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Original Research

Predictive and prognostic value of v-set and transmembrane domain-containing 1 expression in monocytes for coronary artery disease

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The aim of this study was to investigate the correlation between v-set and transmembrane domain-containing 1 (VSTM1) expression and incidence of major adverse cardiac events (MACE) in patients with coronary heart disease (CHD). A total of 310 patients were divided into a non-acute coronary syndrome (non-ACS) group (containing the stable angina group, and the asymptomatic coronary artery diseaseand other patients group) and an ACS group (containing unstable angina and acute myocardial infarction patients). Monocytic VSTM1 expression levels (assessed via average fluorescence intensity derived from antibody binding to VSTM1) in each group were detected and analyzed. The cut-off value of monocytic VSTM1 expression to predict the onset of ACS and MACE was confirmed. VSTM1 expression in monocytes from the ACS group was lower than that of the non-ACS group. The incidence of MACEs in the high VSTM1-expression group was much less than that of those in the low VSTM1 expression group at the 1 year follow-up stage. VSTM1 expression had an independent-inversed association with increased incidence of MACE and ACS. VSTM1 expression in monocytes may help to predict the occurrence of ACS in patients with CHD, and moreover it may provide the means to evaluate MACE prognosis during CHD patient follow-up.

Keywords

Coronary artery disease; VSTM1; Predictive and prognostic value; Acute coronary syndrome; Stable coronary artery disease; Atherosclerosis

1. Introduction

Coronary heart disease (CHD), also known as coronary artery disease (CAD), is one of a series of coronary artery diseases affecting hundreds of millions of people worldwide. It is the leading cause of death in the world and typically develops as the result of atherosclerosis (AS) build-up in the arteries [1, 2]. The unitability of atherosclerotic plaque primarily contributes to acute cardiovascular events, such as ST-elevation acute coronary infarction, non-ST-elevation myocardial infarction and unstable angina, these diseases are collectively known as acute coronary syndrome (ACS) [3, 4]. Monocytes/macrophages are a critical cell type in the pathological process of AS, which involves the initiation, progression and eventually rupturing of atherosclerotic atheroma [5, 6]. The formation of foam cells, which have a monocyte/macrophage origin, are the hallmark of atherogenesis [7]. Recent studies have shown the core status of monocytes/macrophages in the formation and progression of AS. High monocyte/macrophage biological function is one of the principal factors associated with plaque instability which induces ACS [8].

V-set and transmembrane domain-containing 1 (VSTM1), also known as signal inhibitory receptor on leukeocytes-1 (SIRL1), is a recently discovered cell membrane receptor. VSTM1 is located on human chromosome 19q. 13.4, adjacent to the region of the leukocyte receptor complex, which is highly similar to many of the receptor proteins regulating leukocyte function, such as LAIR-1 [9]. As a membrane receptor, VSTM1 consists of extracellular, transmembrane, and intracellular sequences. Its intracellular sequence is highly conserved between different species and genera, suggesting the importance of its function. VSTM1 is composed of two ITIM (immunoreceptor tyrosine-based inhibitory motif) regions, which affect its downstream targets through tyrosine phosphorylation [10]. VSTM1 is mainly distributed in myeloid cells, especially on the surface of monocytes [11–13]. It has been reported that VSTM1 expression is negatively correlated with the severity of the inflammation [9, 14]. Simultaneously, similar phenotypic changes of proinflammatory cytokine-tumor necrosis factor (TNF-α) have been unveiled in monocytes [11]. Coincidentally, an *in vivo* study has also demonstrated that inflammatory activity in pneumonia has phenotypes with the opposite trends to VSTM1 expression [15]. Moreover, VSTM1 in phagocytes sets an activation threshold to prevent inappropriate production of oxygen radicals. Upon

infection, VSTM1 expression is downregulated, allowing microbial killing and clearance of the pathogen [12]. In summary, the formation of a vulnerable plaque is a kind of sterile inflammatory process [16], the hyperfunction of monocytes/macrophages plays a central role, and VSTM1 may effectively modulate monocyte function, especially in aseptic inflammation-related diseases. However, no relevant research has focused on the significance of VSTM1 in the occurrence and aggravation of CHD. Therefore, we hypothesized and conducted a study investigating whether VSTM1 contributes to the formation and stability of coronary atherosclerotic plaques, which could also act as a predictive and prognostic factor when evaluating CHD.

To test this hypothesis, monocytes were isolated from the peripheral blood of CHD patients to assess the relationship between monocytic VSTM1 expression and severity of CHD, in addition to investigating the value of VSTM1 as a tool for prognosis evaluation in ACS patients.

2. Methods

2.1 Study population

Patients were enrolled between October 2018 to March 2019. CHD diagnosis was confirmed according to coronary artery branch diameter loss of $\geq$50% through coronary angiography (CAG), these patients were then enrolled into the study. The diagnosis of ACS and MACE were confirmed in accordance with the 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC) [17] and the 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC) [18]. The diagnosis of chronic coronary syndrome (CCS) was confirmed in accordance with the 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes [19].

