学位論文 博士（医学） 甲

High levels of stromal cell-derived factor-1α predict secondary cardiac events in stable patients with a history of myocardial infarction
（心筋梗塞後安定期の SDF-1α 値で将来の心イベントを予測できる）

松岡 聡志

山梨大学

High levels of stromal cell-derived factor-1α predict secondary cardiac events in stable patients with a history of myocardial infarction

Satoshi Matsuoka (MD)[a], Manabu Uematsu (MD)[a], Takamitsu Nakamura (MD, PhD)[a], Takuya Shimizu (MD)[a], Mika Futamata (MD)[a], Jun-ei Obata (MD, PhD)[a], Daisuke Fujioka (MD, PhD)[a], Kazuto Nakamura (MD, PhD)[a], Toru Yoshizaki (MD)[a], Kiyotaka Kugiyama (MD, PhD, FJCC)[a]

[a] *Department of Internal Medicine II, University of Yamanashi, Faculty of Medicine, Chuo, Yamanashi, Japan*

Key words: stromal cell-derived factor-1α, myocardial infarction, prognosis, cardiac events, inflammatory marker.

This paper was published in the following journal: Journal of Cardiology, 69(1), 320-325. doi.org/10.1016/j.jjcc.2016.06.011

Abstract

Background: We recently showed that stromal cell-derived factor (SDF)-1α, a proinflammatory mediator, is produced in infarcted myocardium and is associated with left ventricular (LV) adverse remodeling and progressive dysfunction following acute myocardial infarction (AMI). The current study examined whether SDF-1α levels in the peripheral vein can provide prognostic information of outcomes in stable patients with a history of MI.

Methods: Plasma levels of SDF-1α in the peripheral vein were measured by enzyme-linked immunosorbent assay in 192 stable patients with a history of MI. All patients were followed prospectively for a period of 90 months or until occurrence of one of the following cardiac events: cardiac death, non-fatal myocardial infarction, unstable angina requiring unplanned coronary revascularization, or worsening heart failure requiring hospital admission.

Results: During the follow-up period (77 ± 26 months), 30 patients had cardiac events. Multivariate Cox analysis revealed that high levels of SDF-1α (≥2162 pg/mL; a cut-off value determined by receiver-operating characteristic analysis) were a significant predictor of cardiac events, independent of traditional risk factors (HR: 1.98; 95% CI: 1.38–2.85; $p < 0.001$). The addition of high levels of SDF-1α to conventional risk factors including brain natriuretic peptide improved net reclassification improvement (NRI) and integrated discrimination improvement (IDI) (NRI 0.90, $p < 0.000$-1αnd IDI 0.05, $p = 0.002$).

Conclusions: High levels of SDF-1α predicted secondary cardiac events in stable patients with a history of MI. SDF-1α levels may be a useful risk assessment tool in patients with a history of MI.

Introduction

Acute myocardial infarction (AMI) induces the production of chemokines and growth factors that recruit neutrophils and phagocytes to the ischemic cardiac tissue, leading to clearance of dead cells and matrix debris [1,2]. This acute, localized inflammatory response is transient, and is followed by resolution of the inflammation [1,3]. However, prolonged inflammation leads to dilative remodeling and progressive dysfunction of the infarcted myocardium through matrix degradation and cardiomyocyte apoptosis [1,2]. Left ventricle (LV) adverse remodeling and dysfunction after MI are predictors of poor outcome following MI, and are associated with ventricular arrhythmias, heart failure, and increased mortality [4,5]. Stromal cell-derived factor (SDF)-1α/CXCL12 is a CXC chemokine with chemotactic effects on CXCR4-expressing progenitor cells [6]. We have recently shown that SDF-1α, a proinflammatory mediator, is produced in the infarcted myocardial lesion and released into the circulation in patients with AMI [7]. In addition, we showed that the myocardial production of SDF-1α in the chronic phase (6 months after MI) but not in the early phase (2 weeks after MI) was associated with LV adverse remodeling and progressive dysfunction in patients with AMI [7]. Thus, prolonged production of SDF-1α in the infarcted myocardium may exert detrimental effects on post-MI LV function in the chronic phase following MI. The prognostic value of SDF-1α levels in the peripheral vein was previously evaluated in patients with AMI/ acute coronary syndrome (ACS) [8,9]. However, it remains unclear whether SDF-1α levels can provide prognostic information in patients in the chronic phase of MI. The current study examined the prognostic value of SDF-1α levels in the peripheral vein in stable patients with a history of MI.

Methods

Study patients

The study screened 386 patients with a history of MI, who underwent cardiac catheterization in Yamanashi University Hospital between February 2003 and March 2009. All patients underwent routine blood testing at the time of discharge. The inclusion criteria were: (1) stable previous MI; (2) no episode of angina at rest and no changes in the frequency of angina in response to sublingual nitroglycerin in the previous 2 months. Exclusion criteria included: (1) ACS, stroke, cardiogenic shock, pulmonary edema, major surgery, trauma or serious infectious disease within 4 weeks prior to enrollment; (2) neoplasm, significant hepatic or inflammatory disease; (3) chronic renal failure or serum creatinine >2.5 mg/dL, congestive heart failure, or left main coronary artery disease; (4) other serious diseases. Finally, a total of 210 patients were enrolled in the study according to these inclusion and exclusion criteria. The study also included 31 control patients without echocardiographic findings of significant valvular disease (moderate or more regurgitation, stenosis), cardiomyopathy [chamber size dilatation, reduced left ventricular ejection fraction (LVEF), ventricular asynergy, ventricular hypertrophy, aneurysm], and pulmonary hypertension, chosen from among the 6-1αngiographically normal patients [without percutaneous coronary intervention (PCI) history] who were evaluated during the study period. Control patients served as a reference group for plasma SDF-1α concentrations. Each of the control patients underwent diagnostic coronary angiography for atypical chest pain at rest at Yamanashi University Hospital during the study period. Control patients fulfilled all of the following inclusion criteria: (1) no significant ST segment changes on 12-lead electrocardiogram (ECG) while having chest pain or on ambulatory ECG; (2) neither chest pain nor ST segment changes during the treadmill test; (3) no coronary artery spasm during provocation with intra-

coronary infusion of acetylcholine [10]. Clinical characteristics of patients with a history of MI and control patients are shown in Table -1αll study participants were ethnic Japanese. All patients gave written, informed consent at the time of enrollment. The study was approved by the ethics committee of Yamanashi University Hospital. The investigation conformed to the principles outlined in the 1975 Declaration of Helsinki.

