	12 (4%)	3.8	1.6–8.8
	Previous CVA	3 (2.1%)	4 (1.3%)	1.6	0.3–7.1
	Previous TIA	8 (6%)	14 (5%)	1.2	0.5–2.9
	Peripheral arterial disease	12 (8%)	14 (5%)	2.0	0.9–4.5
Clinical findings	Systolic blood pressure	147 ± 26	143 ± 23	1.007	0.999–1.016
	Diastolic blood pressure	73 ± 13	72 ± 12	1.005	0.990–1.021
	BMI	27.3 ± 4.4	26.8 ± 4.9	1.02	0.97–1.07
ECG	Acute MI on ECG	13 (9%)	1 (<1%)	29.0	3.8–224
	Ischaemic ECG changes	103 (69%)	60 (20%)	9.1	5.8–14.3
Biomarkers	H-FABP-POC	86 (58%)	61 (20%)	5.4	3.5–8.4
	H-FABP ELISA	7.8 (4.2–22.8)	4.0 (2.6–6.1)	1.05	1.03–1.08
	Dichotomized at 7ng/ml	81 (54%)	58 (19%)	5.1	3.3–7.8
	hs-cTnT	25 (12–81)	3 (1–7)	1.09	1.07–1.12
	Dichotomized at 14pg/ml	106 (71%)	30 (10%)	22.5	13.4–37.7

Values are given as mean (±SD) proportion (%).

atypical chest pain. Of 69 false negative H-FABP ELISA tests, 35 (51.5%) patients had symptom onset within 3 h prior to presentation. However, the diagnostic accuracy of hs-cTnT as single marker for ACS was superior to both H-FABP tests, regardless of time from symptom onset (Table 3).

A multimarker strategy, combining hs-cTnT with H-FABP results into one test (considered positive when either one of two was positive), increased sensitivity and NPV in both early presenters (<3h and <6h) and the total cohort as compared with values for hs-cTnT as single biomarker (Table 4). Of the 25 patients (symptoms <3 h) with ACS not identified by hs-cTnT on admission, eight were identified by additional H-FABP POC testing.

Multivariable analysis

The use of readily available clinical information (clinical model) – age, sex, history of hypertension, hypercholesterolaemia, diabetes mellitus, current and former smoking, family history of cardiovascular disease, history of MI, PCI or CABG – resulted in an AUC of 0.72 (95% confidence interval (CI) 0.69–0.79) (Table 5). The addition of suspect ECG changes (e.g. suggestive of acute STEMI or STT-deviations suggestive of cardiac ischaemia) to this model resulted in a significant increase of the AUC (to 0.82 (95% CI 0.79–0.87), LR-test $p<0.01$). The addition of the rapidly available H-FABP POC test results to the clinical model and ECG did further improve the AUC. In the final model containing all predictors including initial ECG and hs-cTnT, the addition of H-FABP POC or H-FABP ELISA assay did not increase diagnostic accuracy (AUC 0.88 *vs.* 0.88, and LR test p-value=1.00, half NRI (>0) 0.38 and 0.35). The multivariate ROC curves of the models are shown in Figure 2. Essentially comparable results were obtained in the subset of patients with symptom onset within 6 h of presentation.

Discussion

Our prospective single centre study of 453 patients presenting to the ED with acute chest pain confirms the previously reported diagnostic accuracy of H-FABP CardioDetect® POC and ELISA tests in the early recognition of ACS. Reassuringly, the POC test performed as well as the quantitative ELISA at a cut-off of 7ng/ml. Lowering the cut-off

Table 3. Diagnostic performance of individual cardiac biomarkers at presentation for ACS.

