Heart-type fatty acid binding protein and high-sensitivity troponin T are myocardial damage markers that could predict adverse clinical outcomes in patients with peripheral artery disease

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ABSTRACT

Background: Despite many recent advances in endovascular therapy (EVT), peripheral artery disease (PAD) is an increasing health problem with high mortality. Heart-type fatty acid-binding protein (H-FABP) and high-sensitivity troponin T (hsTnT) are markers of ongoing myocardial damage and have been reported to be useful indicators of future cardiovascular events. However, it remains to be determined whether H-FABP and hsTnT can predict adverse clinical outcomes in patients with PAD.
Methods and results: We enrolled 208 de novo PAD patients who underwent EVT. Serum H-FABP and hsTnT were measured in all patients before EVT. During the median follow-up period of 694 days, there were 40 major adverse cardiovascular and cerebrovascular events (MACCEs) including all-cause deaths, and re-hospitalizations due to cardiovascular and cerebrovascular diseases and amputations. H-FABP and hsTnT were found to be higher in patients with critical limb ischemia (CLI) compared to those without this condition. Multivariate Cox proportional hazard regression analysis revealed that both H-FABP and hsTnT were independent predictors of MACCEs after adjustment for confounding factors. Kaplan–Meier analysis demonstrated that patients in the highest tertile according to H-FABP levels, as well as those in the highest hsTnT tertile, were at greatest risk for MACCEs. The net reclassification index was significantly improved by the addition of H-FABP as well as the addition of hsTnT to traditional risk factors.
Conclusion: The myocardial damage markers H-FABP and hsTnT were increased in PAD patients with CLI and could predict MACCEs in PAD patients.

1. Introduction

Peripheral artery disease (PAD) is an athero-occlusive disease of the lower limb arteries resulting in impaired mortality. The presence of PAD is an independent predictor of cardiovascular events [1]. Despite medical advances, patients with PAD have three times higher risk of all-cause mortality in general and of cardiovascular mortality in particular compared to those without PAD [2]. Although reduced lower extremity performance was reported to be a prognostic marker for mortality, little is known about useful biomarkers for identifying PAD patients at high risk.

Cardiac biomarkers have been reported to be useful in predicting an increased risk of death, not only in patients with heart disease, but also for subjects in the general population [3–5]. Heart-type fatty acid-binding protein (H-FABP) is a low molecular weight protein in the cytosol of cardiomyocytes, and is rapidly released into the circulation from

damaged myocardial tissue [6]. Therefore, H-FABP is a marker for myocardial damage. Cardiac troponin T, which is a myofibrillar component of cardiomyocytes, is an established marker for myocardial damage [7]. Myocardial damage markers can be used to risk stratify patients with various types of heart disease [8–11]. However, the prognostic value of these myocardial damage markers has not yet been elucidated in patients with PAD.

The purpose of the present study was to determine whether myocardial damage, assessed by measuring the concentrations of H-FABP and high-sensitivity troponin T (hsTnT) in the bloodstream, can predict major adverse cardiovascular and cerebrovascular events (MACCEs) and risk stratify patients with PAD.

2. Methods

2.1. Study population

We carried out a prospective study of 208 de novo patients who were admitted to our hospital for the treatment of PAD. A diagnosis

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was made by two physicians according to the ankle brachial index (ABI) and computed tomographic angiography. Endovascular therapy (EVT) was performed by experienced cardiologists who followed the Trans-Atlantic Inter-society Consensus II (TASC II) guideline recommendation. The physicians were blinded to the results of the biochemical analyses, and medical therapy was independently administered to optimize improvement of symptoms. The exclusion criteria of the present study were acute coronary syndrome within the 3 months preceding admission, estimated glomerular filtration rate less than 30 mL/min/1.72 m², and malignant disease. Blood samples were obtained in the early morning 1 or 2 days before the first EVT. Demographic and clinical data including age, gender, ABI, and Fontaine class were collected from interviews with the patients, as well as from their medical records. Medications and ABI at discharge were recorded from the hospital medical records.

