Research article

Plasma levels of bactericidal/permeability-increasing protein correlate with systemic inflammation in acute coronary syndrome

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ABSTRACT

Background: Neutrophils play important roles in atherosclerosis and atherothrombosis. Bactericidal/permeability-increasing protein (BPI) is mainly expressed in the granules of human neutrophils in response to inflammatory stress. This observational, cross-sectional study investigated the plasma level of BPI in patients with acute coronary syndrome (ACS) and its correlation with blood neutrophil counts and circulating inflammatory biomarkers.
Methods: A total of 367 patients who had acute chest pain and who were admitted to our hospital for coronary angiography (CAG) and/or percutaneous coronary intervention (PCI) from May 1, 2020 to August 31, 2020 were recruited. Among them, 256 had a cardiac troponin value above the 99th percentile upper reference limit and were diagnosed with ACS. The remaining patients (n = 111) were classified as non-ACS. The TIMI and GRACE scores were calculated at admission. The Gensini score based on CAG was used to determine atherosclerotic burden. Plasma levels of interleukin (IL)-1β, myeloperoxidase-DNA (MPO-DNA), high sensitivity C-reactive protein (hs-CRP), S100A8/A9, and BPI were measured using enzyme-linked immunosorbent assays. Correlations of plasma BPI levels with examination scores and levels of circulating inflammatory biomarkers were explored. Receiver operating characteristic (ROC) curve analysis was used to determine the diagnostic efficacy of BPI for ACS and myocardial infarction.
Results: Patients in the ACS group showed significantly higher plasma BPI levels compared to the non-ACS group (46.42 ± 16.61 vs. 16.23 ± 6.19 ng/mL, $p < 0.05$). Plasma levels of IL-1β, MPO-DNA, hs-CRP, and S100A8/A9 in the ACS group were also significantly higher than those in the non-ACS group (all $p < 0.05$). In addition, plasma BPI levels were positively correlated with the TIMI, GRACE, and Gensini scores (r = 0.176, $p = 0.003$; r = 0.320, $p < 0.001$; r = 0.263, $p < 0.001$, respectively) in patients with ACS. Plasma BPI levels were also positively correlated with blood neutrophil counts (r = 0.266, $p < 0.001$) and levels of circulating inflammatory biomarkers (IL-1β, r = 0.512; MPO-DNA, r = 0.452; hs-CRP, r = 0.554; S100A8/A9, r = 0.434; all $p < 0.001$) in patients with ACS. ROC curve analysis revealed that the diagnostic efficacy of BPI for ACS was not inferior to that of IL-1β, MPO-DNA, hs-CRP, S100A8/A9, or blood neutrophil counts. ROC analysis also showed that the diagnostic efficacy of BPI for myocardial infarction was not inferior to that of creatine kinase (CK)-MB or cardiac troponin I.

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Conclusion: BPI is associated with systemic inflammation in ACS and may be involved in the process of atherosclerosis and atherothrombosis. The potential of BPI as a prognostic and diagnostic biomarker for ACS should be investigated in clinical settings.

1. Introduction

Advances in the sensitivity and precision of the cardiac troponin assay in recent years has improved the diagnostic accuracy of acute chest pain [1,2]. However, a considerable proportion of patients fall in an ambiguous category due to myocardial injury or the absence of coronary plaque rupture/erosion/dissection following coronary angiography (CAG) [2,3]. Thus, identification of novel biomarkers for differentiating these indeterminate patients is of great importance for the early diagnosis of acute coronary syndrome (ACS).

ACS is associated with unstable coronary atherosclerotic plaques (i.e. plaques with rupture and erosion), which may indicate exacerbation of vascular inflammation in both adaptive and innate immunity [4,5]. Exacerbated inflammation induces the recruitment of immune cells including mast cells, eosinophils, macrophages, and neutrophils, to the coronary vasculature [6]. Neutrophils play important roles in atherosclerosis and atherothrombosis by releasing antimicrobial proteins (e.g. S100A8/A9) [7,8] and inflammatory mediators [e.g. interleukin (IL)-1β] [9–11], or forming web-like structures named neutrophil extracellular traps (NETs) [12,13]. S100A8/A9 is a heterodimeric protein complex mainly secreted by activated neutrophils [14–17]. Myeloperoxidase-DNA (MPO-DNA) is a circulating marker of NETs [18,19]. The roles of these circulating inflammatory biomarkers in ACS have been explored in recent years.

Bactericidal/permeability-increasing protein (BPI) is a pluripotent protein (~55 kDa) mainly expressed in the granules of human neutrophils in response to inflammatory stress. BPI plays a key role in the host defense against Gram-negative bacteria [20,21]. In addition to its bactericidal activity, BPI has been identified as a target of anti-neutrophil cytoplasmic antibody (ANCA) in a variety of diseases, such as cystic fibrosis [22], inflammatory bowel disease (IBD) [23,24], reactive arthritis [25], chronic obstructive pulmonary disease (COPD) [26,27], and vasculitis [28,29]. BPI participates in lipid metabolism as its structure is similar to some lipid metabolic proteins [30]. It is also closely related to diabetes, an independent risk factor of atherosclerotic disease. A previous study revealed that plasma BPI levels were significantly associated with insulin sensitivity, glucose metabolism, central obesity, and components of metabolic syndrome in patients with glucose intolerance [31]. It was also found that plasma BPI concentrations positively correlated with levels of high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C), as well as endothelium-dependent vasodilatation [32]. A proteomics study reported that the BPI level was markedly decreased in patients with total coronary atherosclerotic occlusion, indicating that BPI may be a biomarker for severe atherosclerotic coronary stenosis [33]. BPI has also been shown to inhibit angiogenesis by promoting apoptosis of vascular endothelial cells both *in vitro* and *in vivo* [34]. Angiogenesis is an important event in the pathogenesis of coronary heart disease (CHD), especially myocardial infarction (MI). These studies indicate that BPI may facilitate inflammation in varied diseases and have a causal relationship with coronary artery disease risk factors, such as hypercholesterolemia, diabetes mellitus, etc. Our ongoing research shows that BPI may promote inflammation and thrombosis in acute myocardial infarction through inducing NETosis. BPI may be a prognostic marker and a therapeutic target of CHD.

Based on the evidence in previous literature, we hypothesized that BPI may play a regulatory role in atherosclerosis-related inflammatory responses and atherothrombosis. So, we designed this observational study to explore the correlations between plasma BPI level and NETosis biomarkers such as hs-CRP, IL-1β, MPO-DNA, and S100A8/A9 in patients with ACS, then evaluate the association between plasma BPI level and coronary atherosclerotic burden using the Gensini score.