Assay	Sensitivity	Specificity	NPV	PPV	AUC
H-FABP POC					
All patients	58 (50–67)	80 (75–84)	79 (75–84)	59 (50–66)	
<3 h	52 (40–64)	82 (75–88)	77 (70–83)	60 (48–72)	
3–6 h	72 (53–87)	76 (66–85)	90 (81–95)	49 (33–64)	
>6 h	61 (47–74)	78 (69–86)	78 (69–86)	61 (47–74)	
H-FABP ELISA					
All patients	54 (49–66)	81 (76–85)	78 (74–83)	58 (49–66)	0.73 (0.67–0.78)
<3 h	47 (35–59)	85 (78–90)	76 (69–83)	61 (47–73)	0.73 (0.66–0.81)
3–6 h	68 (49–84)	79 (69–87)	89 (80–95)	50 (34–67)	0.78 (0.67–0.89)
>6 h	59 (44–72)	77 (67–85)	77 (67–85)	59 (44–72)	0.73 (0.63–0.82)
hs-cTnT					
All patients	71 (64–78)	90 (86–93)	86 (82–90)	78 (71–84)	0.88 (0.84–0.91)
<3 h	63 (51–74)	92 (87–96)	83 (76–88)	81 (69–90)	0.86 (0.81–0.92)
3–6 h	64 (45–81)	89 (80–94)	89 (80–94)	64 (45–81)	0.86 (0.77–0.95)
>6 h	87 (75–95)	88 (80–94)	92 (85–97)	80 (68–89)	0.91 (0.85–0.96)

Values are given as % (95% CI).
ACS: acute coronary syndrome; AUC: area under the receiver operating characteristics curve; H-FABP: heart-type fatty acid binding protein; hs-cTnT: high sensitivity troponin-T; NPV: negative predictive value; POC: point of care; PPV: positive predictive value

Table 4. Diagnostic performance of the combination of cardiac biomarkers at presentation.

Assay	Sensitivity	Specificity	NPV	PPV
hs-cTnT + H-FABP POC				
All patients	80 (73–86)	76 (71–80)	89 (84–92)	62 (55–68)
<3 h	75 (63–84)	79 (72–86)	86 (79–91)	65 (54–75)
<6 h	74 (64–82)	76 (70–82)	87 (82–91)	58 (49–66)
⩾6 h	91 (81–99)	72 (62–81)	94 (86–98)	65 (53–76)
hs-cTnT + H-FABP ELISA				
All patients	77 (70–83)	77 (72–81)	87 (83–91)	62 (55–69)
<3 h	69 (57–79)	83 (76–89)	84 (77–89)	68 (56–78)
<6 h	71 (61–79)	79 (73–84)	86 (81–90)	59 (50–68)
⩾6 h	87 (75–95)	72 (62–81)	91 (82–96)	64 (51–75)

Values are given as % (95% confidence interval).
H-FABP: heart-type fatty acid binding protein; hs-cTnT: high sensitivity troponin-T; NPV: negative predictive value; POC: point of care; PPV: positive predictive value;

point of H-FABP ELISA towards 5ng/ml, as used by McCann[7] and McMahon,[8] slightly increased the NPV (78.3% *vs*. 80.0%) but did not improve overall diagnostic accuracy due to the associated decrease in the PPV (58.3% *vs*. 45.6%). As a single biomarker, hs-cTnT outperformed both H-FABP assays in the diagnostic accuracy for ACS. A H-FABP POC test might however be of help to rule out ACS when hs-cTnT tests are not available, for example in hospitals with limited access to laboratory techniques or in out of hospital settings to help general practitioners, ambulance personnel and others in deciding whether or not to refer a patient to the hospital. This should, however, be confirmed in studies in these settings (e.g. the RAPIDA-study (ClinicalTrials.gov Identifier: NTC01826994) and preferably with a more robust H-FABP POC test.