2.2. Ethics statement

The Institutional Ethics Committee of the Yamagata University School of Medicine approved the study, and all participants provided written informed consent. The procedures were performed in accordance with the Helsinki Declaration.

2.3. Measurements

Hypertension was defined as systolic blood pressure (BP) ≥140 mm Hg or diastolic BP ≥90 mm Hg or antihypertensive medication use. Diabetes mellitus was defined as glycosylated hemoglobin A1c ≥6.5% (National Glyco-hemoglobin Standardization Program), or anti-diabetic medication use. Hyperlipidemia was defined as total cholesterol ≥220 mg/dL or triglyceride ≥150 mg/dL or anti-hyperlipidemic drug use. Chronic kidney disease (CKD) was defined as a reduced glomerular filtration rate (<60 mL/min/1.73 m²) according to the Kidney Disease Outcomes Quality Initiative clinical guideline [12,13].

2.4. Biochemical markers

Blood samples for measurements of serum H-FABP concentrations were drawn and centrifuged at 2500 g for 15 min at 4 °C within 30 min of collection, and the obtained serum was stored at −70 °C until analysis. H-FABP levels were measured using a two-step sandwich enzyme-linked immunosorbent assay (ELISA) kit (MARKIT-M H-FABP, Dainippon Pharmaceutical Co. Ltd., Tokyo, Japan), as previously described [14]. Concentrations of high-sensitivity troponin T were measured by using a fourth-generation electrochemiluminescence immunoassay on an Elecsys 2010 automatic analyzer (Elecsystroponin-T, Roche Diagnostics, Tokyo, Japan) [15]. Blood samples were also obtained for measuring the concentrations of brain natriuretic peptide (BNP). These samples were transferred to chilled tubes containing 4.5 mg ethylenediaminetetraacetic acid disodium salt and aprotinin (500 U/mL), and centrifuged at 1000 g for 15 min at 4 °C. The clarified plasma samples were frozen, stored at −70 °C, and thawed just before the assay was performed. BNP concentrations were measured using a commercially available radioimmunoassay specific for human BNP (Shiono RIA BNP Assay Kit, Shionogi Co. Ltd., Tokyo, Japan) [16,17].

2.5. Endpoint and follow-up

All subjects were prospectively followed for a median period of 694 days (interquartile range, 349–1070 days). Patients were followed up by telephone or medical records twice a year for 1500 days. The endpoint was MACCEs including all-cause death and rehospitalization due to cardiovascular and cerebrovascular diseases such as stroke, ischemic heart disease, heart failure, abdominal

aortic aneurysm, and the development of critical limb ischemia (CLI) and amputation.

2.6. Statistical analysis

Normality of continuous variables was checked by a Shapiro–Wilk test. Since the concentrations of H-FABP, hsTnT, and BNP were not normally distributed, we used $\log_e$ [H-FABP], $\log_{10}$ [hsTnT], and $\log_{10}$ [BNP] for all analyses. All values are expressed as the mean ± standard deviation. Continuous and categorical variables were compared with t-tests and chi-square tests, respectively. A Cox proportional hazard analysis was performed to determine independent predictors for MACCEs and significant predictors selected in the univariate analysis were entered into a multivariate analysis. Survival curves were constructed with the Kaplan–Meier method and compared using log-rank tests. The receiver operating characteristics (ROC) curves for MACCEs were constructed and used as a measure of the predictive accuracy of H-FABP and hsTnT on MACCEs. The area under the ROC curve was calculated by using the trapezoidal rule [18]. In addition, we calculated the net reclassification index (NRI) and the integrated discrimination index (IDI) to measure the quality of improvement for the correct reclassification according to the addition of H-FABP or hsTnT to the model. A value of P < 0.05 was considered statistically significant. All statistical analyses were performed with a standard program package (JMP version 8; SAS

Table 1
Comparison of clinical characteristics between patients with and without MACCEs.