2. Materials and methods

2.1. Study population

Subjects who had acute chest pain and were clinically suspected of having ACS and who were admitted to our hospital for coronary angiography (CAG) and/or percutaneous coronary intervention (PCI) between May 1, 2020, and August 31, 2020 were screened. Patients who had one of the following characteristics were excluded: cardiogenic shock, acute infection, blood disorders, severe hepatic, or severe renal failure (plasma creatine level >265.2 μmol/L), as well as ongoing chronic inflammatory, autoimmune, or malignant diseases. All patients had clinical indications for CAG. The diagnosis of MI was in accordance with the criteria of the ESC/ACCF/AHA/WHF Fourth Universal Definition of Myocardial Infarction (2018) [35]. For those patients who had a cardiac troponin I value below the 99th percentile upper reference limit and coronary artery stenosis greater than or equal to 50 % judged by CAG, intravascular ultrasound (IVUS) was used to identify coronary plaque rupture/erosion/dissection. If there is plaque rupture/erosion/dissection, the patients were categorized as unstable angina, otherwise stable angina. ACS included MI and unstable angina. The remaining patients were classified as non-ACS. In total, 256 ACS patients and 111 non-ACS patients were included in this study. All patients ranged in age between 30 and 87 years. Among the ACS patients, 159 were diagnosed with MI based on the CAG results, dynamic changes in cardiac troponin I levels, and the IVUS results. Their demographic and clinical records (i.e. age, gender, BMI, smoking history, hyperlipidemia, diabetes mellitus, atrial fibrillation, hypertension, stroke, and medications), as well as biochemical and hematological data (i.e. glucose, creatinine, uric acid, lipid profile, creatine kinase isoenzyme, platelets, neutrophils, leukocytes, etc.), were recorded for statistical analysis. BMI was calculated as weight (kg) divided by height (m) in squared meters. This study was approved by the Ethics Committee of The First Affiliated Hospital of Bengbu Medical College (BYYFY–2020KY115, Bengbu, China). All

participants gave written informed consent. The study was performed in accordance to the declaration of Helsinki.

2.2. CAG and assessment of the severity of coronary atherosclerotic burden

CAG was performed by experienced cardiologists according to the conventional Judkins method. Rapamycin-coated stents and radial artery puncture were used for all patients. During the procedure, 1 mL of 1 % lidocaine was used for local anesthesia. After the artery sheath was implanted, 3000 U heparin and 200 μg nitroglycerin were injected through the sheath. The CAG results were interpreted by experienced physicians following current guidelines [36]. The decision regarding subsequent performance of PCI was made by the same physicians according to current PCI guidelines [36].

Periprocedural medication and management were administered in accordance with current PCI guidelines [37]. Dual antiplatelet therapies and statins were administered to all patients before the procedure. The criteria for successful PCI were in accordance with international practice guidelines: TIMI grade 3 blood flow and residual stenosis ≤20 % after the procedure. The Gensini score was used to quantitatively assess the severity of coronary atherosclerotic burden according to the distributions and degree of stenosis shown in CAG [38].

2.3. Blood samples

Blood samples (5 mL) were collected from each subject through the median cubital vein and tested within 30 min after admission. These samples were collected in tubes with heparin sodium or EDTA. Samples with heparin sodium were used for a series of blood tests

Table 1
Clinical characteristics of the study population.

	non-ACS (n = 111)	ACS (n = 256)
Medical history		
Age, years	60.63 ± 10.74	61.88 ± 11.21
Male sex, n (%)	68 (61.26)	178 (69.53)
Heart rate, bpm	70.98 ± 8.92	72.72 ± 11.87
Systolic pressure, mmHg	131.87 ± 15.93	134.07 ± 19.29
BMI, kg/m^2	24.83 ± 3.80	24.88 ± 3.54
Smoker, n (%)	19 (17.12)	50 (19.53)
Hypertension, n (%)	62 (55.86)	138 (53.91)
Hyperlipidemia, n (%)	35 (31.53)	65 (25.39)
Diabetes mellitus, n (%)	15 (13.51)	46 (17.97)
Atrial fibrillation, n (%)	2 (1.80)	5 (1.95)
Stroke, n (%)	6 (5.41)	15 (5.86)
Medication		
Antiplatelet, n (%)	101 (90.99)	244 (95.31)
Anticoagulant, n (%)	61 (54.95)	196 (76.56)
Lipid-lowering agent, n (%)	98 (88.29)	232 (90.63)
ACE inhibitor/ARB, n (%)	35 (31.53)	107 (41.80)
β-blockers, n (%)	49 (44.14)	155 (60.55)
Nitrate esters, n (%)	35 (31.53)	125 (48.83)
Proton pump inhibitors, n (%)	87 (78.38)	172 (67.19)
Biochemical and hematological data		
Glucose, mmol/L	5.42 ± 1.97[a]	6.17 ± 2.94
Creatinine, μmol/L	69.52 ± 12.93[a]	74.41 ± 21.19
Uric acid, μmol/L	338.15 ± 83.775	334.01 ± 98.38
Cholesterol, mmol/L	4.49 ± 1.83	4.08 ± 1.28
High Density Lipoprotein, mmol/L	1.08 ± 0.29[a]	0.98 ± 0.27
Low Density Lipoprotein, mmol/L	2.45 ± 0.85	2.37 ± 0.89
Apolipoprotein A, mmol/L	1.20 ± 0.29[a]	1.11 ± 0.27
Apolipoprotein B, mmol/L	0.84 ± 0.28	0.81 ± 0.29
LP (a), mmol/L	334.66 ± 26.74[a]	385.89 ± 33.75
Triglycerides, mmol/L	1.73 ± 0.56	2.02 ± 0.33
MB isoform of creatine kinase, IU/L	12.81 ± 1.20[a]	38.47 ± 6.05
Troponin I, ng/mL	0.40 ± 0.03[a]	3.43 ± 0.83
Platelets,[a]10^9/L	206.57 ± 60.49	210.45 ± 69.00
Leukocytes,[a]10^9/L	6.42 ± 1.76	7.56 ± 2.50
Neutrophils,[a]10^9/L	3.88 ± 1.40[a]	5.09 ± 2.45
Lymphocytes (%)	30.56 ± 8.54[a]	25.49 ± 9.56
Neutrophils (%)	59.13 ± 9.38[a]	65.17 ± 11.02
Monocytes (%)	6.82 ± 1.98	6.71 ± 2.10
Eosinophils (%)	2.39 ± 0.17	2.32 ± 0.21
Basophils (%)	0.34 ± 0.08	0.28 ± 0.05

Data are presented as mean ± SD.

[a] $p < 0.05$ was statistically significant when comparisons were made among the non-ACS and ACS group.

following a standard protocol from the Department of Clinical Laboratory at our hospital, which included routine blood analysis, blood glucose, coagulation function, lipid profile, uric acid, renal function, creatine kinase isoenzyme, and cardiac troponin I. Plasma samples were collected by centrifugation at $2500 \times g$ for 15 min and stored at $-80\ ^\circ$C until subsequent analysis. Additional morning fasting blood samples were collected within 24 h of admission for monitoring fasting blood glucose, creatine kinase isoenzyme, and cardiac troponin I.