Prospective study

Patients were followed every month in the hospital or with a clinic visit for 90 months, or until the occurrence of cardiac death, non-fatal MI, refractory unstable angina pectoris (uAP) requiring unplanned coronary revascularization, or decompensated heart failure. The time to the first event was evaluated prospectively. Cardiac death was confirmed by hospital records. Acute MI and uAP were diagnosed by the presence of acute ischemic symptoms lasting $\geq$20 min within 48 h prior to hospital admission, and ECG changes. Acute MI was diagnosed when creatine kinase-MB levels increased to at least 2 times the upper limit of normal or when troponin T levels were >0.1 ng/mL [11]. The diagnoses of MI and uAP were confirmed by coronary angiography. Decompensated heart failure was defined as resting dyspnea with progressive fluid retention requiring hospitalization and treatment with an intravenous diuretic. Follow-up data were collected from the patients' primary physicians every 3 months by two blinded investigators (T.N., J.O.). During the follow-up period, standard medications were prescribed to all patients according to the guidelines of the American Heart Association [12], as shown in Table 1. Diet and lifestyle recommendations were continued throughout the follow-up period.

Laboratory measurements

Venous blood was obtained from all patients on the morning of discharge after a 12-h overnight fast. The initial volumes of each blood sample, including those forcibly drawn, were discarded. Serum and EDTA-plasma were aliquoted and stored at –80℃ until time of analysis. Plasma SDF-1α levels were measured by enzyme-linked immunosorbent assay using a commercial kit (R & D Systems, Minneapolis, MN, USA). In our laboratory, the intra- and inter-assay coefficients of variation were 1.9% and 3.5%, respectively. The minimal detection limit of this assay was 18 pg/mL. Serum C-reactive protein (CRP) levels were assayed by rate nephelometry (Dade Behring, Tokyo, Japan). Plasma levels of brain natriuretic peptide (BNP) were measured by immunoradiometric assay (Shionogi Pharmaceutical, Osaka, Japan). Estimated glomerular filtration rate (eGFR) was calculated by the Modification of Diet in Renal Disease study equation. Echocardiographic LVEF was calculated by the motion-mode method using the Teichholz formula [13].

Statistical analysis

All descriptive data were expressed as mean ± SD, median, or frequency (%). The Shapiro–Wilk test showed that age, body mass index (BMI), heart rate, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), hemoglobin A1c (HbA1c), LVEF, SDF-1α, BNP, and CRP were not normally distributed, hence, these variables were expressed as the median and inter-quartile ranges (25th and 75th percentiles). Non-normally distributed variables were log-transformed before the analysis. Continuous variables were compared between the two groups using the unpaired t-test or the Mann–Whitney U-test, as appropriate. Frequencies were compared using the chi-square test. For comparisons of the mean value of ≥3 groups, one-way analysis of variance (ANOVA) followed by a Scheffe test for post hoc comparisons was employed. The correlation between

the two groups was examined using univariate and multivariate linear regression analyses. Kaplan–Meier analysis was performed on the 2 groups and was based on a cut-off SDF-1α level. The cut-off level of SDF-1α (2162 pg/mL) was determined by receiver-operating characteristic (ROC) analyses of SDF-1α in the study patients with a history of MI (Fig. 1). The predictive values were assessed by univariate or forward stepwise multivariate Cox proportional hazards analysis. For a forward stepwise multivariate Cox hazard analysis, the significance level was set at 0.1. In the univariate and multivariate Cox proportional hazards analyses, continuous variables were estimated for 1-SD change. Dichotomous variables were coded as 1 for the presence of, and 0 for the absence of, each factor. The following factors were included as dichotomous variables: systemic hypertension (≥140/90 mmHg or use of an antihypertensive medication) [14], diabetes mellitus (DM) (as defined by the American Diabetes Association or use of an antidiabetic medication) [15,16], smoking history (defined as smoking ≥10 cigarettes/day for ≥10 years), atrial fibrillation (including paroxysmal atrial fibrillation recorded with an ECG), and multivessel disease. The Cox models included only variables that showed proportionality in a Schoenfeld residuals test. The category-free net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) were used to examine the additive effects of SDF-1α and BNP levels on the predictive value of the baseline model that consisted of conventional cardiovascular risk factors. Conventional risk factors included age, male gender, DM, hypertension, smoking, levels of LDL-C, HDL-C, CRP, LVEF, and multivessel disease. All probability values were presented as 2-tailed with statistical significance inferred at $p < 0.05$. All confidence intervals were computed at the 95% level. Statistical analyses were performed using STATA 10.0 (StataCorp, College Station, TX, USA).

Power analysis

Our previous study showed that the composite endpoints occurred in approximately 52% of stable coronary artery disease (CAD) patients with high coronary risk factors and higher macrophage inhibitory factor (MIF) levels as an inflammatory marker, and in 18% of patients with lower MIF levels during 5 years of follow-up [17]. On the basis of that study, a total of 72 patients were required for a two-sided statistical analysis with sufficient statistical power of 0.90 (b = 0.10 and a = 0.05). This justified the number of patients (n = 220) included in the current prospective study.

Results

Over the course of the study, 7 patients were lost to follow up, and 11 patients were excluded due to non-cardiac related death during follow-up. A total of 192 patients completed the follow-up study (2–90 months, mean = 77 ± 26 months). During the follow-up period, 30 (15.6%) cardiac events occurred, including 5 cardiac deaths, 1 non-fatal MI, 12 uAPs requiring unplanned coronary revascularization, and 12 episodes of worsening heart failure. Patient characteristics are shown in Table 1.

Patients and SDF-1α levels

The time between blood sampling for SDF-1α level and the previous MI ranged from 3 to 26 months, with a mean of 7.7 ± 2.1 months. The time between onset of previous MI and blood sampling did not correlate with SDF-1α levels (r = –0.08, p = 0.28). As shown in Table 2, SDF-1α levels were positively correlated with age, BNP, and CRP but inversely correlated with eGFR in the univariate linear regression analysis (Table 2). SDF-1α levels did not significantly correlate with the frequency of any cardiovascular medication (Table 2).

Prevalence of PCI-related variables was similar between patients with high and low SDF-1α levels (Supplementary Table 1).