The patient inclusion criteria were: (1) Age $\geq$18 years and $<$85 years; (2) Acute coronary syndrome (unstable angina, non-ST elevated myocardial infarction, ST elevated myocardial infarction), with an onset of symptoms during the previous 24 hours and positive troponin-I or troponin-T; (3) Where an initial invasive strategy was chosen (the patient was expected to undergo coronary angiography from admission). (4) Patient agreed to comply with follow-up evaluations. The exclusion criteria were: (1) Patients with severe hepatic insufficiency (defined as Child Pugh Class C); (2) An estimated glomerular filtration rate of less than 50 mL/min per 1.73 m^2; (3) Complicated infection present; (4) Major surgery undertaken within the last 2 months; (5) History of trauma; (6) Known hypersensitivity/contraindication to aspirin, clopidogrel, prasugrel, ticagrelor, heparin or bivalirudin, or sensitivity to contrast media, which can't be adequately pre-medicated; (7) Other medical illness (e.g., cancer or congestive heart failure) or known history of substance abuse (alcohol, cocaine, heroin etc.), as per physician judgment, that may cause non-compliance with the protocol or confound the data interpretation or could be associated with a limited life expectancy. Unless a contraindication existed, the patients received coronary revascularization, aspirin, clopidogrel, cilostazol, tirofiban, low-molecular-weight heparin (LMWH), ACEI/ARB, beta-blockers, calcium channel blockers, nitrates, digitalis, and diuretics according to the clinical conditions (see Fig. 1).

2.2 Definitions

A total of 310 patients that met the inclusion criteria but not the exclusion criteria were included in this study. These patients were divided into a non-ACS group and an ACS group based on clinical symptoms, laboratory examination and CAG results. The non-ACS group included the stable angina group, and the asymptomatic coronary artery disease and other patients, whereas the ACS group included unstable angina (UA) and acute myocardial infarction (AMI) patients. The AMI group contained non-ST-elevation myocardial infarction (NSTEMI) and ST-elevation-myocardial infarction (STEMI) patients.

Stable angina was defined as a rapid, temporary, reversible ischemia-hypoxia syndrome due to increased myocardial load on the basis of coronary artery stenosis. Asymptomatic coronary artery disease or 'others' were defined as patients who did not have chest pain and myocardial ischemia when conducting daily activities while the CAG showed coronary stenosis and an insufficient coronary blood supply. Unstable angina was defined as angina at rest, accelerating angina, or new-onset angina, without concomitant elevation of cardiac markers. NSTEMI was defined as ischemic symptoms with elevated cardiac markers in the absence of ST-elevation on ECG. STEMI was defined as continuous chest pain lasting $>$30 min, arrival at the hospital within 12 h from the onset of chest pain, ST-segment elevation $>$0.1 mV in $\geq$2 contiguous leads or new left bundle-branch block on the 12-lead ECG, and elevated cardiac markers (creatine kinase-MB or troponin T/I).

The one year follow-up MACE events included cardiac death, non-fatal myocardial infarction, target vessel revascularization (TVR) and target lesion revascularization, (TLR). Cardiac death was defined as any death attributable to a cardiovascular event (such as myocardial infarction, low output failure, fatal arrhythmias). Non-fatal MI was defined as, after 8 hours of PPCI, a two-fold elevation of the upper limit of normal creatine kinase myocardial band (CK-MB) or troponin levels after 8 hours of PPCI. In addition, this was accompanied by one or more of the following symptoms: new/recurrent sustained ischemic chest pain, hemodynamic decompensation, or new/recurrent ST elevation/depression of $\geq$0.1 mV. TVR was defined as any repeat percutaneous intervention or surgical bypass of any segment of the target ves-