2.4. Enzyme-linked immunosorbent assay (ELISA)

The MPO-bound DNA complexes in plasma samples were detected using ELISA as previously described [39–41]. Briefly, a 96-well microtiter plate (Corning) was coated with anti-MPO antibody (4 µg/mL, Bio-Rad) at 4 °C overnight, followed by 2-h incubation with 1 % bovine plasma albumin in phosphate buffered saline (PBS) at room temperature. Plasma samples were added to the plate and incubated overnight at 4 °C. For detection, anti-dsDNA-HRP antibody (1:100 dilution, Roche Diagnostic) was added to the samples and incubated at room temperature for 2 h. The reaction was developed with 3,3′,5,5′-tetramethylbenzidine (BD Biosciences) and terminated by the addition of 2 N sulfuric acid. Absorbance was detected at 450 nm using a plate reader (Synergy, BioTek). The plasma levels of BPI, S100A8/A9, hs-CRP, and IL-1β were also measured using ELISA kits according to the manufacturer's instructions (Cusabio Biotech, China). The absorbance was read at 450 nm in a microplate reader. The means were used to calculate the levels of BPI, S100A8/A9, hs-CRP, and IL-1β in corresponding samples based on established standard curves. ELISA experiments were performed in triplicate.

2.5. Statistical analysis

Shapiro-Wilk test was used to check normal distribution of the continuous variables. Numerical data are shown as means ± standard deviation (SD). Student's *t*-test or one-way analysis of variance followed by Bonferroni test was used to determine statistical significance. Pearson's or Spearman's correlation analysis was used to determine the correlation between parameters. If both variables x and y are normally distributed, we calculate Pearson's correlation coefficient (r). If normality assumption is not met for one or both variables in a correlation analysis, a rank correlation coefficient, such as Spearman's rho (ρ) may be calculated. Linear regression analysis was performed to identify independent influencing factors of BPI. A *p*-value <0.05 was considered statistically significant. Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic efficacy of plasma BPI levels for ACS and MI. The optimal cut-off of plasma BPI levels for ACS and MI were calculated. In the ROC analysis, the maximum Youden index corresponds to the optimal cutoff. Youden Index calculated as equivalently max (sensitivity + specificity - 1). All data analyses were performed using SPSS 22.0 software (IBM Corp.).

3. Results

3.1. Demographic and clinical characteristics of participants at baseline

There were no significant differences in age, gender, BMI, smoking history, hypercholesterolemia, diabetes mellitus, atrial fibrillation, stroke, hypertension, and medications between the ACS and non-ACS groups (Table 1).

There were no significant differences in the levels of uric acid, cholesterol, LDL, apolipoprotein B, triglycerides, numbers of platelets and leukocytes, and percentages of monocytes, eosinophils, and basophils between the ACS and non-ACS groups. The level of creatinine in the non-ACS group was significantly lower than that of the ASC group (69.52 ± 12.93 *vs.* 74.41 ± 21.19 µmol/L, $p < 0.05$). Patients with ASC had significantly higher levels of glucose (6.17 ± 2.94 *vs.* 5.42 ± 1.97 mmol/L) and Lp (a) (385.89 ± 33.75 *vs.* 334.66 ± 26.74 mmol/L), an increased number of neutrophils (5.09 ± 2.45 *vs.* 3.88 ± 1.40 * 10^9/L), and a higher percentage of

Table 2
The level of inflammation biomarkers and scores.

	non-ACS	ACS
	(n = 111)	(n = 256)
Plasma BPI (ng/mL)	16.23 ± 6.19[a]	46.42 ± 16.61
Hs-CRP (pg/mL)	17699.58 ± 1650.11[a]	40355.17 ± 8389.56
IL-1β (pg/mL)	2509.66 ± 168.08[a]	4843.68 ± 107.67
MPO-DNA (ng/mL)	456.39 ± 30.43[a]	890.63 ± 38.26
S100A8/A9 (ng/mL)	11.74 ± 4.61[a]	19.54 ± 3.71
TIMI score	1.73 ± 0.13[a]	3.24 ± 0.45
GRACE score	107.41 ± 23.84[a]	129.10 ± 33.47
Gensini score	21.31 ± 3.22[a]	56.97 ± 3.56

BPI, bactericidal/permeability increasing protein.
IL-1β, interleukin-1 β
Hs-CRP, high sensitivity C-reactive protein.
Data are presented as mean ± SD.
[a] p < 0.05 was statistically significant when comparisons were made among the non-ACS and ACS group.

neutrophils (65.17 ± 11.02 *vs.* 59.13 ± 9.38), but lower levels of LDL (2.37 ± 0.89 *vs.* 2.45 ± 0.85 mmol/L) and apolipoprotein A (1.11 ± 0.27 *vs.* 1.20 ± 0.29 mmol/L), and a lower percentage of lymphocytes (25.49 ± 9.56 *vs.* 30.56 ± 8.54) compared with the non-ACS group (all $p < 0.05$) (Table 1).

3.2. The association of BPI with inflammatory markers, risk scores, and coronary artery disease severity

The ACS group showed significantly higher plasma levels of BPI compared with the non-ACS group (46.42 ± 16.61 *vs.* 16.23 ± 6.19 ng/mL, $p < 0.05$) (Table 2, Fig. 1). Similarly, plasma levels of hs-CRP, IL-1β, MPO-DNA, and S100A8/A9 in the ACS group were significantly higher compared with the non-ACS group (all $p < 0.05$) (Table 2). To investigate the clinical relevance of high plasma BPI levels in ACS, we examined the correlations of BPI levels with 14 clinical characteristics, 15 blood parameters, four inflammation biomarkers, and five CAG indexes in ACS patients. We adjusted p-values using the LD-adjusted Bonferroni correction to decrease the probability of Type I errors. The significance level was set as $p < 0.005$ after correction.

No significant correlations were found between plasma BPI levels and blood platelet counts, although recent RNA-seq data analysis reported the upregulation of BPI in the platelets of patients with STEMI/NSTEMI [42]. Plasma BPI levels positively correlated with blood neutrophil counts ($r = 0.266$, $p < 0.001$) in patients with ACS in Spearman's correlation analysis, and this correlation was more significant in the whole cohort ($r = 0.316$, $p < 0.001$) (Fig. 2A and B). After correction, the correlations between plasma BPI levels and blood neutrophil counts were confirmed in patients with ACS ($p < 0.001$) (Tables 3 and 4). In addition, we found that plasma BPI levels positively correlated with glucose levels ($r = 0.138$, $p = 0.0027$) in patients with ACS, and this correlation was also significant in the whole cohort ($r = 0.135$, $p = 0.007$). After correction, the correlations between plasma BPI levels and glucose levels were confirmed in patients with ACS ($p < 0.001$) (Tables 3 and 4).