A dual biomarker strategy of adding the H-FABP POC results to hs-cTnT measurements at presentation slightly increased the NPV and sensitivity for diagnosis of ACS in a univariable analysis, although it was associated with a decrease in the PPV and specificity. In our multivariable analysis, the addition of the H-FABP POC or ELISA test to routine clinical decision making at the ED (represented by the clinical model with ECG and hs-cTnT measurement (AUC 0.88)) did not improve diagnostic accuracy (AUC 0.88 and 0.88 respectively, LR-test *p*-value=1.00, half NRI (>0) 0.38 and 0.35). Similar results were observed for patients presenting within 6 h of symptom onset. These findings are in line with the observations of Collinson et al.,[14] who reported a dual biomarker strategy with H-FABP to improve diagnostic sensitivity for the diagnosis of

Table 5. Stepwise multivariable analysis: models with and without (dichotomized) H-FABP.

Model	AUC	95% CI	LR test *p*-value	Half NRI (>0)
All patients (*n*=453)				
Clinical model	0.718	0.671–0.765		
Clinical model with ECG	0.824	0.783–0.865	<0.001	0.494
Clinical model with ECG and H-FABP POC	0.835	0.796–0.874	<0.001[a]	0.377[a]
Clinical model with ECG and H-FABP ELISA	0.836	0.797–0.875	<0.001[a]	0.353[a]
Clinical model with ECG and hs-cTnT	0.878	0.845–0.911	<0.001[a]	0.613[a]
Clinical model with ECG, hs-cTnT and H-FABP POC	0.877	0.843–0.910	0.461[b]	0.377[b]
Clinical model with ECG, hs-cTnT and H-FABP ELISA	0.876	0.843–0.910	0.554[b]	0.353[b]
Symptom onset <6 h (*n*=301)				
Clinical model	0.707	0.646–0.768		
Clinical model with ECG	0.807	0.754–0.860	<0.001	0.453
Clinical model with ECG and H-FABP POC	0.820	0.769–0.871	0.002[a]	0.376[a]
Clinical model with ECG and H-FABP ELISA	0.823	0.772–0.874	<0.001[a]	0.360[a]
Clinical model with ECG and hs-cTnT	0.852	0.805–0.899	<0.001[a]	0.540[a]
Clinical model with ECG, hs-cTnT and H-FABP POC	0.846	0.799–0.893	0.292[b]	0.376[b]
Clinical model with ECG, hs-cTnT and H-FABP ELISA	0.848	0.801–0.895	0.217[b]	0.360[b]

Adjusted for over-optimism.
[a]As compared with clinical model with ECG.
[b]As compared with clinical model with ECG and hs-cTnT.
Clinical model: age, sex, hypertension, hypercholesterolaemia, family history of CVD, current and former smoking, diabetes mellitus, and history of myocardial infarction, PCI or CABG.
ACS: acute coronary syndrome; AUC: area under the receiver operating characteristics curve; CABG: coronary artery bypass grafting; CVD: cardiovascular disease; ECG: electrocardiography; H-FABP: heart-type fatty acid binding protein; hs-cTnT: high sensitivity troponin-T; LR: likelihood ratio; NRI: net reclassification improvement; PCI: percutaneous coronary intervention; POC: point of care

NSTEMI. In contrast, Reiter et al.[12] reported the combined testing of H-FABP and hs-cTnT to decrease the diagnostic accuracy for NSTEMI compared with hs-cTnT testing alone, as quantified by the AUC (0.88 (95% CI 0.86–0.91) *vs*. 0.94 (95% CI 0.92–0.95). A dual biomarker strategy with copeptin instead of H-FABP as early biomarker in addition to hs-cTnT has also been proposed. A recent meta-analysis reported the addition of copeptin to hs-cTnT to increase the overall sensitivity for the diagnosis of AMI (thus excluding unstable angina from the primary endpoint) from 0.91 to 0.98 (difference 0.07, 95% CI 0.03–0.11, p=0.002) at the cost of a 25% decrease in overall specificity (to 0.50 (95% CI 0.42–0.58)).[21] Unfortunately, the diagnostic value of copeptin for unstable angina seems to be poor.[22]