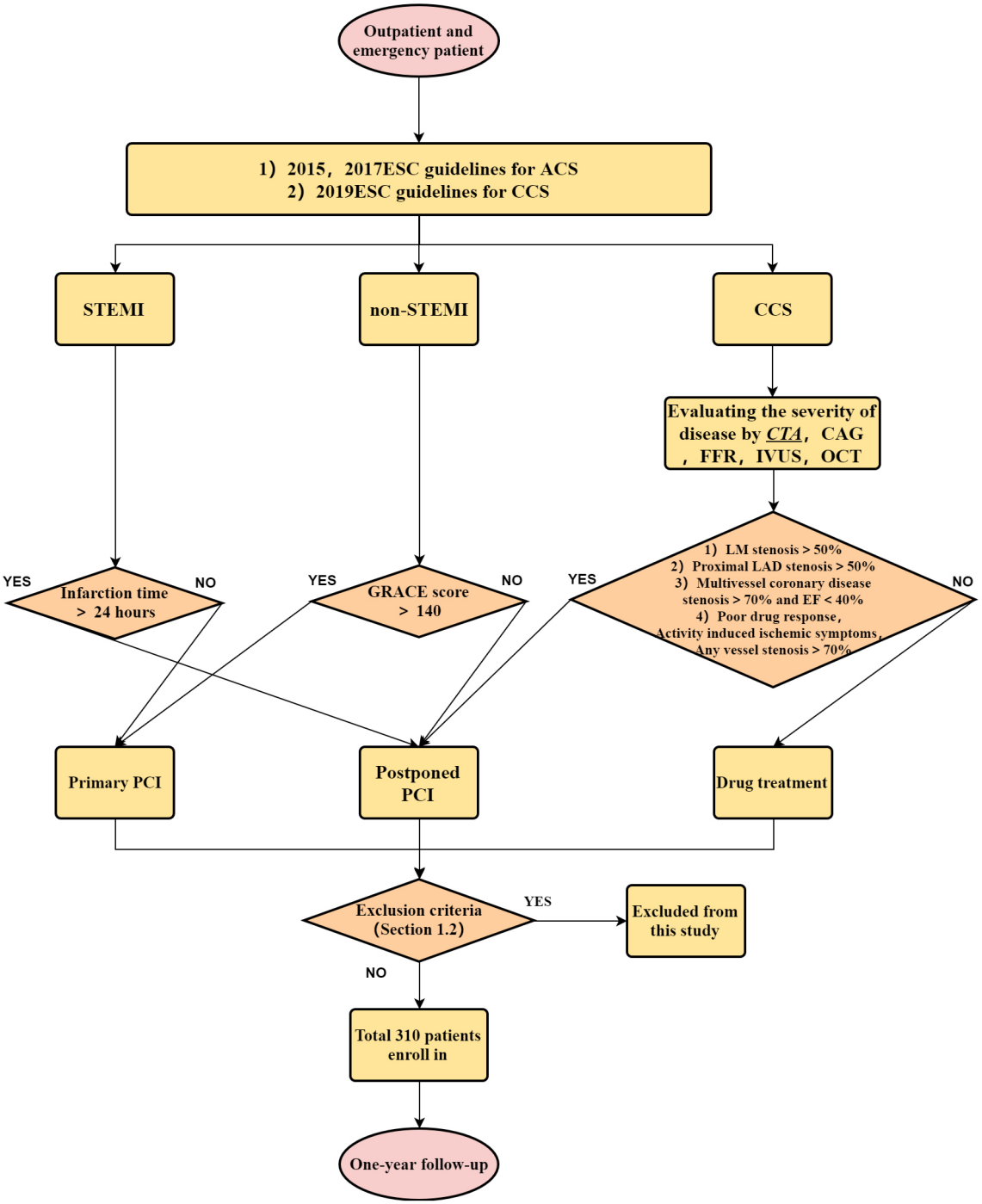

Fig. 1. Flow chart of the study.

sel despite the stent restenosis. TLR was defined as in-stent restenosis, which involves revascularization of the lesion site of the target vessel.

2.3 Data collection

The basic data about each patient was retrieved from the medical records, this included gender, age, history of hypertension, diabetes, cerebral infarction, smoking, glycosylated hemoglobin, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and other details pertinent to the study. CAG was performed using an FD20 cardiovascular angiography instrument (Philips, the Netherlands). The standard Judkins technique or right radial artery approach was used to determine the location and degree of stenosis.

2.4 Determination of VSTM1

The expression of VSTM1 in monocytes was detected using flow cytometry. After admission, the relevant clinical data collection and biochemical index examination were conducted for each patient. Briefly, 2 mL of venous blood