We next examined the correlations of plasma BPI levels with the levels of hs-CRP, IL-1β, MPO-DNA, and S100A8/A9. Plasma BPI levels positively correlated with the levels of hs-CRP, IL-1β, MPO-DNA, and S100A8/A9 ($r = 0.746$; $r = 0.741$; $r = 0.611$; $r = 0.529$, all $p < 0.001$) in whole cohort patients in Spearman's correlation analysis [Fig. 3(A–D)]. The correlations of BPI with MPO-DNA and S100A8/A9 were more significant in the ACS compared to the non-ACS subgroup (Table 3) (Fig. 3C and D). To identify factors independently associated with BPI, we performed linear regression analysis of plasma BPI levels with the concentrations of hs-CRP, IL-1β, MPO-DNA, and S100A8/A9 using Spearman's correlation analysis. The p-values were corrected using the LD-adjusted Bonferroni correction. After correction, the correlations of BPI levels with the concentrations of hs-CRP, IL-1β, MPO-DNA, and S100A8/A9 persisted in patients with ACS (all $p < 0.001$). These results indicate that plasma BPI levels positively correlate with the concentrations of hs-CRP, IL-1β, MPO-DNA, and S100A8/A9 (Tables 3 and 4).

The TIMI, GRACE, and Gensini scores of the ACS group were significantly higher compared with the non-ACS group (all $p < 0.05$) (Table 2) [Fig. 4(A–C)]. We found that plasma BPI levels positively correlated with the TIMI and GRACE scores in Spearman's correlation analysis ($r = 0.176$, $p = 0.003$; $r = 0.320$, $p < 0.001$) in patients with ACS, and this correlation was more significant in the whole cohort ($r = 0.486$, $p < 0.001$; $r = 0.384$, $p < 0.001$) (Fig. 4B and C). After correction, the correlations of plasma BPI levels with the TIMI and GRACE scores were confirmed in patients with ACS (all $p < 0.001$) (Tables 3 and 4).

The severity of coronary atherosclerosis was assessed using the Gensini scoring system based on the CAG results. The Gensini scoring system is a well-recognized and widely used system that evaluates the severity of coronary atherosclerosis in the clinic [38,43, 44]. We also evaluated the correlation between BPI levels and the Gensini score and found that higher plasma BPI levels were associated with higher Gensini scores in patients with ACS in Spearman's correlation analysis ($r = 0.263$, $p < 0.001$) (Fig. 4A), as well as in the whole cohort ($r = 0.605$, $p < 0.001$) (Table 3). After correction, the correlation between plasma BPI levels and the Gensini score was confirmed in patients with ACS ($p < 0.001$) (Tables 3 and 4). We further investigated the correlations of BPI levels with CAG results, including "the number of diseased coronary arteries", "calcified lesions, chronic total occlusion", "in-stent restenosis", and
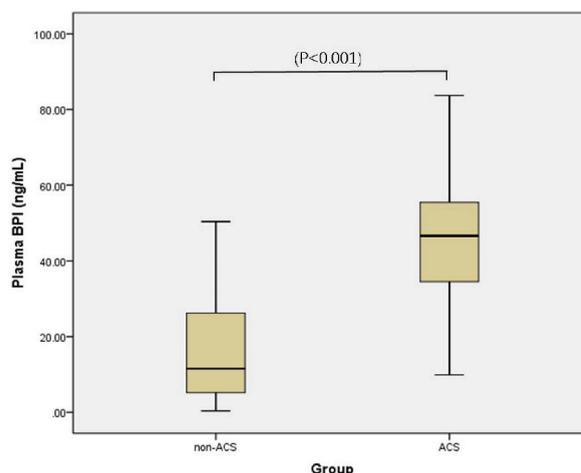
Fig. 1. Comparison of plasma BPI levels between non-ACS and ACS groups.
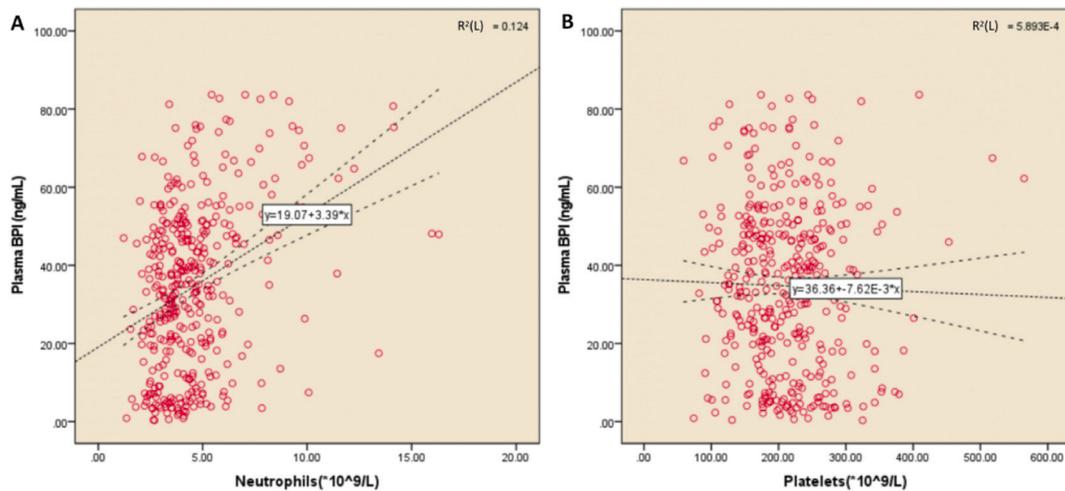
Fig. 2. Correlations of plasma BPI levels with blood neutrophil and platelet counts.

"total number of stents in coronary". We found that plasma BPI levels positively and significantly correlated with "the number of diseased coronary arteries", "calcified lesions, chronic total occlusion", and "total number of stents in coronary" in the whole cohort in Spearman's correlation analysis (r = 0.489, p < 0.001; r = 0.128, p = 0.011; r = 0.264, p < 0.001; r = 0.277, p < 0.001), but not in patients with ACS. However, after LD-adjusted Bonferroni correction, the correlations of plasma BPI levels with the above indexes were not significant (Tables 3 and 4).

3.3. ROC analysis of the diagnostic efficacy of plasma BPI levels for ACS and MI

ROC curve analysis revealed that the optimal cut-off of plasma BPI levels for ACS was 29.01 ng/mL. The ROC curves for BPI, hs-CRP, IL-1β, MPO-DNA, S100A8/A9, and neutrophils are compared (Fig. 5A and B). We found that BPI, hs-CRP, IL-1β, MPO-DNA, S100A8/A9, and neutrophils all had diagnostic efficacy for ACS, with area under the curve (AUC) values of 0.93 (0.90–0.95), 0.87 (0.84–0.91), 0.87 (0.84–0.91), 0.81 (0.76–0.85), 0.76 (0.71–0.81), and 0.68 (0.62–0.73), respectively (Fig. 5A and B). The pairwise comparison of ROC curves using the Z-Test showed that the diagnostic efficacy of BPI levels for ACS was significantly different from that of hs-CRP, IL-1β, MPO-DNA, S100A8/A9, and neutrophils (BPI *vs.* hs-CRP: z = 2.856, *p* = 0.0043; BPI *vs.* IL-1β: z = 3.241, *p* = 0.0012; BPI *vs.* MPO-DNA: z = 5.316, *p* < 0.0001; BPI *vs.* S100A8/A9: z = 6.397, *p* < 0.0001; BPI *vs.* neutrophils: z = 8.186, *p* < 0.0001).