Prospective study

Patients (n = 82) with higher SDF-1α levels (≥2162 pg/mL, a cut-off value determined by ROC analysis) had 26 events during follow-up, whereas patients (n = 110) with lower SDF-1α levels (<2162 mg/dL) had 4 events ($p < 0.01$). The cut-off value of SDF-1α levels as 2162 pg/mL, determined by ROC analysis, provided sensitivity of 86.7%, specificity of 65.4%, and accuracy of 68.8% for prediction of future events (Fig. 1). Kaplan–Meier analysis demonstrated a significantly higher probability of a cardiac event in patients with higher SDF-1α levels compared with those with lower SDF-1α levels (Fig. 2). A univariate Cox proportional hazards analysis revealed that SDF-1α levels (HR 1.87; 95% CI 1.35–2.60), age (HR 1.83; 95% CI 1.21–2.78), DM (HR 2.73; 95% CI 1.30–5.75), atrial fibrillation (HR 2.93; 95% CI 1.02–8.42), multivessel disease (HR 2.50; 95% CI 1.02–6.12), LVEF (HR 0.66; 95% CI 0.47–0.94), eGFR (HR 0.60; 95% CI 0.40–0.88), and BNP levels (HR 1.37; 95% CI 1.17–1.60) were significantly associated with future cardiac events, as shown in Table 3. A forward stepwise multivariate Cox proportional hazards analysis demonstrated that SDF-1α levels remained significantly associated with future events after adjustment for potential confounding variables (HR 1.98; 95% CI 1.38–2.85).

Incremental effects of SDF-1α levels on the predictive value of traditional risk factors

Category-free NRI and IDI demonstrated the additive value of SDF-1α to the baseline conventional risk factors consisting of age, male gender, DM, hypertension, smoking, levels of LDL-C, HDL-C, and CRP, LVEF, and multivessel disease (NRI 0.77, $p = 0.0001$;

IDI 0.07, p = 0.001) (Table 4). When BNP was added to the baseline model of risk factors, SDF-1α had significant additive effect on the predictive value of the model (NRI 0.90, p < 0.0001; IDI 0.05, p = 0.002) (Table 4). In contrast, BNP had no significant additive effect on the predictive value of a risk factor model with SDF-1α (Table 4).

Discussion

The present study showed that high SDF-1α levels are a predictor of adverse outcomes in patients with a history of MI. Moreover, high SDF-1α levels had incremental effects on prognostic value of traditional risk factors in this patient population. Thus, SDF-1α levels may aid in risk assessment in stable patients with a history of MI. We have previously shown that SDF-1α is released from the infarcted myocardium into the coronary circulation in both the early (2 weeks after MI) and chronic phases (6 months after MI) following AMI [7]. The myocardial production and release of SDF-1α in the chronic phase of MI, rather than in the early phase, was associated with post-MI LV adverse remodeling and progressive dysfunction [7]. These results support the present finding that SDF-1α levels can predict secondary cardiac events in patients with a history of MI. Animal experiments have demonstrated that local infusion or over-expression of SDF-1α attenuates myocardial ischemic injury following LV dysfunction [18–22]. In contrast, other experimental studies have shown that SDF-1α/CXCR4 signaling worsens post-MI LV function due to increased inflammatory injury and apoptotic mechanisms [23–26]. Thus, the effect of SDF-1α on post-MI LV function in animal models remains unclear. In animal models, the effects of endogenous SDF-1α on post-MI cardiac tissue may be balanced between angiogenic/ reparative and proinflammatory actions of SDF-1α/CXCR4 signaling [21]. In humans, our previous [7] and present studies suggested that endogenous SDF-1α in the infarcted

myocardium and in the peripheral vein might exert detrimental effects on post-MI cardiac tissue.

Previous clinical reports have demonstrated that, when SDF-1α levels were measured in the acute phase of ischemic events, high SDF-1α levels in the peripheral vein correlated with recurrent cardiac events in patients with AMI and non-ST elevation ACS [8,9]. However, our previous study showed that myocardial production of SDF-1α in the early phase of AMI did not reflect post-MI LV adverse remodeling and dysfunction [7]. These results suggest that SDF-1α in the infarcted myocardium and in the peripheral vein might reflect different tissues of origin. That is, SDF-1α levels in the peripheral vein during the early phase of cardiac ischemic events may not originate from the infarcted myocardium. SDF-1α is produced in the ischemic myocardium through induction of hypoxia-inducible factor [27]. Also, SDF-1α may be induced by proinflammatory stimuli in various systemic tissues [28,29] as systemic activation of proinflammatory responses occurs in the chronic phase of MI [30,31]. However, the precise source of SDF-1α in the peripheral vein remains undefined. A previous report showed that SDF-1α levels in the peripheral vein increased during the acute phase of MI [9]. In the present stable patients with a history of MI, SDF-1α levels were higher in the patients with future secondary cardiac events than in control subjects. The mechanism by which high SDF-1α levels were sustained in the peripheral vein during the chronic phase of MI is unclear. A systemic inflammatory response occurs during the acute phase of MI, but is resolved in the chronic phase [1,3]. The mechanisms leading to resolution, including inhibition of proinflammatory cytokine synthesis [1], may be impaired in the patients who have recurrent cardiac events. For example, post-MI LV dysfunction might induce systemic activation of the renin– angiotensin–aldosterone system, leading to prolonged proinflammatory responses in the chronic phase of MI [32–34]. Thus, sustained elevation of SDF-1α levels may reflect

persistent LV adverse remodeling and dysfunction after MI. In support of this, we observed that SDF-1α levels positively correlated with BNP levels in the present study. In the present study, BNP did not have a significant predictive value in the multivariate Cox proportional hazard analysis. This was explained by the strong correlation of BNP with LVEF which was included in the co-variables of the multivariate Cox hazard analysis. The present study included a relatively small number of patients evaluated at a single center. The present results could not be generalized in other cohorts including patients with acute coronary syndrome or heart failure. A large prospective trial is required to understand the precise role of SDF-1α in the pathogenesis of chronic phase of MI. In conclusion, high levels of SDF-1α in the peripheral vein predicted secondary cardiac events in stable patients with a history of MI. The high SDF-1α levels had incremental effects on the prognostic value of the traditional risk factors in this patient population. Hence, SDF-1α levels may be a useful risk assessment tool in patients with a history of MI.

Funding

This study was supported by JSPS KAKENHI Grant Number B2- 19390209 and B-22390158.

Disclosures

The authors declare that there is no conflict of interest.