Variables	All patients n = 208	Event free n = 168	MACCE n = 40	P value
Age (years old)	74 ± 8	73 ± 8	77 ± 6	0.0022
Men/women	175/33	140/28	35/5	0.5168
Hypertension, n (%)	172 (83%)	140 (84%)	32 (80%)	0.6165
Diabetes mellitus, n (%)	98 (47%)	77 (46%)	21 (53%)	0.5350
Hyperlipidemia, n (%)	134 (64%)	111 (66%)	23 (58%)	0.3088
Previous IHD, n (%)	65 (31%)	42 (25%)	23 (58%)	<0.0001
Previous cerebrovascular disease	38 (18%)	26 (15%)	12 (30%)	0.0326
Fontaine II/III/IV	157/27/24	137/20/11	20/7/13	<0.0001
CLI, n (%)	24 (10%)	11 (7%)	13 (28%)	0.0004
CKD, n (%)	81 (39%)	60 (35%)	21 (53%)	0.0384
Endovascular therapy data				
Iliac artery, n (%)	142 (68%)	120 (71%)	22 (55%)	0.0448
Femoropopliteal artery, n (%)	123 (59%)	96 (57%)	27 (68%)	0.2311
Tibial or peroneal artery, n (%)	29 (14%)	18 (11%)	11 (28%)	0.0059
Stent, n (%)	175 (90%)	140 (90%)	35 (88%)	0.5999
Pre ABI	0.58 ± 0.18	0.58 ± 0.18	0.58 ± 0.16	0.9609
Post ABI	0.89 ± 0.19	0.90 ± 0.18	0.84 ± 0.25	0.1811
Blood examination				
Log$_{10}$ BNP (pg/mL)	1.62 ± 0.51	1.52 ± 0.46	1.99 ± 0.51	<0.0001
Log$_e$ H-FABP (ng/mL)	1.50 ± 0.57	1.4 ± 0.49	1.87 ± 0.68	<0.0001
Log$_{10}$ hsTnT (pg/mL)	1.07 ± 0.37	1.00 ± 0.33	1.25 ± 0.46	0.0002
Crea (mg/dL)	0.86 ± 0.27	0.83 ± 0.24	0.97 ± 0.36	0.0031
Medication				
Aspirin, n (%)	146 (70%)	117 (70%)	29 (73%)	0.7226
Clopidogrel, n (%)	117 (57%)	96 (57%)	21 (53%)	0.5947
Cilostazol, n (%)	69 (33%)	53 (33%)	16 (40%)	0.3075
Other antiplatelet drug, n (%)	44 (21%)	34 (20%)	10 (25%)	0.5075
ACEIs and/or ARBs, n (%)	132 (63%)	106 (63%)	26 (65%)	0.8221
Calcium channel blockers, n (%)	118 (57%)	99 (59%)	19 (48%)	0.1898
Statin, n (%)	103 (50%)	85 (57%)	18 (45%)	0.5247

Data are expressed as mean ± SD, number (percentage).
ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; BNP, brain natriuretic peptide; CKD, chronic kidney disease; CLI, critical limb ischemia; H-FABP, heart-type fatty acid binding protein; hsTnT, high-sensitivity troponin T; MACCEs, major adverse cardiovascular and cerebrovascular events.

Institute Inc., Cary, NC, USA and R 3.0.2 with additional packages including Rcmdr, Epi, pROC, and PredictABEL).

3. Results

3.1. Comparison of clinical characteristics between patients with and without MACCEs

The baseline characteristics of the patients are shown in Table 1. There were 175 men and 33 women. The mean $\log_e$ [H-FABP] and $\log_{10}$ [hsTnT] were 1.50 ng/mL and 1.07 pg/mL, respectively. Patients with MACCEs during the course of the study were older and had higher prevalence rates of CLI, previous ischemic heart disease (IHD), previous cerebrovascular disease, and CKD than those without MACCEs. The patients with MACCEs also had higher levels of BNP, H-FABP, hsTnT, and creatinine compared to those without it. There was no significant difference in EVT data excluding occlusion of tibial or peroneal artery and medications at discharge between patients with and without MACCEs.