Using the same method, we found that the optimal cut-off of plasma BPI levels for MI was 38.71 ng/mL. The ROC curves for BPI, CK-MB, and cardiac troponin I (TnI) are compared (Fig. 6). ROC curve analysis showed that BPI, CK-MB, and TnI all demonstrated diagnostic efficacy for MI, with AUC values of 0.88 (0.84–0.93), 0.71 (0.64–0.79), and 0.83 (0.78–0.89), respectively. Using the Z-Test, the diagnostic efficacy of BPI for MI was significantly different from that of CK-MB (BPI *vs.* CK-MB: z = 3.896, *p* = 0.0001), but no significant difference was observed between BPI and TnI (BPI *vs.* TnI: z = 1.448, *p* = 0.1475). The above findings indicate that the diagnostic value of BPI for MI is not inferior to that of TnI, and may be superior to that of CK-MB.

4. Discussion

In the present study, we measured plasma BPI levels in patients with ACS and assessed their correlation with clinical characteristics and circulating inflammatory biomarkers. The major findings of this study are as follows: 1) the ACS group had significantly higher plasma BPI levels than the non-ACS group; 2) plasma BPI levels positively correlated with hs-CRP, IL-1β, MPO-DNA, and S100A8/A9 levels, as well as blood neutrophil counts in patients with ACS; 3) plasma BPI levels positively correlated with the TIMI and GRACE scores, as well as the Gensini score, which indicated the severity of coronary atherosclerosis in patients with ACS; 4) the diagnostic efficacy of plasma BPI levels for ACS was not inferior to that of hs-CRP, IL-1β, MPO-DNA, S100A8/A9, and blood neutrophil counts, and also not inferior to CK-MB and TnI for the diagnosis of MI. Therefore, plasma BPI levels may serve as a biomarker for ACS.

Atherosclerosis is a chronic inflammatory disease of the vessel wall and a major cause of death worldwide. Inflammation substantially contributes to the initiation, progression, and destabilization of atherosclerosis. Although the pathogenic mechanisms underlying atherosclerosis vary across patients, neutrophils are considered the major immune cell that drives the development of this disease [45]. Neutrophils are activated in response to a variety of stimuli, such as inflammatory cytokines, and initiate subsequent effector functions. S100A8/A9 [7,8] and IL-1β [9–11] are important inflammatory mediators in the process of atherosclerosis, and hs-CRP is a canonical biomarker well studied in CHD.

In the current study, we found that patients with ACS had significantly higher plasma BPI levels compared with non-ACS cases. In our study entitled "Positive correlations between plasma BPI level and MPO-DNA and S100A8/A9 in myocardial infarction" (PMID:

Table 3
Spearman correlation between BPI with clinical characteristics and inflammation Biomarkers.

Variable	non-ACS (n = 111)		ACS (n = 256)		Whole Cohort (n = 367)	
	rs	p-value	rs	p-value	rs	p-value
Clinical characteristics						
Age (years)	0.06	0.478	0.039	0.534	0.140[a]	0.005
Gender (F = 1, M = 0)	−0.151	0.071	−0.005	0.941	−0.177[a]	<0.001
Heart rate (bpm)	−0.142	0.092	0.081	0.194	0.029	0.56
Systolic pressure (mmHg)	0.155	0.065	−0.074	0.237	0.029	0.56
BMI (kg/m^2)	0.037	0.66	−0.132	0.061	−0.061	0.26
Hypertension	0.188[b]	0.024	−0.07	0.262	0.034	0.496
Hyperlipidemia	−0.124	0.139	−0.033	0.598	−0.026	0.608
Diabetes mellitus	−0.106	0.208	−0.041	0.509	0.056	0.261
Smoking	0.027	0.747	0.08	0.201	0.113[b]	0.024
Atrial fibrillation	0.049	0.557	0.106	0.091	0.093	0.062
Stroke	−0.12	0.15	0.004	0.945	0.073	0.145
Previous stenosis≥50 %	0.217[a]	0.009	−0.305[a]	<0.001	0.017	0.729
PreMI	0.134	0.11	−0.092	0.143	0.019	0.707
PrePCI	0.112	0.181	−0.212[a]	0.001	0.025	0.62
TIMI score	0.194[a]	0.02	0.176[a]	0.003	0.486[a]	<0.001
Grace score	−0.135	0.108	0.320[a]	<0.001	0.384[a]	<0.001
Blood parameters						
Blood neutrophils counts	−0.001	0.986	0.266[a]	<0.001	0.316[a]	<0.001
Blood leukocytes counts	−0.043	0.607	−0.1	0.12	−0.111[b]	0.029
Neutrophils (%)	0.006	0.947	0.263[a]	<0.001	0.290[a]	<0.001
Blood platelets counts	−0.147	0.08	0.024	0.706	−0.056	0.268
Glucose (mmol/L)	−0.077	0.362	0.138[b]	0.027	0.135[a]	0.007
Cholesterol (mmol/L)	−0.06	0.477	0.116	0.075	0.004	0.944
Triglycerides (mmol/L)	0.061	0.473	−0.084	0.2	0.004	0.937
HDL (mmol/L)	0.044	0.603	−0.002	0.971	−0.114[b]	0.027
LDL (mmol/L)	−0.054	0.524	0.127	0.051	0.006	0.907
Apolipoprotein A (mmol/L)	0.03	0.726	−0.018	0.78	−0.097	0.06
Apolipoprotein B (mmol/L)	−0.051	0.547	0.137[b]	0.036	0.01	0.843
LP (a) (mmol/L)	0.293[a]	0.002	−0.049	0.517	0.117[b]	0.048
CK-MB (IU/L)	−0.021	0.829	0.208[a]	0.002	0.268[a]	<0.001
Creatinine (μmol/L)	0.182[b]	0.029	−0.094	0.136	0.092	0.065
Uric acid (μmol/L)	0.119	0.158	0.021	0.742	0.058	0.248
New biomarkers						
Hs-CRP (pg/mL)	0.700[a]	<0.001	0.554[a]	<0.001	0.746[a]	<0.001
IL-1β(pg/mL)	0.638[a]	<0.001	0.512[a]	<0.001	0.741[a]	<0.001
MPO-DNA (ng/mL)	0.403[a]	<0.001	0.452[a]	<0.001	0.611[a]	<0.001
S100A8/A9 (ng/mL)	0.211[b]	0.011	0.434[a]	<0.001	0.529[a]	<0.001
Coronary angiography						
Number of diseased vessels	0.665[a]	<0.001	0.047	0.450	0.489[a]	<0.001
Calcified lesions	0.122	0.144	0.048	0.440	0.128[b]	0.011
Chronic total occlusion	0.258[a]	0.002	0.111	0.077	0.264[a]	<0.001
In-stent restenosis	0.305[a]	<0.001	−0.173[a]	0.005	0.043	0.397
Total number of stents	0.111	0.184	−0.059	0.347	0.277[a]	<0.001
Gensini score	0.701[a]	<0.001	0.263[a]	<0.001	0.605[a]	<0.001

ACS, acute coronary syndrome. HDL, high density lipoprotein. LDL, low density lipoprotein.
CK-MB, MB isoform of creatine kinase. BMI, body mass index.
BPI, bactericidal/permeability increasing protein.
Hs-CRP, high sensitivity C-reactive protein.
IL-1β, interleukin-1 β
[a] Correlation was statistically significant at 0.01 level (Two-tailed).
[b] Correlation was statistically significant at 0.05 level (Two-tailed).