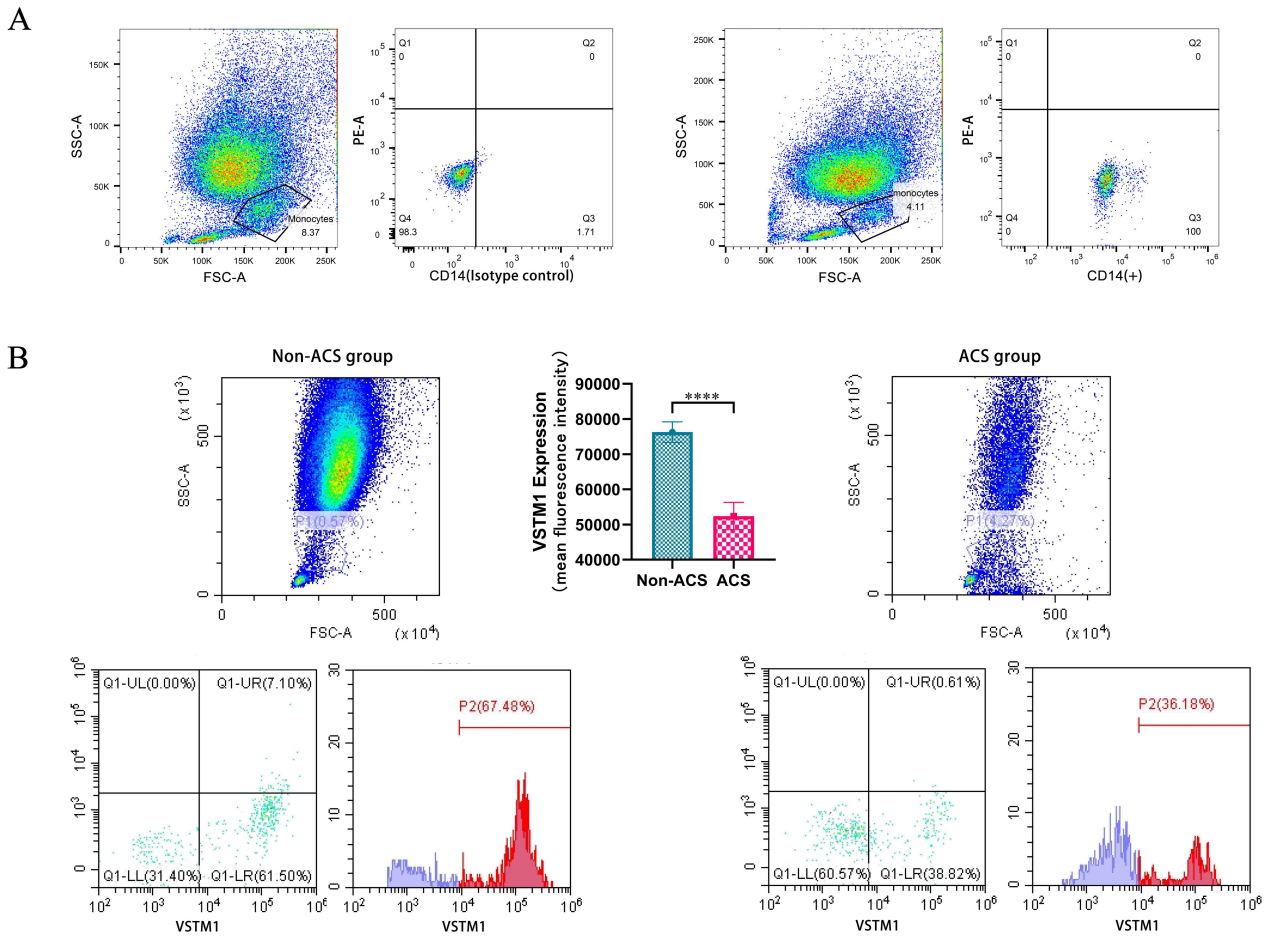

Fig. 2. Monocyte identification and VSTM1 expression detection in monocytes using FCM. (A) Identification of monocytes using anti-CD14 and its isotype control. (B) Differential expression of VSTM1 in monocytes from the non-ACS and ACS groups was assessed by FCM (mean fluorescence intensity) using anti-VSTM1. The results revealed that VSTM1 expression in the non-ACS group was higher than the ACS group ($P < 0.0001$, *$P < 0.05$, **$P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

was collected from each patient on their admission day, this was mixed with EDTA-K anticoagulants. Following centrifugation for 5 min, the upper aqueous phase containing the leukocytes was collected. Thereafter VSTM1 within/on the monocytes was labeled using an indirect immunofluorescence antibody. The leukocyte suspension was mixed with human anti-CD14, followed by incubation at 4 °C for 30 min. The reaction was terminated by adding phosphate buffer saline (PBS), the mixture was centrifuged at 400 g (1500 rpm) for 5 min, then the supernatant was discarded. The cell pellet was washed twice with PBS, followed by centrifugation for 5 min, and the supernatant was again discarded. Next, the cell suspension was mixed with 1 uL secondary antibody, followed by incubation at 4 °C for 30 min. The labeled leukocytes were subjected to flow cytometry within 1 h.

The VSTM1 fluorescence intensity associated with the monocytes was determined via flow cytometry using a Becton Dickinson FACSCalibur. The excitation wavelength was set at 488 nm. Primarily, leukocytes with strained with anti-CD14 (ab183322, Abcam, Boston, MA, US) and isotype control (SA00006-2, proteintech, Chicago, IL, US), were sorted according to cell size, intracellular particle density, forward angle scattering (FSC), and lateral angle scattering (SSC), the leukocytes were divided into three subgroups as follows: neutrophils, lymphocytes, and monocytes. The monocytes gate underwent further study and the purity of monocytes was confirmed based on the fluorescence intensity of CD14. Subsequently leukocytes strained with anti-VSTM1 (ab189494, Abcam, Boston, MA, US) were used to detect the degree of VSTM1 protein expression on the cell membrane, and mean fluorescence intensity was applied to assess the expression of VSTM1 in the monocytes subgroup. Approximately 10,000 monocytes were analyzed using this predetermined gate. The average fluorescence intensity was visualized using a histogram.

2.5 Data statistics

Statistical analysis was performed using SPSS software (version 22.0, IBM Corp., Chicago, IL, USA). Categorical variables were shown as frequencies and compared using the

Table 1. Baseline data in each group [measurement data (values represent mean ± SD or n%)].