3.2. Myocardial damage marker levels in CLI, IHD, and CKD settings

As shown in Fig. 1, patients with CLI as well as those with CKD had higher levels of H-FABP compared to those without either of these conditions. There was, however, no significant difference in H-FABP levels between patients with and without previous IHD. hsTnT levels were also higher in patients with CLI as well as those with CKD compared to those without either of these conditions, but in contrast to the case of H-FABP, hsTnT levels were also higher in patients with previous IHD than in those without IHD (Fig. 2).

3.3. Myocardial damage and MACCEs in patients with PAD

During the follow-up period, there were 40 MACCEs including 9 all-cause deaths, 25 re-hospitalizations due to cardiovascular and cerebrovascular events, and 6 re-hospitalizations due to the development of CLI and amputation.

To determine the risk factors for MACCEs, we performed univariate and multivariate Cox proportional hazard regression analyses. In the univariate analysis, H-FABP and hsTnT were significantly associated with MACCEs (Table 2). Age, previous cerebrovascular disease, previous IHD, CLI, CKD, and BNP were also related to MACCEs. Significant predictors in univariate analysis were entered into multivariate Cox proportional hazard regression analysis in a stepwise manner. Both H-FABP and hsTnT were found to be independent predictors of MACCEs in patients with PAD after adjustment of previous IHD and BNP (H-FABP, hazard ratio, 2.61; 95% confidence interval, 1.81–3.76; P < 0.0001 and hsTnT, hazard ratio, 1.77; 95% confidence interval, 1.12–2.69; P = 0.0083; Fig. 3A). Since myocardial damage markers were affected by CLI, CKD, and previous IHD, we entered these risk factors into the multivariate Cox proportional hazard regression analysis. Both H-FABP and hsTnT were found to be independent predictors of MACCEs after adjusting for CLI, previous IHD, and CKD (H-FABP, hazard ratio, 2.75; 95% confidence interval, 1.94–3.87; P < 0.0001 and hsTnT, hazard ratio, 2.14; 95% confidence interval, 1.42–3.23; P = 0.0003; Fig. 3B).

3.4. Risk stratification

All subjects were divided into tertiles according to their H-FABP levels: first tertile (<1.28 ng/mL, n = 69), second tertile (1.28–1.65 ng/mL, n = 69), and third tertile (>1.65 ng/mL, n = 70). A Kaplan–Meier analysis demonstrated that the ratio of MACCEs was highest in the 3rd tertile of H-FABP than in the other two groups (Fig. 4A). All subjects were also divided into tertiles according to their hsTnT levels: first tertile (<0.90 pg/mL, n = 69), second tertile (0.90–1.23 ng/mL, n = 69), and third tertile (>1.23 ng/mL, n = 70). As was found for H-FABP, the Kaplan–Meier analysis of the hsTnT tertiles also demonstrated that the ratio of MACCEs was highest in the 3rd tertile (Fig. 4B).

3.5. Comparison of the prognostic values of H-FABP and hsTnT

To compare the prognostic capacity of H-FABP with that of hsTnT, an ROC analysis was performed. AUC of H-FABP, sensitivity, and specificity
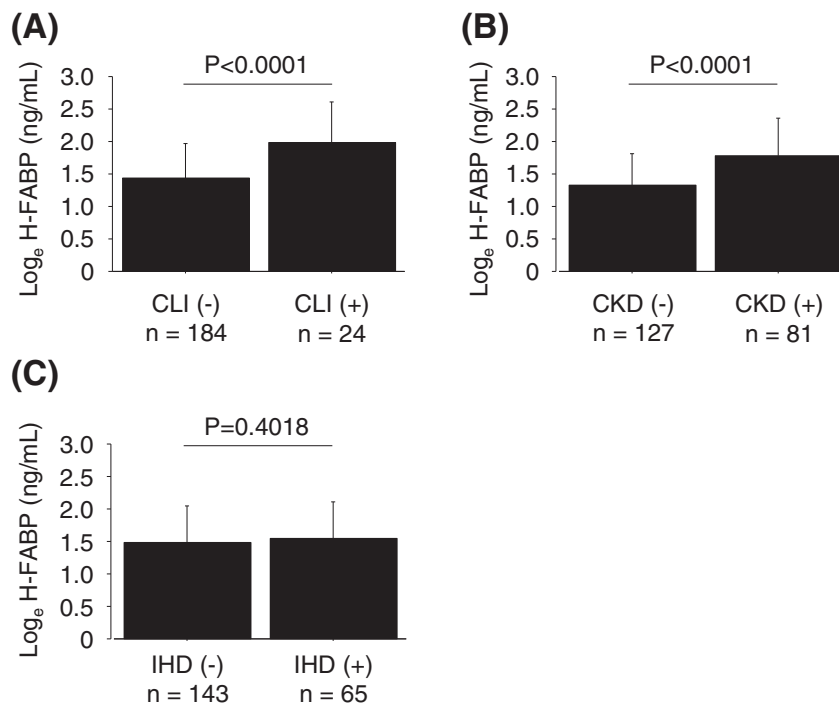