Table 1. Characteristics of study patients with a history of MI and control subjects.

	Patients with events (n=30)	Patients without events (n=162)	Control (n=31)
Age (yr)	72 (61-79)*†	65 (56-75)	63 (58-70)
Male gender, *n* (%)	26 (87)†	128 (79)†	15 (48)
Smoking history, *n* (%)	21 (70)†	119 (73)†	11 (35)
Hypertension, *n* (%)	18 (60)	106 (65)	15 (48)
Diabetes mellitus, *n* (%)	19 (63)*†	58 (36)†	1 (3)
Atrial fibrillation, *n* (%)	4 (13)	7 (4)	4 (13)
Multivessel disease, *n* (%)	24 (80)*	95 (59)	-
BMI (kg/m^2)	23 (22-25)	24 (22-26)	24 (21-26)
Systolic BP (mmHg)	131 ± 28	138 ± 25	145 ± 29
Heart Rate (beats/min)	65 (56-71)	65 (60-74)	64 (60-78)
LDL-C (mg/dL)	95 (81-129)†	103 (84-126)	116 (101-135)
HDL-C (mg/dL)	43 (36-55)†	44 (37-50)†	58 (51-67)
HbA1c (%)	6.7 (5.9-7.4)†	6.1 (5.8-6.8)†	5.8 (5.5-6.0)
LVEF (%)	52 (38-61)*†	57 (49-66)†	68 (62-75)
eGFR (mL/min)	58 ± 18*†	67 ± 17	70 ± 15
SDF-1α (pg/mL)	2346 (2221-2504)*†	2030 (1829-2300)	1948 (1715-2086)
BNP (pg/mL)	74 (41-207)*†	38 (20-80)	15 (6-29)
CRP (mg/L)	0.7 (0.4-1.3)*	0.5 (0.2-1.0)	0.3 (0.2-1.1)
Medications, *n* (%)			
Aspirin	30 (100)†	162 (100)†	3 (10)
Thienopyridines	26 (87)†	125 (77)†	0 (0)
β-blocker	8 (27)†	44 (27)†	3 (10)
ACEI / ARB	25 (83)†	119 (73)†	7 (23)
Statin	18 (60)†	117 (72)†	6 (19)

Data are expressed either as the mean value ± SD, median and range (25th and 75th percentile), or number (%) of patients. MI, myocardial infarction; BMI, body mass index; BP, blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; SDF-1α, stromal cell-derived factor-1α; BNP, brain natriuretic peptide; CRP, C-reactive protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

* $p < 0.05$ vs. patients without events.

† $p < 0.05$ vs. control patients.

Table 2. Univariate and multivariate linear regression analysis of the correlations between SDF-1α and baseline clinical parameters in patients with a history of MI.

	Univariate analysis		Multivariate analysis	
	r	p value	β	p value
Age	0.179	0.01	0.043	0.59
Male gender	- 0.004	0.95	-	-
Smoking	0.043	0.56	-	-
Hypertension	0.053	0.46	-	-
Diabetes mellitus	- 0.010	0.16	-	-
Atrial fibrillation	0.138	0.06	-	-
Multivessel disease	0.010	0.89	-	-
BMI	- 0.095	0.19	-	-
Systolic BP	- 0.131	0.08	-	-
Heart rate	0.036	0.62	-	-
LDL-C	- 0.014	0.85	-	-
HDL-C	0.007	0.93	-	-
HbA1c	- 0.142	0.05	-	-
LVEF	0.084	0.25	-	-
eGFR	- 0.214	0.003	- 0.124	0.12
BNP	0.346	< 0.0001	0.295	< 0.0001
CRP	0.149	0.04	0.088	0.20
Medications			-	-
Thienopyridines	0.079	0.27	-	-
β-blocker	0.050	0.49	-	-
ACEI / ARB	0.005	0.94	-	-
Statin	- 0.070	0.34	-	-

The multivariate analysis consisted of covariates that demonstrated a significant correlation in the univariate analysis. r = regression coefficient. β = standardized regression coefficient. Aspirin was not included in this analysis because it was used by all patients. SDF-1α, stromal cell-derived factor-1α; MI, myocardial infarction; BMI, body mass index; BP, blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; BNP, brain natriuretic peptide; CRP, C-reactive protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angio-tensin II receptor blocker.

Table 3. Univariate and stepwise multivariate Cox hazard analysis of the risk factors for future cardiac events.

	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Age	1.83	1.21-2.78	< 0.01	1.83	1.16-2.91	0.01
Male gender	1.57	0.55-4.49	0.40			
Smoking	0.82	0.37-1.79	0.62			
Hypertension	0.82	0.39-1.70	0.59			
Diabetes mellitus	2.73	1.30-5.75	< 0.01	2.79	1.28-6.10	0.01
Atrial fibrillation	2.93	1.02-8.42	0.04			
Multivessel disease	2.50	1.02-6.12	0.04			
BMI	0.84	0.57-1.22	0.35			
Systolic BP	0.78	0.53-1.13	0.19			
Heart Rate	0.77	0.52-1.15	0.20			
LDL-C	0.85	0.58-1.23	0.38			
HDL-C	1.03	0.73-1.47	0.86			
HbA1c	1.14	0.84-1.53	0.41			
LVEF	0.66	0.47-0.94	0.02	0.69	0.50-0.97	0.03
eGFR	0.60	0.40-0.88	0.01			
SDF-1α	1.87	1.35-2.60	< 0.001	1.98	1.38-2.85	< 0.001
BNP	1.37	1.17-1.60	< 0.001			
CRP	1.25	0.96-1.62	0.10			
Medications						
Thienopyridines	1.78	0.62-5.10	0.28			
β-blocker	0.99	0.44-2.23	0.98			
ACEI / ARB	1.55	0.59-4.04	0.37			
Statin	0.66	0.32-1.37	0.27			

The hazard ratios and 95% CI for continuous variables were estimated by a 1-SD increase. Dichotomous variables were coded as 1 for the presence of, and 0 for the absence of, each factor. BMI, body mass index; BP, blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; SDF-1α, stromal cell-derived factor-1α; BNP, brain natriuretic peptide; CRP, C-reactive protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

Table 4. Incremental effects of SDF-1α levels on the predictive value of traditional risk factors.