Since both H-FABP and copeptin are known to be less heart-specific than troponins, a possible positive effect of a dual biomarker strategy on (early) diagnostic NPV and sensitivity will always be counterbalanced by a decrease in PPV and specificity, and thus may not be recognized as an improvement when measured in terms of the AUC or NRI. However, in the evaluation of patients suspected of having an ACS, a high NPV is crucial, because of the detrimental consequences of a missed diagnosis (including possible sudden cardiac death). In our study, 10 (9.3%) of 107 early presenters (<6 h) with both negative initial hs-cTnT and negative H-FABP ELISA in combination with an ECG lacking evidence of ST-elevation or ST-depression had a discharge diagnosis of ACS (n=3 NSTEMI, n=7 unstable angina). In day to day practice a NPV of around 90% is too low to exclude ACS without a further diagnostic assessment.

Diagnostic strategies to overcome the current sensitivity deficit of single blood drawing at admission require either improvement of existing (early) biomarkers (e.g. a more robust H-FABP assay, or a low hs-cTnT threshold rule-out strategy[23]) or the addition of a clinical decision rule to admission (dual) biomarker testing. Early discharge of low- to intermediate-risk patients with suspected ACS has recently been reported to be safe, using a strategy incorporating dual biomarker testing on admission (using either H-FABP[24] or copeptin[25,26] along with hs-cTnT) together with clinical risk-stratification. As obviating repeated hs-cTnT testing is likely to shorten length of stay at the ED and to reduce (over)admission of chest pain patients to the hospital for further evaluation, implementation of an 'early rule out' strategy should result in substantial reduction of healthcare costs, as costs involved with these patients at the ED are primarily due to admission time.[27] Clearly, the efficacy, safety and potential economical benefits of such a strategy need to be validated prospectively before clinical implementation.

Strengths of our study include the relatively large number of patients included and the short median time since symptom onset, the exclusion of clear cut ST elevation ACS (in