Fig. 1. H-FABP levels in patients with and without CLI, CKD and previous IHD. (A) H-FABP levels were higher in patients with CLI than those without CLI. (B) H-FABP levels were higher in patients with CKD than those without CKD. (C) There was no significant difference in H-FABP levels between patients with and without IHD. H-FABP, heart-type fatty acid binding protein; CLI, critical limb ischemia; CKD, chronic kidney disease and IHD, ischemic heart disease.
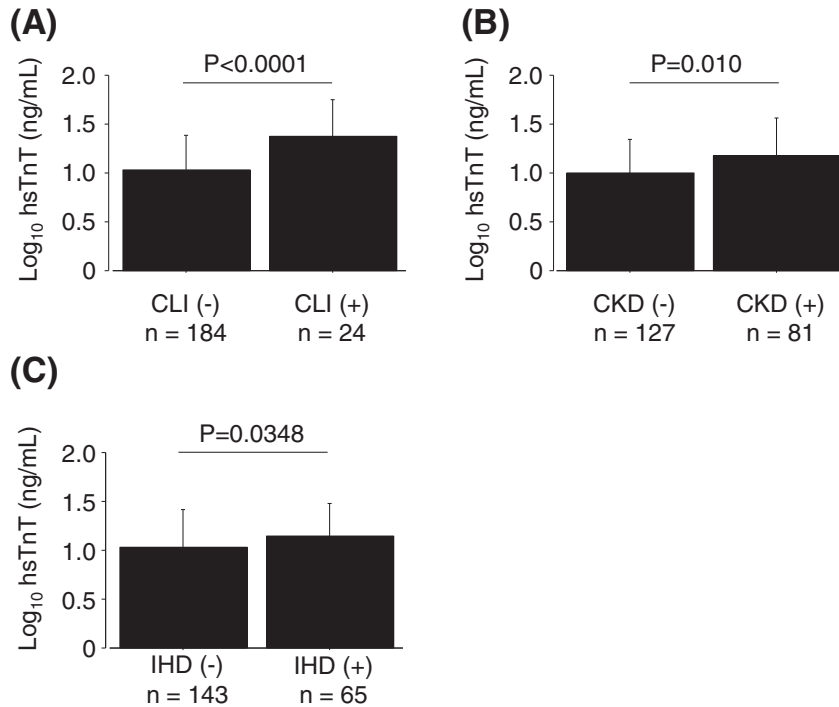
(A)

(B)

(C)

Fig. 2. hsTnT levels in patients with and without CLI, CKD and previous IHD. (A) hsTnT levels were higher in patients with CLI than those without CLI. (B) hsTnT levels were higher in patients with CKD than those without CKD. (C) hsTnT levels were higher in patients with IHD than those without IHD. hsTnT, high-sensitivity troponin T; CLI, critical limb ischemia; CKD, chronic kidney disease and IHD, ischemic heart disease.

were found to be 0.746, 88%, and 62%, respectively. The cut-off value of $\log_e$ [H-FABP] was 1.46 ng/mL. AUC of hsTnT, sensitivity, and specificity were 0.681, 65%, and 71%, respectively. The cut-off value of $\log_{10}$ [hsTnT] was 1.2 pg/mL. AUC of H-FABP was thus significantly greater than that of hsTnT (P = 0.0223).