	Category-free NRI		IDI	
	NRI value	*p* value	IDI value	*p* value
Traditional risk factors				
+ BNP	0.44	0.03	0.03	0.31
+ SDF-1α	0.77	0.0001	0.07	0.001
Traditional risks + BNP				
+ SDF-1α	0.90	< 0.0001	0.05	0.002
Traditional risks + SDF-1α				
+ BNP	0.10	0.62	0.004	0.69

NRI, net reclassification improvement; IDI, integrated discrimination improve-ment; BNP, brain natriuretic peptide; SDF-1α, stromal cell-derived factor-1α. The traditional risk factors consisted of age, male gender, diabetes mellitus, hypertension, smoking, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, C-reactive protein, left ventricular ejection fraction, and multivessel disease.

Supplementary Table 1. Comparison of clinical variables between patients with high and low SDF-1α.

	High SDF-1α (≥ 2162 pg/mL) (n=82)	Low SDF-1α (< 2162 pg/mL) (n=110)
Age (years)	70 (60-77) *	63 (54-73)
Male gender, *n* (%)	65 (79)	89 (81)
Smoking history, *n* (%)	59 (72)	81 (74)
Hypertension, *n* (%)	55 (67)	69 (63)
Diabetes mellitus, *n* (%)	34 (41)	43 (39)
Atrial fibrillation, *n* (%)	8 (10)	3 (3)
Multivessel disease, *n* (%)	51 (62)	68 (62)
BMI (kg/m^2)	23 (22-25)	24 (22-26)
Systolic BP (mmHg)	134 ± 27	139 ± 24
Heart Rate (beats/min)	65 (60-74)	65 (59-73)
LDL-C (mg/dL)	97 (78-123)	106 (87-129)
HDL-C (mg/dL)	44 (38-52)	44 (37-50)
HbA1c (%)	6.1 (5.8-6.8)	6.2 (5.8-7.0)
LVEF (%)	57 (45-64)	56 (49-66)
eGFR (mL/min)	60 ± 18 *	69 ± 17
BNP (pg/mL)	66 (33-105) *	32 (17-72)
CRP (mg/L)	0.6 (0.3-1.4)	0.5 (0.2-1.0)
Medications, *n* (%)		
Aspirin	82 (100)	110 (100)
Thienopyridines	66 (80)	85 (77)
β-Blocker	25 (30)	27 (25)
ACEI / ARB	64 (78)	80 (73)
Statin	52 (63)	83 (75)
PCI-related variables		
Pre-dilatation, *n* (%)	37 (45)	49 (45)
Post-dilatation, *n* (%)	12 (15)	15 (14)
BMS use, *n* (%)	42 (51)	51 (46)
DES use, *n* (%)	16 (20)	23 (21)
Total stent length (mm)	20 (16-25)	18 (16-24)
Thrombus aspiration, *n* (%)	41 (50)	64 (58)

Data are expressed either as the mean value ± SD, median and range (25th and 75th percentile), or number (%) of patients. The cut-off value of SDF-1α levels as 2162 pg/mL

was determined by ROC analysis. SDF-1α, stromal cell-derived factor-1α; BMI, body mass index; BP, blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; BNP, brain natriuretic peptide; CRP, C-reactive protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; PCI, percutaneous coronary intervention that was performed in the culprit coronary lesion at the occurrence of MI; BMS, bare metal stent; DES, drug-eluting stent.
* $p<0.05$ vs. low SDF-1α patients.

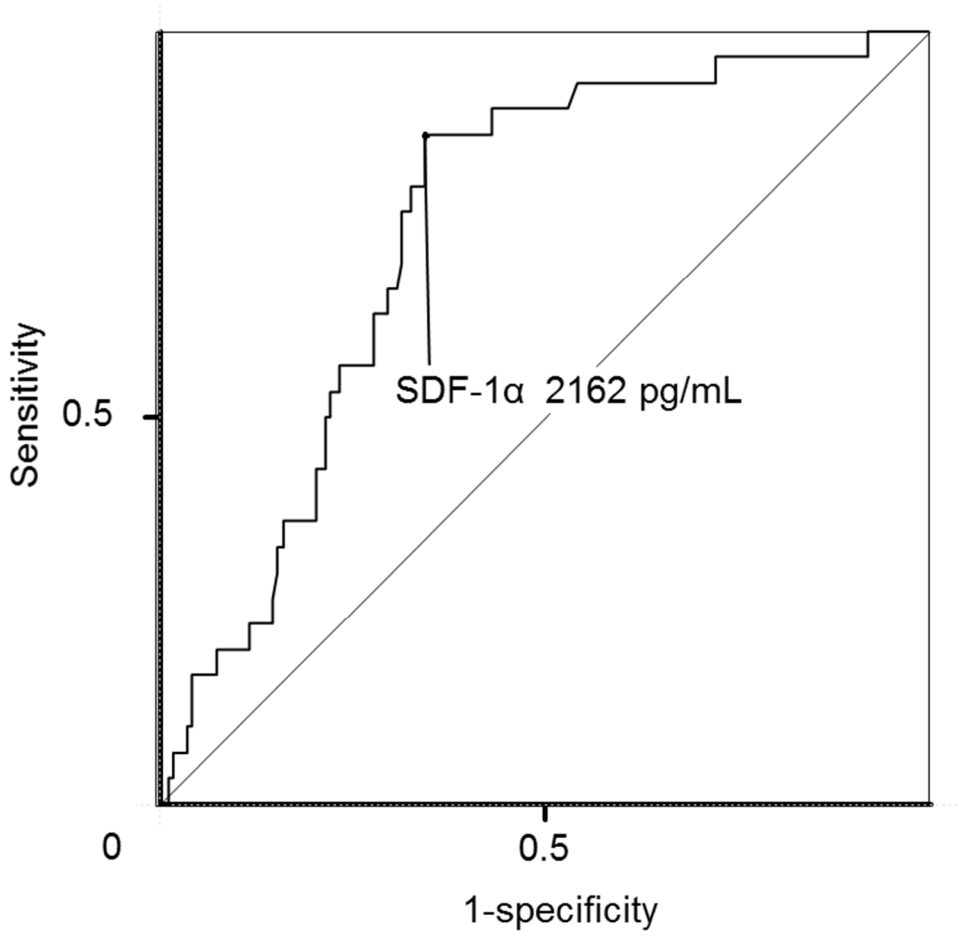

Fig. 1. Receiver operator characteristic curve to obtain optimal cut-off level of stromal cell-derived factor-1α (SDF-1α) for the prediction of cardiac events.