	Non-ACS				ACS				*P*
	SA (N = 154)	N%	Asyp (N = 51)	N%	UA (N = 49)	N%	AMI (N = 56)	N%	
Age	64.6 ± 12.47	/	68.18 ± 10.36	/	70.06 ± 10.83	/	64.80 ± 10.28	/	0.229
Male	88 (57.14%)	28.39	33 (64.71%)	10.65	30 (61.22%)	9.68	40 (71.43%)	12.90	0.661
Smoke	31 (20.13%)	10.00	19 (37.25%)	6.13	18 (36.73%)	5.81	27 (48.21%)	8.71	0.024
Hypertension	97 (62.99%)	31.29	27 (52.94%)	8.71	30 (61.22%)	9.68	30 (53.57%)	10.00	0.631
Diabetes	31 (20.13%)	10.00	16 (31.37%)	5.16	15 (30.61%)	4.84	19 (33.93%)	6.13	0.422
Total cholesterol (mg/dL)	172.9 ± 41.8	/	168.7 ± 44.1	/	170.7 ± 47.6	/	190.5 ± 39.4	/	0.031
Triglycerides (mg/dL)	124.2 ± 88.1	/	130.9 ± 91.3	/	132.8 ± 93.0	/	137.8 ± 98.3	/	0.682
LDL cholesterol (g/dL)	95.5 ± 20.1	/	91.2 ± 6.0	/	109.4 ± 25.3	/	119.5 ± 30.9	/	0.004
HDL holesterol (mg/dL)	41.4 ± 10.8	/	39.8 ± 8.1	/	39.4 ± 9.7	/	37.9 ± 6.6	/	0.401

ACS, acute coronary syndrome; SA, stable angina group; Asyp, asymptomatic coronary artery disease or others group; UA, unstable angina group; AMI, acute myocardial infarction group; LDL, low density lipoprotein; HDL, high density lipoprotein; the same abbreviations are used in the other tables.

Table 2. VSTM1 expression levels in each group (values represent mean ± SD).

	Non-ACS		ACS		*P*
	SA (N = 154)	Asyp (N = 51)	UA (N = 49)	AMI (N = 56)	
VSTM1	76284.52 ± 29920.60	77704.48 ± 25361.91	52396.69 ± 28109.62	53179.35 ± 20116.80	<0.001
	76637.78 ± 27812.41		52814 ± 24111.32		

chi-square or Fisher exact test as appropriate. Normally distributed continuous variables are shown as mean and standard deviation, and the groups were compared using a Student's *t*-test. Non-normally distributed continuous variables were shown as median with interquartile ranges, and compared using a Mann-Whitney U test. A 2-sided *P*-value of <0.05 was considered statistically significant. A LSD (least significant difference) method and homogeneity test of variance were conducted prior to ANOVA which was used for selected for multiple comparisons. A receiver operating characteristic (ROC) curve was used to identify the cut-off value of VSTM1 expression using differentiated patients comparing patients with and without MACE. Time-to-event rates are shown as Kaplan-Meier estimates and were compared with the log-rank test. Odds ratio (OR) between CHD risk factors (including VSTM1, LDL-c, diabetes, hypertension, smoking status, gender, and age) and risk of ACS were summed up.

3. Results

3.1 Baseline characteristics

The baseline demographic characteristics and laboratory data for both the non-ACS and ACS groups are displayed in Table 1. There were no significant differences in any of the factors such as age, gender, history of hypertension, history of diabetes, and triglyceride between the groups ($P > 0.05$). The number of smokers, the levels of total cholesterol and LDL in the ACS group were higher compared with the non-ACS group ($P < 0.05$).

3.2 VSTM1 expression levels in each group

As shown in Fig. 2, anti-CD14 and its isotype control were initially used to identify monocytes with the aid of flow cytometry (FCM) (Fig. 2A). In subsequent studies, anti-VSTM1 was applied to detect the differential expression of VSTM1 in monocytes from the non-ACS and ACS groups. The FCM results revealed that VSTM1 expression was higher in the non-ACS than the ACS group ($P < 0.0001$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, Fig. 2B). In Table 2, the expression of VSTM1 in the stable angina group, the asymptomatic coronary artery disease or 'others' group, and the UA and AMI groups were observed as 76284.52 ± 29920.60, 77704.48 ± 25361.91, 52396.69 ± 28109.62, 53179.35 ± 20116.80, respectively. The expression of VSTM1 in the ACS group was significantly lower when compared to the non-ACS group ($P < 0.01$).

3.3 Cut-off value of VSTM1 expression for predicting patients with MACE

In Fig. 3, it was found out that the cut-off value of VSTM1 expression to differentiate patients with versus those without MACE was 66657 (area under the curve =0.658 (0.619–0.747), $P < 0.001$, sensitivity =83.3%, specificity =46.9%). According to the cut-off value, the VSTM1 expression was divided into two groups as follows: a low-VSTM1 group (VSTM1 <66657) and a high-VSTM1 group (VSTM1 >66657). Table 3 shows the baseline characteristics grouped according to the cut-off value. Compared with the higher value group, smoking and high LDL-C patients were lower in number in the low value group. The incidence rate of non-ACS (SA and Aysp) in the high value group was higher than that observed in the low value group (57.7% vs.31.6%, $P < 0.001$; 20.0% vs.8.4%, $P = 0.011$, respectively). Inversely, the number of ACS group patients in the low-value group increased significantly when compared to those in the high-value group (33.3% vs.6.5%, $P < 0.001$; 50.5% vs.3.7%, $P <$

Table 3. The patient baseline characteristics grouped according to the cut-off value.