34387532), we have presented the correlated data about plasma BPI levels in STEMI and NSTEMI cases (63.54 ± 11.783 ng/mL in STEMI and 39.04 ± 11.303 ng/mL in NSTEMI). The plasma BPI levels increased by 8.76 times in the STEMI group and 5.38 times in the NSTEMI group compared to the control group. Plasma BPI levels are increased in myocardial infarction patients and positively correlated with MPO-DNA and S100A8/A9. In this study, we then sought to determine if plasma BPI levels correlated with the levels of hs-CRP, IL-1β, and S100A8/A9, as well as blood neutrophil counts in ACS. We found that plasma BPI levels positively correlated with the concentrations of circulating inflammatory biomarkers, as well as blood neutrophil counts in whole cohort patients. The correlations of BPI with MPO-DNA and S100A8/A9 were more significant in the ACS compared to the non-ACS subgroup (Tables 3 and 4). These data indicate that BPI is involved in the pathogenesis of atherosclerosis and that BPI facilitates inflammation/thrombosis through inducing NETosis, but to better understand how BPI affects atherosclerotic plaques we assessed the severity of coronary atherosclerosis in ACS using the Gensini scoring system [43]. We further examined the correlation between plasma BPI levels and the severity of coronary atherosclerosis and found that higher plasma BPI levels correlated with more severe coronary atherosclerosis in

Table 4

Unary linear regression of BPI.

Index	B	S.E.	Beta	T	p
Hs-CRP (pg/mL)[a]	0.001	0	0.778	24.744	<0.001
IL-1β (pg/mL)[a]	0.009	0	0.767	23.86	<0.001
MPO-DNA (ng/mL)[a]	0.032	0.002	0.627	16.046	<0.001
S100A8/A9 (ng/mL)[a]	1.264	0.098	0.543	12.893	<0.001
Neutrophils (*10^9/L)[a]	3.392	0.459	0.353	7.385	<0.001
Platelets (*10^9/L)	−0.008	0.016	−0.024	−0.476	0.634
Glucose (mmol/L)[a]	1.807	0.399	0.222	4.528	<0.001
LP (a) (mmol/L)	0.007	0.004	0.113	1.906	0.058
Previous coronary artery disease≥50 %	0.069	2.449	0.001	0.028	0.978
In-stent restenosis	3.663	5.615	0.033	0.652	0.515
TIMI score[a]	6.826	0.638	0.473	10.703	<0.001
GRACE score[a]	0.276	0.03	0.424	9.342	<0.001
Gensini score[a]	0.293	0.024	0.53	12.459	<0.001

BPI, bactericidal/permeability increasing protein.

Hs-CRP, high sensitivity C-reactive protein.

IL-1β, interleukin-1 β

[a] $p < 0.005$.
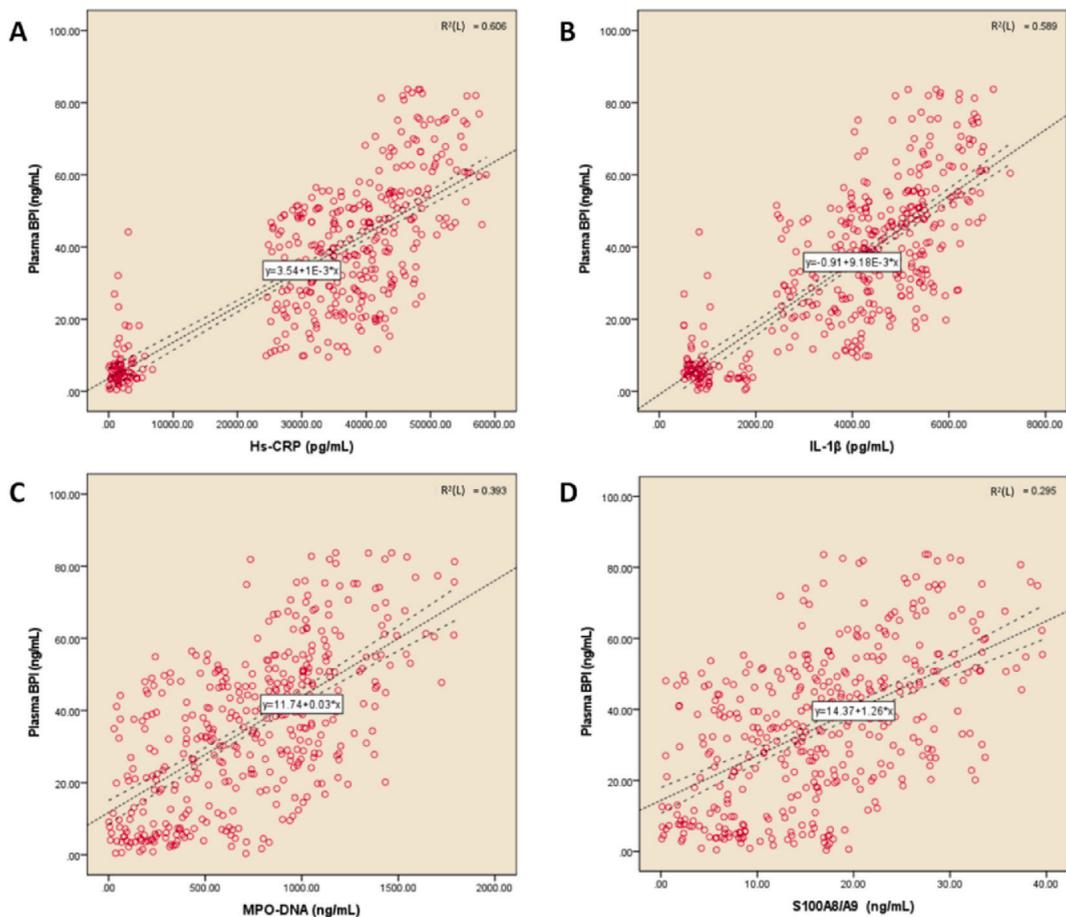
Fig. 3. Correlations of plasma BPI levels with the levels of hs-CRP, IL-1β, MPO-DNA, and S100A8/A9.