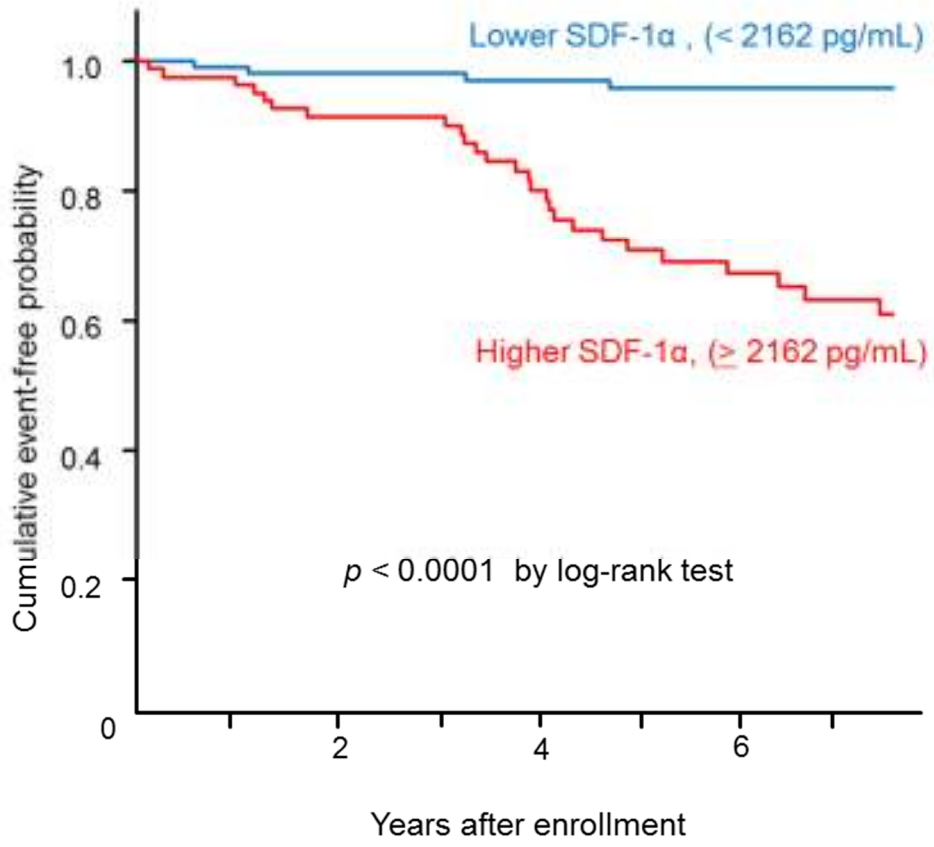

Fig. 2. Kaplan–Meier analysis according to stromal cell-derived factor-1α (SDF-1α) levels. The cut-off value of SDF-1α (2162 pg/mL) was determined by receiver-operating characteristic analysis in the study patients. High levels of SDF-1α (≥2162 pg/mL, n = 82) resulted in higher probability of future cardiac events than the lower one (<2162 pg/mL, n = 110) ($p < 0.0001$).

References

[1] Frangogiannis NG. Regulation of the inflammatory response in cardiac repair. Circ Res 2012;110:159–73.

[2] Nahrendorf M, Pittet MJ, Swirski FK. Monocytes: protagonists of infarct inflammation and repair after myocardial infarction. Circulation 2010;121: 2437–45.

[3] Nikolaos G, Frangogiannis Dewald O, Ren G, Duerr GD, Zoerlein M, Klemm C, Gersch C, Tincey S, Michael LH, Entman ML, Frangogiannis NG. Of mice and dogs: species-specific differences in the inflammatory response following myocardial infarction. Am J Pathol 2004;164:665–77.

[4] Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. Circulation 1990;81: 1161–72.

[5] White HD, Norris RM, Brown MA, Brandt PW, Whitlock RM, Wild CJ. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. Circulation 1987;76:44–51.

[6] Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, Nagler A, Ben-Hur H, Many A, Shultz L, Lider O, Alon R, Zipori D, Lapidot T. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science 1999;283:845–8.

[7] Uematsu M, Yoshizaki T, Shimizu T, Obata JE, Nakamura T, Fujioka D, Watanabe K, Watanabe Y, Kugiyama K. Sustained myocardial production of stromal cell-derived factor-1α was associated with left ventricular adverse remodelling in patients with myocardial infarction. Am J Physiol Heart Circ Physiol 2015;309:H1764–71.

[8] Fortunato O, Spinetti G, Specchia C, Cangiano E, Valgimigli M, Madeddu P. Migratory activity of circulating progenitor cells and serum SDF-1α predict adverse events in patients with myocardial infarction. Cardiovasc Res 2013;100:192–200.

[9] Tong G, Wang N, Zhou Y, Leng J, Gao W, Tong X, Shen Y, Yang J, Ye X, Zhou L, Gao Y. Role of stromal cell-derived factor-1 in patients with non-ST elevation acute coronary syndrome. Int Heart J 2014;55:219–27.

[10] Kugiyama K, Motoyama T, Hirashima O, Ohgushi M, Soejima H, Misumi K, Kawano H, Miyao Y, Yoshimura M, Ogawa H, Matsumura T, Sugiyama S, Yasue H. Vitamin C attenuates abnormal vasomotor reactivity in spasm coronary arteries in patients with coronary spastic angina. J Am Coll Cardiol 1998;32: 103–9.

[11] Braunwald E, Antman EM, Beasley JW, Califf RM, Cheitlin MD, Hochman JS, Jones RH, Kereiakes D, Kupersmith J, Levin TN, Pepine CJ, Schaeffer JW, Smith 3rd EE, Steward DE, Theroux P, et al. ACC/AHA guidelines for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction: executive summary and recommendations. A report of the American College of Cardiology/American Heart Association task force on practice guidelines (committee on the management of patients with unstable angina). Circulation 2000;102:1193–209.

[12] Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143–421.

[13] Teichholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of asynergy. Am J Cardiol 1976;37:7–11.

[14] Norimatsu K, Miura S, Suematsu Y, Shiga Y, Miyase Y, Nakamura A, Yamada M, Matsunaga A, Saku K. Associations between glycated albumin or hemo-globin A1c and the presence of coronary artery disease. Int J Cardiol 2015;65:487–93.