	<66657 (N = 95)	>66657 (N = 215)	*P*-value
Age	67.21 ± 13.72	66.54 ± 9.87	0.627
Male	66 (69.5%)	125 (58.1%)	0.059
Smoke	46 (48.4%)	49 (22.8%)	<0.001
Hypertension	62 (65.3%)	122 (56.7%)	0.159
Diabetes	22 (23.2%)	59 (27.4%)	0.429
LDL cholesterol, mg/dL	116.8 ± 26.7	91.9 ± 24.5	<0.001
Triglycerides, mg/dL	136.0 ± 98.4	130.0 ± 97.0	0.618
SA	30 (31.6%)	124 (57.7%)	<0.001
Asyp	8 (8.4%)	43 (20.0%)	0.011
UA	35 (33.3%)	14 (6.5%)	<0.001
AMI	48 (50.5%)	8 (3.7%)	<0.001

0.001, respectively).

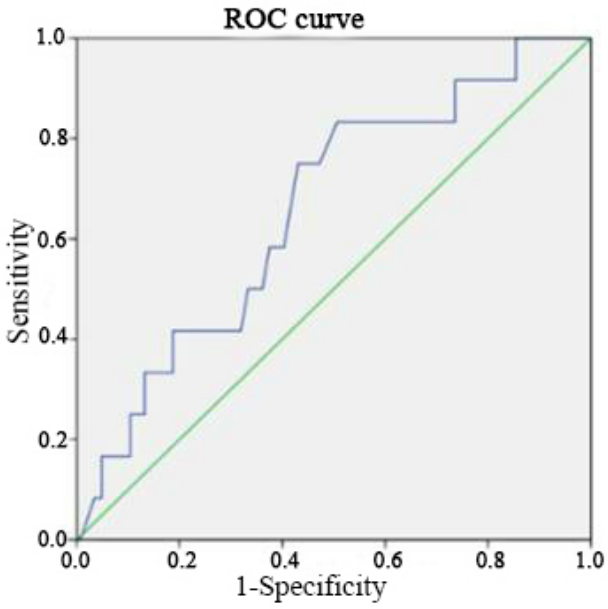

Fig. 3. The receiver operating characteristic (ROC) curve to detect the best cut-off value of VSTM1 expression in predicting patients with and without MACE.

Best cut-off: 66657; AUC: 0.658; Sensitivity: 83.3%; Specificity: 49.3%; PPV: 61.94%; NPV: 74.7%.

After the traditional risk factors had been adjusted for, smoking and serum LDL-c level were risk factors that increased the incidence of ACS (odds ratio [or]: 2.325; 95% confidence interval [CI]: 1.409–3.837, $P < 0.001$; odds ratio [or]: 1.876; 95% confidence interval [CI]: 1.212–2.321, $P = 0.007$). VSTM1 showed a protective effect in alleviating the incidence of ACS (odds ratio [or]: 0.78; 95% confidence interval [CI]: 0.589–0.924, $P = 0.041$; Fig. 4).

3.4 MACE at 1-year follow-up

A total of 11 patients experienced MACE during their 1 year of follow-up (Table 4). The Kaplan-Meier curve (Fig. 5) showed that the high-VSTM1 group had a lower incidence of MACE compared with the low-VSTM1 group at the 1 year follow up stage ($P = 0.04$). In accordance with the presence or absence of MACE, the patients were divided into two groups as follows, MACE group and non-MACE group. Table 5 shows the baseline characteristics of the two groups. Compared with the non-MACE group, the proportion of smokers, and the levels of LDL, observed in the MACE group were significantly higher in comparison to the non-MACE group ($P < 0.005$). The expression of VSTM1 in the MACE group was significantly lower in comparison to the non-MACE group (53989.84 ± 20349.05 vs.75599.42 ± 22271.97, $P = 0.002$).

Table 4. MACE at 1-year follow-up.

	Non-ACS		ACS	
	SA (N = 154)	Asyp (N = 51)	UA (N = 49)	AMI (N = 56)
Cardiac death	0	0	0	0
Non-fatal MI	0	0	0	1
TVR	0	0	0	0
TLR	0	2	3	5

MACE, major adverse cardiac event; MI, myocardial infarction; TVR, target vessel revascularization; TLR, target lesion revascularization.

4. Discussion

In the present study, the independent correlation between monocyte expression of VSTM1 and the severity of the lesion natures in different stages of CHD, that is, the predictive value of VSTM1, were explored. VSTM1 provided an objective and effective method by which prognosis could be estimated in CHD patients undergoing PCI, and could therefore act as a potential biomarker in detecting plaque vulnerability and assessing the incidence of MACE events in CHD patients.