3.6. Improving reclassification by addition of H-FABP or hsTnT to predict MACCEs

To determine whether model fit and discrimination improve with addition of H-FABP or of hsTnT to the basic predictors such as age, previous IHD, CKD, and CLI, we evaluated the improvement of NRI and IDI. As shown in Table 3, both NRI and IDI were significantly improved

Table 2
Univariate and multivariate Cox proportional hazard analysis of predicting MACCEs in patients with peripheral artery disease.

Variables	Hazard ratio	95% confidence interval	P value
Univariate analysis			
Age (years old)	1.07	1.02–1.12	0.0034
Men/women	1.01	0.39–2.59	0.9815
Hypertension	1.44	0.66–3.14	0.3568
Diabetes mellitus	1.22	0.65–2.27	0.5368
Hyperlipidemia)	1.36	0.72–2.58	0.3461
Previous IHD	3.57	1.92–6.66	<0.0001
Previous cerebrovascular disease	2.44	1.23–4.83	0.0108
CLI	4.08	2.03–8.19	<0.0001
CKD	1.97	1.06–3.68	0.0324
Stent, n (%)	2.87	0.67–12.17	0.1522
Pre ABI	0.98	0.13–7.69	0.9836
Post ABI	0.24	0.04–1.50	0.1278
$\log_{10}$ BNP	2.44	1.75–3.42	<0.0001
$\log_e$ H-FABP	2.48	1.86–3.28	<0.0001
$\log_{10}$ hsTnT	2.54	1.78–3.65	<0.0001

Abbreviation as in Table 1.

by the addition of H-FABP. NRI was also significantly improved by the addition of hsTnT to the basic predictors, but the addition of hsTnT did not yield any significant difference in IDI.

4. Discussion

The results of this study revealed five novel findings: 1) patients with MACCEs had higher levels of H-FABP and hsTnT compared to those without MACCE; 2) patients with CLI had higher levels of H-FABP and hsTnT than those without CLI; 3) multivariate Cox proportional hazard regression analysis revealed that both H-FABP and hsTnT levels independently predicted the occurrence of MACCEs; 4) the Kaplan–Meier analysis demonstrated that the prevalence of MACCEs was greatest in patients in the highest H-FABP tertile and in the highest hsTnT tertile; and 5) a model including H-FABP as well as one including hsTnT showed significantly improved abilities to predict MACCEs in patients with PAD.