ACS, which was consistent with the results of the whole cohort. The correlation of BPI with Gensini score was more significant in the non-ACS compared to the ACS subgroup, somewhat inconsistent with the results of correlations of BPI with NETosis biomarkers. The Gensini score calculated the severity of coronary atherosclerosis in terms of the degree and extent of coronary artery stenosis, which are not always consistent with the level of inflammation. All the above findings support that BPI is an inflammatory marker involved in the pathogenesis of atherosclerosis.
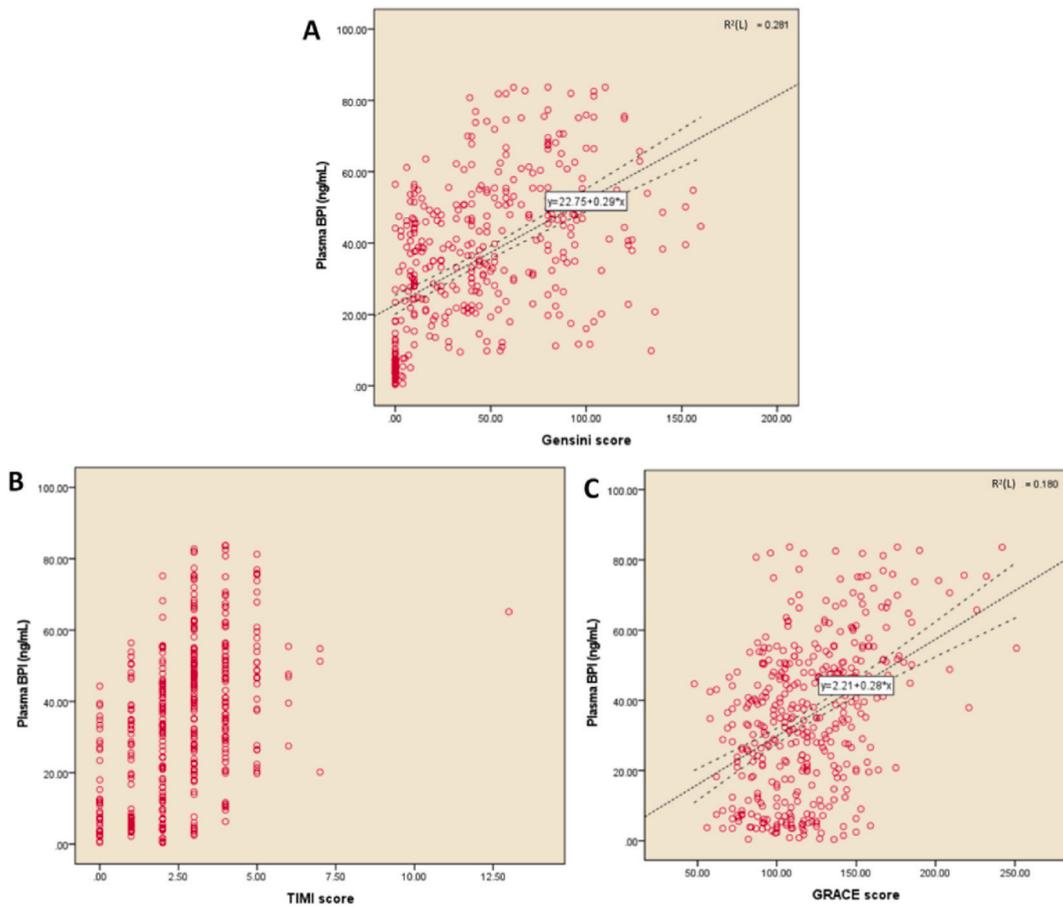
Fig. 4. Correlations of plasma BPI levels with the TIMI, GRACE, and Gensini scores.
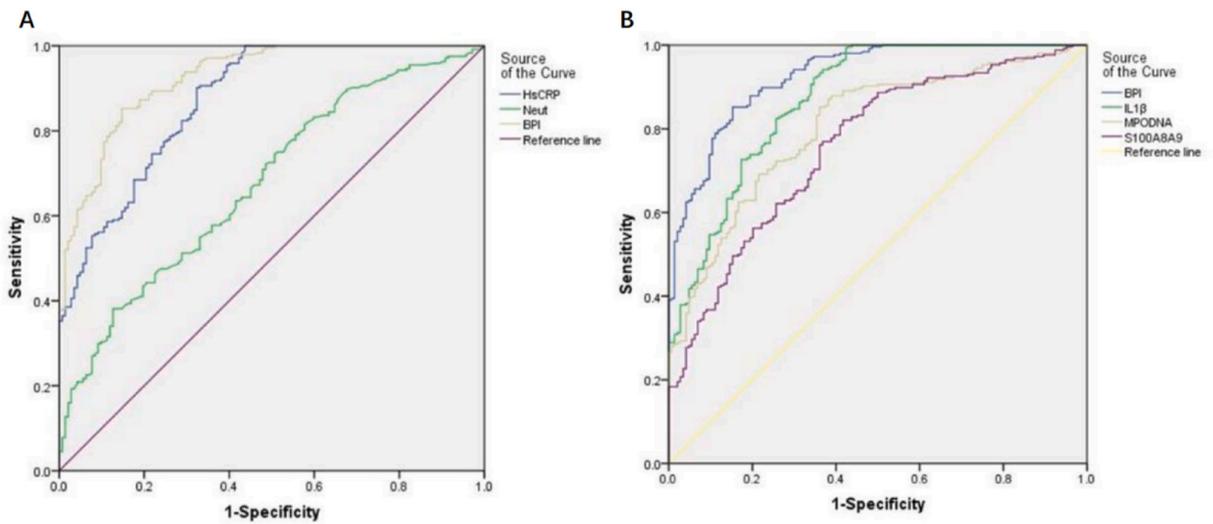
Fig. 5. ROC analysis of the diagnostic efficacy of plasma BPI for ACS. Compared with hs-CRP and blood neutrophil counts (A). Compared with IL-1β, MPO-DNA, and S100A8/A9 (B).
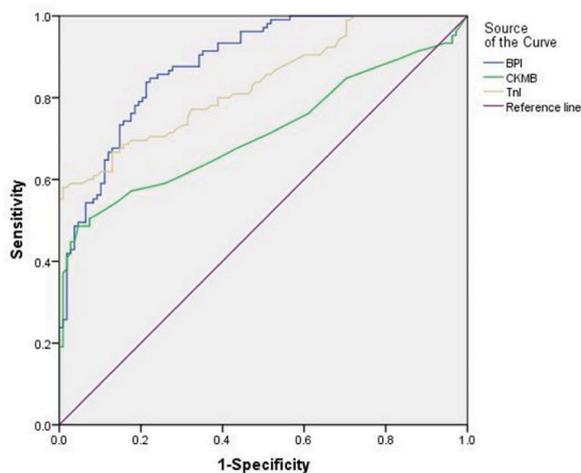
Fig. 6. ROC analysis of the diagnostic efficacy of plasma BPI, CK-MB, and TnI for MI.