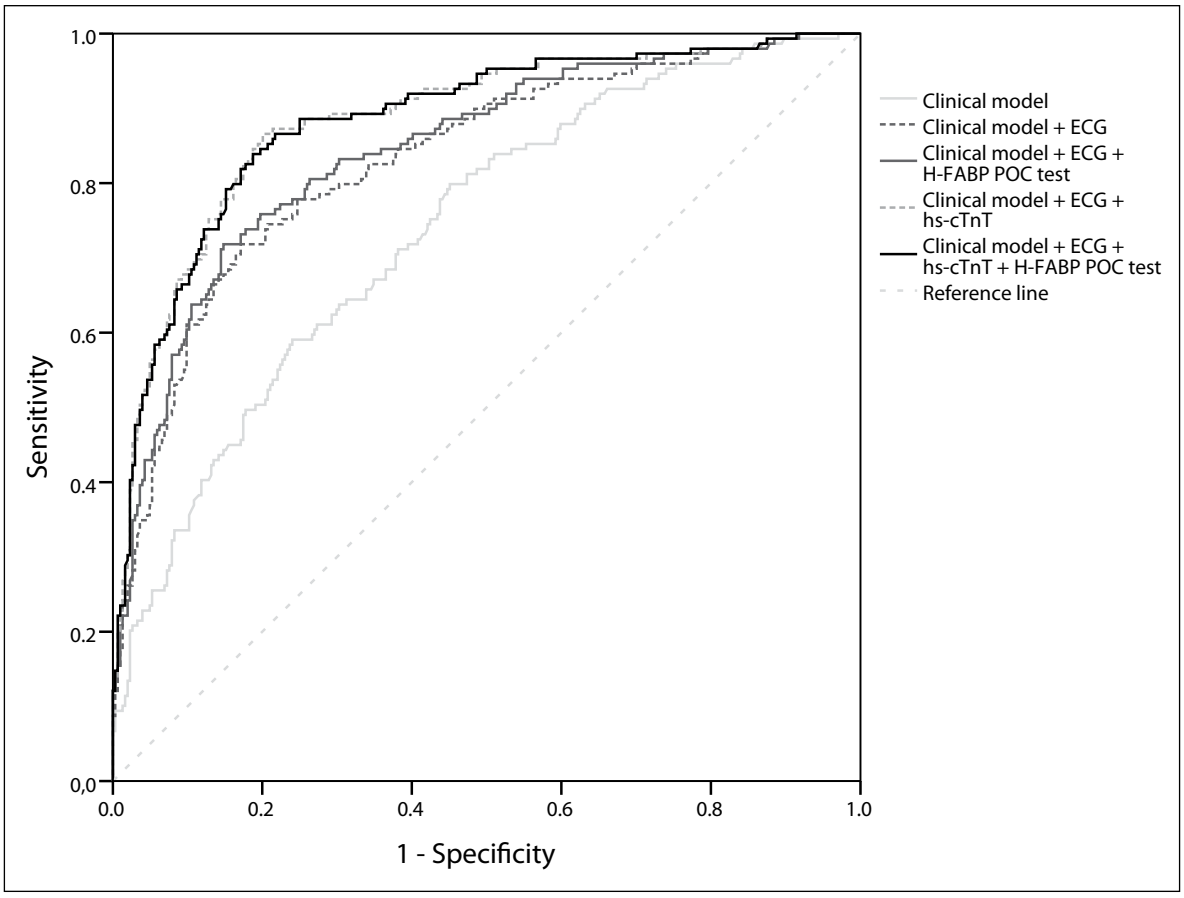

Figure 2. Receiver operating characteristics curve multivariable logistic regression analysis with clinical model, ECG and biomarkers.
hs-cTnT: high sensitivity troponin-T; ECG: electrocardiography; H-FABP: heart-type fatty acid binding protein; POC: point of care

whom there is no diagnostic uncertainty), the inclusion of patients with unstable angina pectoris, the use of both an H-FABP POC test and ELISA techniques and the comparison with hs-cTnT. To our knowledge this is the first study to assess the diagnostic value of rapid H-FABP POC testing and H-FABP ELISA in *addition* to readily available information from clinical evaluation (history, physical examination and ECG) of patients with chest pain presenting to the ED, a study population with large diagnostic uncertainty, with the use of a logistic regression model based on daily practice. Although diagnostic accuracy of biomarkers is overall higher for AMI as compared with ACS (comprising both AMI and unstable angina), and therefore has more appealing results in terms of diagnostic accuracy for biomarkers, we decided to broaden our study to diagnostic accuracy of biomarkers for the complete ACS scope, since the diagnosis of unstable angina (by definition troponin negative) carries considerable short term risk on events.

Several limitations of our study need to be mentioned. First, the POC test did not show an appropriate control line in 47 (10%) patients. In addition, 28 test results were uninterpretable (the control line turned red, but the result line was uninterpretable). To err on the side of caution we considered unclear POC test results to be positive, as safely ruling out ACS is of paramount importance in daily clinical practice (i.e. negative test results should be unquestionably negative). This might have caused an underestimation of PPVs and an overestimation of NPVs, as compared with an improved POC test lacking unclear test results. Secondly, we excluded patients (n = 44) without baseline hs-cTnT, thus lacking a reliable panel diagnosis. A sensitivity analysis, however, including those patients lacking hs-cTnT (total cohort n=486) with panel diagnosis adjustment based on imputed hs-cTnT showed no differences in diagnostic accuracy of the biomarkers.

We conclude that although H-FABP testing for ACS in patients presenting with chest pain to the ED improves diagnostic accuracy in addition to clinical findings and ECG, in our study H-FABP has no additional diagnostic value when hs-cTnT testing is available. Nevertheless, H-FABP might play a role as an early biomarker in an early rule-out strategy for low- to medium-risk patients alongside hs-cTnT together with risk-stratification based on clinical information.

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Conflict of interest

JFCG became chief scientific officer of FABPulous b.v. (rapid in vitro diagnostics, Maastricht) but only after this study had been completed. The other authors report no relationships that could be construed as a conflict of interest.

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