4.1. Myocardial damage in PAD

Previously, troponin leakage detected by a highly sensitive assay kit (hsTnT >0.003 ng/mL) was reported to occur in 20–25% of the general population and 60% of elderly adults [19–21]. In the present study, troponin leakage was observed in 85% of PAD patients. On the other hand, H-FABP was detected in all PAD patients examined, with a mean $\log_e$ [H-FABP] of 1.50 ng/mL. We previously reported a mean $\log_e$ [H-FABP] level of 1.25 ng/mL in the general population [5]. These findings suggest that the patients with PAD in our study had a greater level of myocardial damage than that of the general population. H-FABP was reported to be more sensitive than TnT for detection of latent myocardial damage in patients with CHF [17]. Since, in contrast to troponin, H-FABP is a low molecular weight protein, cytosolic H-FABP is easily released into the circulation through the porous membranes of damaged myocardial cells in the absence of cardiomyocyte necrosis
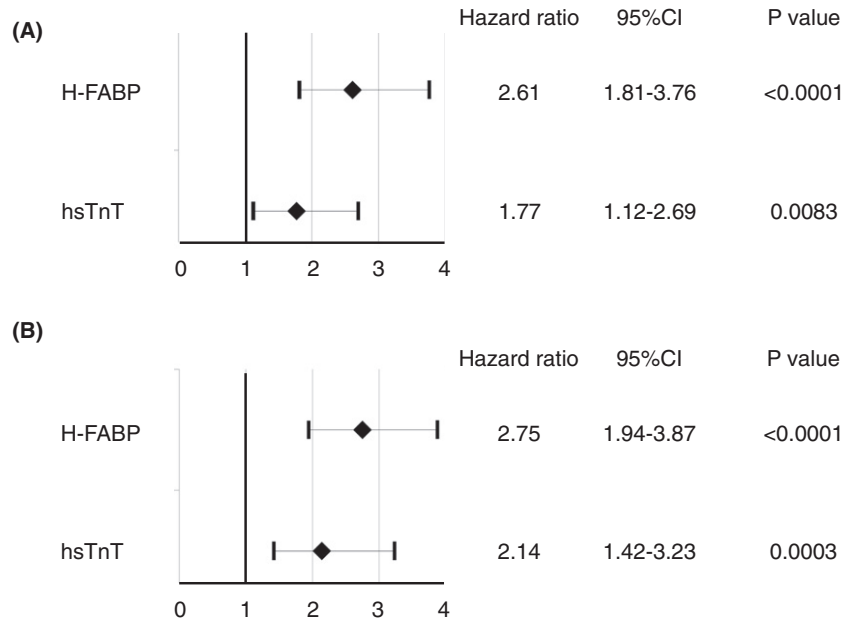
Fig. 3. Multivariate Cox proportional hazard regression analysis for predicting MACCEs in patients with PAD. (A) Hazard ratio after adjustment for IHD and BNP. (B) Hazard ratio after adjustment for CLI, CKD, and IHD. BNP, brain natriuretic peptide; CLI, critical limb ischemia; CKD, chronic kidney disease; IHD, ischemic heart disease and MACCEs, major adverse cardiovascular and cerebrovascular events.

[22,23]. In this way, measuring H-FABP, but not troponin, appears to have detected latent myocardial damage in all patients with PAD in the present study.

In accordance with previous reports [19,24], the presence of CKD and IHD was associated with myocardial damage in patients with PAD. Troponin levels were reported to be elevated in PAD patients with CLI [25,26]. In the present study, we showed that patients with CLI had higher levels of H-FABP and hsTnT compared to those without CLI. Although the precise mechanism is unclear, these findings suggest an association of CLI with myocardial damage.

4.2. Clinical outcomes related to myocardial damage in patients with PAD

Previous studies have not fully determined whether biochemical markers could be useful for identification and risk stratification of these high-risk PAD patients, although BNP was reported to be useful for the diagnosis of PAD in patients with diabetes mellitus and to predict MACCEs in patients with PAD [27,28]. Our results showed, for the first time, that H-FABP and hsTnT, diagnostic markers of myocardial infarction [22], are the feasible markers for MACCEs in patients with PAD.

Linnemann et al. reported that troponin leakage, detected with a conventional cardiac TnT assay kit (>0.01 ng/mL), occurred in 21.3% of PAD patients that were studied, and was significantly associated with MACCEs [26]. We also showed that troponin leakage, detected by high-sensitivity cardiac TnT, can predict MACCEs in patients with PAD. The abnormal cut-off value of 1.2 pg/mL (0.016 ng/mL) for $\log_{10}$ [hsTnT] in the present study indicates that it is possible that troponin leakage detected by both high-sensitivity cardiac TnT and conventional TnT could identify PAD patients at high risk.