It is known that inflammation contributes greatly to the formation of atherosclerotic plaques [20, 21]. AS is amonocyte/macrophage inflammatory response to the "pathogenic" lipoproteins invading the arterial wall, and these cells promote the initiation and progression of AS, and dysfunction of monocytes/macrophages act as a critical factor in the pathological progression and vulnerability of atherosclerotic plaques [6, 7, 22–24]. Therefore, the migration and invasiveness of macrophages both play critical roles in the occurrence and development of AS [25, 26]. Furthermore, it is now generally believed that the enhancement of intimal monocytes/macrophages accumulation can deteriorate the vulnerability of the plaques, or make the plaques prone to rupture, leading to thrombosis in atherosclerosis [27, 28]. Based on these factors, vulnerable atherosclerotic plaques in humans present with a thin-cap fibroatheroma (TCFA) and a large lipid-rich core with monocyte/macrophage infiltration [29, 30], and the hyperfunction of phagocytosis, migration, chemotactic responses of monocytes/macrophages are prone to enlarging the lipid core and weaken the fibrous cap. Mechanically, the formation of TCFA and a large lipid-rich core

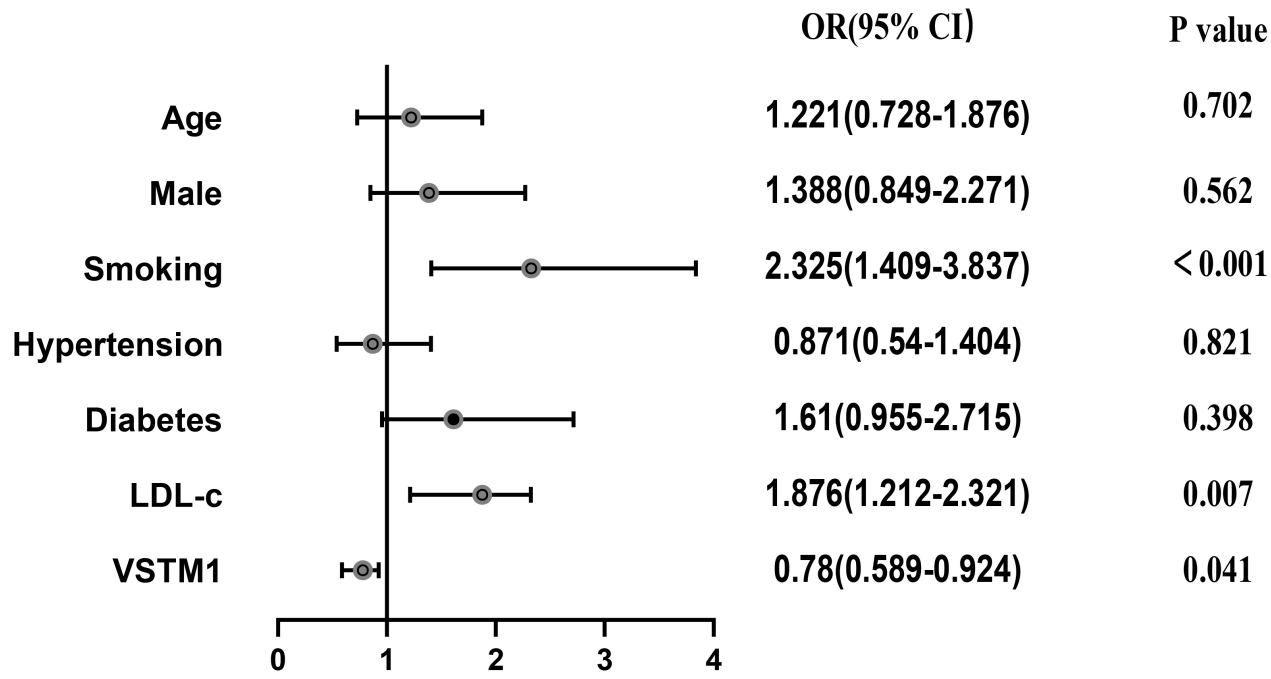

Fig. 4. Coronary heart disease risk factors. Smoking and serum LDL-c levels were considered risk factors that increased the incidence of ACS (odds ratio [or]: 2.325; 95% confidence interval [CI]: 1.409–3.837, $P < 0.001$; odds ratio [or]: 1.876; 95% confidence interval [CI]: 1.212–2.321, $P = 0.007$). VSTM1 showed a protective effect in alleviating incidence of ACS (odds ratio [or]: 0.78; 95% confidence interval [CI]: 0.589–0.924, $P = 0.041$).

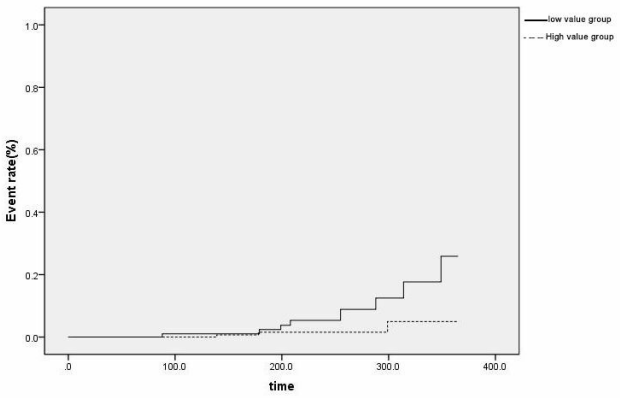

Fig. 5. Kaplan–Meier graphs comparing event rate between the study groups. The Kaplan-Meier curves for the high VSTM1 and low VSTM1 groups ($P = 0.04$).

are the most important determinants of plaque rupture in the occurrence of ACS, which may be interpreted as a process of plaque stabilization [8, 31–33].