[15] American Diabetes Association. Standards of medical care in diabetes – 2006. Diabetes

Care 2006;29(Suppl. 1):S4–2.

[16] Ikeda N, Hara H, Hiroi Y. 1,5-Anhydro-D-glucitol predicts coronary artery disease prevalence and complexity. Int J Cardiol 2014;64:297–301.

[17] Makino A, Nakamura T, Hirano M, Kitta Y, Sano K, Kobayashi T, Fujioka D, Saito Y, Watanabe K, Watanabe Y, Kawabata K, Obata JE, Kugiyama K. High plasma levels of macrophage migration inhibitory factor are associated with adverse long-term outcome in patients with stable coronary artery disease and impaired glucose tolerance or type 2 diabetes mellitus. Atherosclerosis 2010;213:573–8.

[18] Abbott JD, Huang Y, Liu D, Hickey R, Krause DS, Giordano FJ. Stromal cellderived factor-1α plays a critical role in stem cell recruitment to the heart after myocardial infarction but is not sufficient to induce homing in the absence of injury. Circulation 2004;110:3300–5.

[19] Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, Rovner A, Ellis SG, Thomas JD, DiCorleto PE, Topol EJ, Penn MS. Effect of stromal-cellderived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 2003;362:697–703.

[20] Elmadbouh I, Haider HKh, Jiang S, Idris NM, Lu G, Ashraf M. Ex vivo delivered stromal cell-derived factor-1α promotes stem cell homing and induces angiomyogenesis in the infarcted myocardium. J Mol Cell Cardiol 2007;42: 792–803.

[21] Frangogiannis NG. The stromal cell-derived factor-1/CXCR4 axis in cardiac injury and repair. J Am Coll Cardiol 2011;58:2424–6.

[22] Saxena A, Fish JE, White MD, Yu S, Smyth JW, Shaw RM, DiMaio JM, Srivastava D. Stromal cell-derived factor-1α is cardioprotective after myocardial infarction. Circulation 2008;117:2224–31.

[23] Chen J, Chemaly E, Liang L, Kho C, Lee A, Park J, Altman P, Schecter AD, Hajjar RJ,

Tarzami ST. Effects of CXCR4 gene transfer on cardiac function after ischemiareperfusion injury. Am J Pathol 2010;176:1705–15.

[24] Jujo K, Hamada H, Iwakura A, Thorne T, Sekiguchi H, Clarke T, Ito A, Misener S, Tanaka T, Klyachko E, Kobayashi K, Tongers J, Roncalli J, Tsurumi Y, Hagiwara N, et al. CXCR4 blockade augments bone marrow progenitor cell recruitment to the neovasculature and reduces mortality after myocardial infarction. Proc Natl Acad Sci U S A 2010;107:11008–13.

[25] Liehn EA, Tuchscheerer N, Kanzler I, Drechsler M, Fraemohs L, Schuh A, Koenen RR, Zander S, Soehnlein O, Hristov M, Grigorescu G, Urs AO, Leabu M, Bucur I, Merx MW, et al. Double-edged role of the CXCL12/CXCR4 axis in experimental myocardial infarction. J Am Coll Cardiol 2011;58:2415–23.

[26] Proulx C, El-Helou V, Gosselin H, Clement R, Gillis MA, Villeneuve L, Calderone A. Antagonism of stromal cell-derived factor-1α reduces infarct size and improves ventricular function after myocardial infarction. Pflugers Arch 2007;455:241–50.

[27] Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, Capla JM, Galiano RD, Levine JP, Gurtner GC. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004;10:858–64.

[28] Jin C, Fu WX, Xie LP, Qian XP, Chen WF. SDF-1α production is negatively regulated by mouse estrogen enhanced transcript in a mouse thymus epithelial cell line. Cell Immunol 2003;223:26–34.

[29] Hohensinner PJ, Kaun C, Rychli K, Niessner A, Pfaffenberger S, Rega G, Furnkranz A, Uhrin P, Zaujec J, Afonyushkin T, Bochkov VN, Maurer G, Huber K, Wojta J. The inflammatory mediator oncostatin M induces stromal derived factor-1 in human adult cardiac cells. FASEB J 2009;23:774–82.

[30] Fang L, Moore XL, Dart AM, Wang LM. Systemic inflammatory response following acute

myocardial infarction. J Geriatr Cardiol 2015;12:305–12.

[31] Libby P, Nahrendorf M, Swirski FK. Leukocytes link local and systemic inflammation in ischemic cardiovascular disease: an expanded cardiovascular continuum. J Am Coll Cardiol 2016;67:1091–103.

[32] Rouleau JL, de Champlain J, Klein M, Bichet D, Moye´ L, Packer M, Dagenais GR, Sussex B, Arnold JM, Sestier F, et al. Activation of neurohumoral systems in postinfarction left ventricular dysfunction. J Am Coll Cardiol 1993;22:390–8.

[33] Sigurdsson A, Held P, Swedberg K. Short- and long-term neurohormonal activation following acute myocardial infarction. Am Heart J 1993;126: 1068–76.

[34] Nabah YN, Mateo T, Estelle´ s R, Mata M, Zagorski J, Sarau H, Cortijo J, Morcillo EJ, Jose PJ, Sanz MJ. Angiotensin II induces neutrophil accumulation in vivo through generation and release of CXC chemokines. Circulation 2004;110: 3581–6.

High levels of stromal cell-derived factor-1α predict secondary cardiac events in stable patients with a history of myocardial infarction

Satoshi Matsuoka (MD)[a], Manabu Uematsu (MD)[a], Takamitsu Nakamura (MD, PhD)[a], Takuya Shimizu (MD)[a], Mika Futamata (MD)[a], Jun-ei Obata (MD, PhD)[a], Daisuke Fujioka (MD, PhD)[a], Kazuto Nakamura (MD, PhD)[a], Toru Yoshizaki (MD)[a], Kiyotaka Kugiyama (MD, PhD, FJCC)[a]

[a] *Department of Internal Medicine II, University of Yamanashi, Faculty of Medicine, Chuo, Yamanashi, Japan*

Key words: stromal cell-derived factor-1α, myocardial infarction, prognosis, cardiac events, inflammatory marker.

This paper was published in the following journal: Journal of Cardiology, 69(1), 320-325. doi.org/10.1016/j.jjcc.2016.06.011

Abstract

Background: We recently showed that stromal cell-derived factor (SDF)-1α, a proinflammatory mediator, is produced in infarcted myocardium and is associated with left ventricular (LV) adverse remodeling and progressive dysfunction following acute myocardial infarction (AMI). The current study examined whether SDF-1α levels in the peripheral vein can provide prognostic information of outcomes in stable patients with a history of MI.