We also showed the clinical usefulness of H-FABP in patients with PAD. The abnormal cut-off value of 1.46 ng/mL (4.3 ng/mL) for $\log_e$ [H-FABP] in the present study is equal to that shown to predict cardiac events in patients with CHF and implantable cardioverter defibrillators
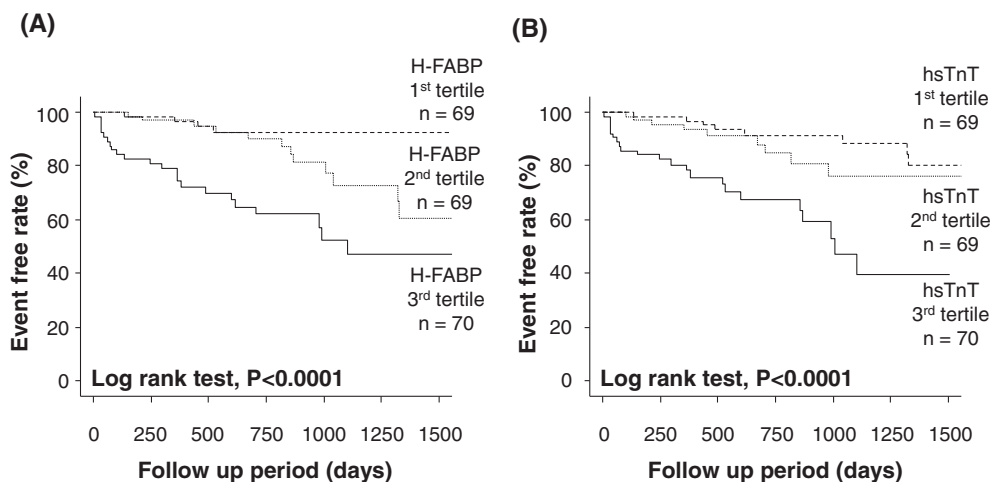

Fig. 4. (A) Kaplan–Meier analysis of MACCEs among patients in all H-FABP tertiles. (B) Kaplan–Meier analysis of MACCEs among patients in all hsTnT tertiles. H-FABP, heart-type fatty acid binding protein; hsTnT, high-sensitivity troponin T and MACCEs, major adverse cardiovascular and cerebrovascular events.

Table 3
Statistics for model fit and improvement with the addition of H-FABP or hsTnT on the prediction of MACCE.

	AUC (P value)	NRI (95% CI, P value)	IDI (95% CI, P value)
Age + CLI + IHD + CKD	0.811	Reference	Reference
+ H-FABP	0.837	0.5032	0.0549
	(P = 0.1834)	(0.1651–0.8413, P = 0.0035)	(0.0047–0.1051, P = 0.0321)
+ hsTnT	0.812	0.3929	0.0152
	(P = 0.9184)	(0.0545–0.7312, P = 0.0228)	(−0.0074–0.0378, P = 0.1882)

AUC, area under the curve; 95% CI, 95% confidence interval; NRI, net reclassification index; IDI, integrated discrimination index; CLI, critical limb ischemia; CKD, chronic kidney disease; IHD, ischemic heart disease; H-FABP, heart-type fatty acid binding protein and hsTnT, high-sensitivity troponin T.

[8,16]. Latent myocardial damage detected by H-FABP, in particular at concentrations greater than 4.3 ng/mL, could provide additional clinical information about MACCEs in patients with PAD.

Myocardial damage is associated with the development of heart failure, ischemic heart disease, and cerebrovascular disease [22,29–31]. In addition, myocardial damage is associated with CLI, which is a risk for mortality and amputation in patients with PAD [32]. Therefore, the myocardial damage markers H-FABP and hsTnT could be useful for predicting MACCEs including amputations in patients with PAD.

4.3. Limitations

First, this study collected baseline information at a single time point. Subsequent medical interventions may have affected serum H-FABP and hsTnT levels. Second, since H-FABP levels in patients with CKD are elevated due to impaired elimination of H-FABP, we could not completely remove the effect of kidney dysfunction on H-FABP levels. Third, although acute coronary syndrome was excluded in the present study, hsTnT levels were higher in patients with IHD than in those without IHD. We could not completely eliminate the impact of IHD on hsTnT levels. Finally, the study population was relatively small in the present investigation. A further study with a larger population is needed to determine precisely what constitutes abnormal levels of H-FABP and hsTnT in patients with PAD.

5. Conclusions

Latent myocardial damage detected by H-FABP and hsTnT is increased in PAD patients with CLI. Notably, both H-FABP and hsTnT could predict MACCEs in patients with PAD, suggesting that they are promising markers for early identification of PAD patients at high risk.

Transparency document

The Transparency document associated with this article can be found in the online version.

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