Importantly, VSTM1 expression in the ACS group was significantly lower when compared with the non-ACS patients (Table 2). A total of 310 CAD patients were followed up for 1 year, and they were sub-divided into a low-VSTM1 group (VSTM1 <66657) and a high-VSTM1 group (VSTM1 >66657) for analysis. The incidence rate of acute coronary events leading to the occurrence of ACS in the high-value group was lower than that observed in the low-value group. Inversely, the incidence rate of acute coronary events in the low value group increased significantly compared with the high value group (Table 5). Besides this, the incidence of MACE in the high-VSTM1 group was much lower when compared with the low-VSTM1 group (Fig. 4).

Our study unveiled that the objective assessment of reduced VSTM1 expression was independently associated with an increased incidence of AMI after the other cardiovascular risk factors had been considered. At the 1 year follow-up stage, the ratio of revascularization and combined adverse events in the ACS group was higher in comparison to the non-ACS group. This might be attributed to VSTM1 acting as a negative index of inflammation. In other words, the more unstable the plaque, the lower the level of VSTM1 expression. Studies have previously found that VSTM1 expression in ACS patients was significantly lower than that in patients with stable angina pectoris. The pathological basis of ACS is atherosclerotic plaque rupture and thrombosis, which may not occur even in severe cases of patients with stable CAD. Our study showed that VSTM1 acts as a protective factor and is independently associated with the prognosis of CHD even after other cardiovascular risk factors were adjusted and accounted for. This novel study reported the impacts of VSTM1 expression on AS and its association with prognosis. Our findings suggested that VSTM1 expression was an important marker, which reflected the development of unstable plaques in CAD.

Table 5. The patient baseline characteristics in the MACE group and the non-MACE group.

	Total	MACE group	Non-MACE group	*P*
	(310 patients)	(11 patient)	(299 patients)	
Age	68.04 ± 12.05	69.30 ± 8.92	67.73 ± 12.78	0.687
Man	191	8	183	0.440
Smoke	95	8	87	0.006
Hypertension	184	7	177	0.768
Diabetes	81	6	75	0.067
Total cholesterol, mg/dL	177.2 ± 43.5	199.4 ± 54.8	175.5 ± 42.6	0.072
HDL cholesterol, mg/dL	39.0 ± 15.0	37.5 ± 15.0	39.1 ± 15.0	0.728
LDL cholesterol, mg/dL	107.1 ± 36.9	119.7 ± 20.6	106.2 ± 16.2	0.008
Triglycerides, mg/dL	134.0 ± 80.5	144.2 ± 94.7	133.2 ± 79.4	0.654
HbA1c, %	6.5 ± 1.4	6.7 ± 1.2	6.5 ± 1.5	0.663
Creatinine, mg/dL	1.1 ± 0.8	1.11 ± 1.4	1.1 ± 0.7	0.965
LVEF, %	59.9 ± 11.2	58.4 ± 8.6	60.1 ± 11.4	0.625
Prior MI	15	0	15	0.446
Prior PCI	56	4	52	0.108
Prior CABG	4	0	4	0.699
VSTM1	61093.51 ± 27000.11	53989.84 ± 20349.05	75599.42 ± 22271.97	0.002

5. Limitations

Limitations existed within our study: (1) In our heart center, due to strict follow-up and medication management (including DAPT, statin, ezetimibe, PCSK9 inhibitors and other interventions), the incidence of MACE was kept to a relatively low degree, which led to the sample size being relatively small in this study. But the sample of MACEs derived from the CHD patient population, which was overall relatively large, ensures this information was relatively complete. (2) It is not clear whether monocytic VSTM1 expression or monocytic dysfunction induced by abnormal expression of VSTM1 may interact with other CHD risk factors such as smoking, diabetes mellitus, CKD and so on. Therefore, the interaction between VSTM1 and related risk factors of CHD require further research.

6. Conclusions

Our study revealed that monocytic VSTM1 expression may reflect the severity of CHD, therefore it may be possible to partly predict the occurrence of ACS based on CCS. In addition, monocytic VSTM1 expression levels could act as predictors of MACE events, which may be an effective method of preventing and treating CHD.

Author contributions

JFZ, CQW, YQF and XQQ conceived, designed and funded the study. XFW, MCX, CYM performed the experiments and wrote the paper. EZ and QH reviewed and edited the manuscript. All authors read and approved the manuscript.

Ethics approval and consent to participate

The clinical study was approved by the hospital ethics review board of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine (SH9H-2019-H21-2). All experimental contents were in accordance with principles outlined in the Declaration of Helsinki.

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Conflict of interest

The authors declare no conflict of interest.

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