Further analysis revealed that plasma BPI levels were positively correlated with the TIMI and GRACE scores (r = 0.176, *p* = 0.003; r = 0.320, *p* < 0.001) in patients with ACS and the whole cohort (r = 0.486, *p* < 0.001; r = 0.384, *p* < 0.001), indicating that BPI was correlated with traditional risk factors and may predict the prognosis of ACS. BPI has been shown to participate in lipid metabolism [32,46] owing to its lipid protein structure [47]. Plasma BPI concentrations were also positively associated with the levels of TC, LDL-C, and HDL-C [32]. It has also been suggested that BPI is related to insulin sensitivity and glucose tolerance [31]. In our study, plasma BPI levels were positively correlated with glucose levels (r = 0.138, *p* = 0.0027) in patients with ACS and the whole cohort (r = 0.135, *p* = 0.007), which was consistent with the findings by Gubern et al. [31]. Future investigation of the causal relationship between BPI and traditional risk factors is needed.

When unstable coronary atherosclerotic plaque is accompanied by rupture/erosion in ACS or an acute coronary event, consequences may vary depending on the severity of concomitant inflammation and segmenting coronary artery thrombosis, namely, the interactions among injured endothelium, activated neutrophils, and platelets [48,49]. NETs are strongly implicated in thrombosis by inducing platelet activation and coagulation [50], as well as thrombus formation [51]. During inflammatory stress, NETs are released by neutrophils as structures composed of DNA fibers lined with histones and granule proteins, such as neutrophil elastase and MPO. The net-like structures have been detected in atherosclerotic lesions and arterial thrombi in humans and mice. Functionally, NETs induce the activation of endothelial cells, antigen-presenting cells, and platelets [52], resulting in proinflammatory immune response. Overall, these findings suggest that NETs are not only present in plaques and thrombi, but also play an important role in triggering atherosclerotic plaque formation and arterial thrombosis [53]. Previous studies have shown that MPO-DNA is a component of coronary thrombi in acute MI [54], suggesting a prothrombotic state [18], and is correlated with the prognosis of STEMI [19], severity of cardiovascular disease [55,56], and coronary atherosclerosis [57]. S100A8/A9 is bound to NETs both *in vitro* and *in vivo* [7,8,58] and is implicated in the pathogenesis of MI. Increased expression of S100A8/A9 was observed in the atherosclerotic plaques of unstable angina pectoris patients [15,59]. In line with these findings, we found that the ACS group exhibited significantly higher levels of MPO-DNA and S100A8/A9 compared with the non-ACS group. Moreover, plasma BPI levels were positively correlated with the levels of MPO-DNA and S100A8/A9. Although MPO-DNA and S100A8/A9 play important roles in NETs formation, the role of BPI in the formation of NETs warrants further exploration.

In recent years, advances in the sensitivity and precision of the cardiac troponin assay have improved the diagnosis of acute chest pain. However, some patients with ACS have elevated cardiac troponin levels due to myocardial injury rather than atherosclerotic plaque rupture/erosion with thrombosis. The combined used of biomarkers related to the pathogenesis of ACS and those for myocardial necrosis may contribute to the early identification of these patients for appropriate treatment. In the present study, ROC analysis showed that the diagnostic efficacy of BPI for MI was not inferior to that of CK-MB or TnI. BPI is not a marker of myocardial necrosis, and an elevation in plasma BPI levels only indicates increased aseptic inflammation in patients with MI. Consistently, we found that BPI, hs-CRP, IL-1β, MPO-DNA, S100A8/A9, and blood neutrophil counts all had diagnostic efficacy for ACS, and the diagnostic value of BPI for ACS was superior to that of circulating inflammatory biomarkers. Early measurement of hemostatic plasma markers in patients with ACS may provide pathophysiological information of an acute coronary event [60]. In this study, the subgroup analysis showed that plasma BPI levels were positively correlated with the hemostatic markers in MI, such as D-dimer and fibrinogen. This may be consistent with the concept that some ruptured/erosional coronary atherosclerotic plaques do not evolve into MI due to lower degrees of inflammation and thrombosis. Hence, BPI may be used to differentiate MI from myocardial injury in ACS.

In the present observational study, we first confirmed the inflammatory characteristics of BPI by determining if plasma BPI levels correlated with levels of hs-CRP, IL-1β, MPO-DNA, and S100A8/A9 in patients with ACS. We then evaluated the association between plasma BPI levels and coronary atherosclerotic burden using the Gensini score. Lastly, we compared the diagnostic efficacy of BPI with other circulating inflammatory biomarkers for ACS and MI, and determined the prognostic value of BPI based on the TIMI and GRACE

scores. The findings presented here highlight the potential of BPI as a diagnostic and prognostic marker for ACS.

Several limitations to this study should be addressed. First, this study had a relatively small sample size. Second, although we excluded patients with acute infection, ongoing chronic inflammatory, autoimmune, or malignant diseases in this observational, cross-sectional study, the increase in plasma BPI levels in patients with ACS needs to be cautiously interpreted. Finally, we did not compare BPI with other risk factors for predicting the prognosis of ACS because we did not analyze major adverse cardiovascular events during follow-up. Future studies are needed to validate our findings of BPI as a biomarker in ACS and to elucidate the mechanisms underlying BPI-induced inflammation and thrombosis.

5. Conclusions

In conclusion, this is the first study that assessed plasma BPI levels in patients with ACS. We showed that circulating BPI levels were increased in patients with ACS and positively correlated with blood neutrophil counts and levels of hs-CRP, IL-1β, MPO-DNA, and S100A8/A9. ROC curve analysis shows that BPI has diagnostic value for both ACS and MI. Also, plasma BPI levels were positively correlated with the severity of coronary atherosclerosis in ACS. Therefore, BPI was associated with systemic inflammation in ACS and may be involved in NET- and neutrophil-mediated atherosclerosis and atherothrombosis. The potential role of BPI as a diagnostic and prognostic biomarker for ACS warrants further investigations.

Funding

Availability of data and material

The datasets generated and analyzed during the present study are available from the corresponding author upon reasonable request.

Ethics approval

This study was approved by the Ethics Committee of The First Affiliated Hospital of Bengbu Medical College (Bengbu, China). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/ or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent to participate

All participants gave written informed consent.

Consent for publication (include appropriate statements)

Not applicable.

CRediT authorship contribution statement

Shicheng Yu: Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition, Conceptualization. **Haoxuan Jia:** Visualization, Validation, Formal analysis. **Zheng Li:** Project administration, Methodology, Investigation. **Shengkai Ding:** Visualization, Supervision. **Fengyun Li:** Supervision, Project administration. **Pan Xu:** Investigation, Data curation. **Yuan Tian:** Software, Resources. **Lingling Ma:** Project administration, Methodology. **Fudong Qian:** Validation, Supervision, Software. **Miaonan Li:** Methodology, Investigation. **Nana Zhang:** Formal analysis, Data curation. **Hongju Wang:** Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32470.

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