Methods: Plasma levels of SDF-1α in the peripheral vein were measured by enzyme-linked immunosorbent assay in 192 stable patients with a history of MI. All patients were followed prospectively for a period of 90 months or until occurrence of one of the following cardiac events: cardiac death, non-fatal myocardial infarction, unstable angina requiring unplanned coronary revascularization, or worsening heart failure requiring hospital admission.

Results: During the follow-up period (77 ± 26 months), 30 patients had cardiac events. Multivariate Cox analysis revealed that high levels of SDF-1α (≥2162 pg/mL; a cut-off value determined by receiver-operating characteristic analysis) were a significant predictor of cardiac events, independent of traditional risk factors (HR: 1.98; 95% CI: 1.38–2.85; $p < 0.001$). The addition of high levels of SDF-1α to conventional risk factors including brain natriuretic peptide improved net reclassification improvement (NRI) and integrated discrimination improvement (IDI) (NRI 0.90, $p < 0.000$-1αnd IDI 0.05, $p = 0.002$).

Conclusions: High levels of SDF-1α predicted secondary cardiac events in stable patients with a history of MI. SDF-1α levels may be a useful risk assessment tool in patients with a history of MI.

Introduction

Acute myocardial infarction (AMI) induces the production of chemokines and growth factors that recruit neutrophils and phagocytes to the ischemic cardiac tissue, leading to clearance of dead cells and matrix debris [1,2]. This acute, localized inflammatory response is transient, and is followed by resolution of the inflammation [1,3]. However, prolonged inflammation leads to dilative remodeling and progressive dysfunction of the infarcted myocardium through matrix degradation and cardiomyocyte apoptosis [1,2]. Left ventricle (LV) adverse remodeling and dysfunction after MI are predictors of poor outcome following MI, and are associated with ventricular arrhythmias, heart failure, and increased mortality [4,5]. Stromal cell-derived factor (SDF)-1α/CXCL12 is a CXC chemokine with chemotactic effects on CXCR4-expressing progenitor cells [6]. We have recently shown that SDF-1α, a proinflammatory mediator, is produced in the infarcted myocardial lesion and released into the circulation in patients with AMI [7]. In addition, we showed that the myocardial production of SDF-1α in the chronic phase (6 months after MI) but not in the early phase (2 weeks after MI) was associated with LV adverse remodeling and progressive dysfunction in patients with AMI [7]. Thus, prolonged production of SDF-1α in the infarcted myocardium may exert detrimental effects on post-MI LV function in the chronic phase following MI. The prognostic value of SDF-1α levels in the peripheral vein was previously evaluated in patients with AMI/ acute coronary syndrome (ACS) [8,9]. However, it remains unclear whether SDF-1α levels can provide prognostic information in patients in the chronic phase of MI. The current study examined the prognostic value of SDF-1α levels in the peripheral vein in stable patients with a history of MI.

Methods

Study patients

The study screened 386 patients with a history of MI, who underwent cardiac catheterization in Yamanashi University Hospital between February 2003 and March 2009. All patients underwent routine blood testing at the time of discharge. The inclusion criteria were: (1) stable previous MI; (2) no episode of angina at rest and no changes in the frequency of angina in response to sublingual nitroglycerin in the previous 2 months. Exclusion criteria included: (1) ACS, stroke, cardiogenic shock, pulmonary edema, major surgery, trauma or serious infectious disease within 4 weeks prior to enrollment; (2) neoplasm, significant hepatic or inflammatory disease; (3) chronic renal failure or serum creatinine >2.5 mg/dL, congestive heart failure, or left main coronary artery disease; (4) other serious diseases. Finally, a total of 210 patients were enrolled in the study according to these inclusion and exclusion criteria. The study also included 31 control patients without echocardiographic findings of significant valvular disease (moderate or more regurgitation, stenosis), cardiomyopathy [chamber size dilatation, reduced left ventricular ejection fraction (LVEF), ventricular asynergy, ventricular hypertrophy, aneurysm], and pulmonary hypertension, chosen from among the 6-1αngiographically normal patients [without percutaneous coronary intervention (PCI) history] who were evaluated during the study period. Control patients served as a reference group for plasma SDF-1α concentrations. Each of the control patients underwent diagnostic coronary angiography for atypical chest pain at rest at Yamanashi University Hospital during the study period. Control patients fulfilled all of the following inclusion criteria: (1) no significant ST segment changes on 12-lead electrocardiogram (ECG) while having chest pain or on ambulatory ECG; (2) neither chest pain nor ST segment changes during the treadmill test; (3) no coronary artery spasm during provocation with intra-

coronary infusion of acetylcholine [10]. Clinical characteristics of patients with a history of MI and control patients are shown in Table -1αll study participants were ethnic Japanese. All patients gave written, informed consent at the time of enrollment. The study was approved by the ethics committee of Yamanashi University Hospital. The investigation conformed to the principles outlined in the 1975 Declaration of Helsinki.

Prospective study

Patients were followed every month in the hospital or with a clinic visit for 90 months, or until the occurrence of cardiac death, non-fatal MI, refractory unstable angina pectoris (uAP) requiring unplanned coronary revascularization, or decompensated heart failure. The time to the first event was evaluated prospectively. Cardiac death was confirmed by hospital records. Acute MI and uAP were diagnosed by the presence of acute ischemic symptoms lasting $\geq$20 min within 48 h prior to hospital admission, and ECG changes. Acute MI was diagnosed when creatine kinase-MB levels increased to at least 2 times the upper limit of normal or when troponin T levels were >0.1 ng/mL [11]. The diagnoses of MI and uAP were confirmed by coronary angiography. Decompensated heart failure was defined as resting dyspnea with progressive fluid retention requiring hospitalization and treatment with an intravenous diuretic. Follow-up data were collected from the patients' primary physicians every 3 months by two blinded investigators (T.N., J.O.). During the follow-up period, standard medications were prescribed to all patients according to the guidelines of the American Heart Association [12], as shown in Table 1. Diet and lifestyle recommendations were continued throughout the follow-up period.

Laboratory measurements

Venous blood was obtained from all patients on the morning of discharge after a 12-h overnight fast. The initial volumes of each blood sample, including those forcibly drawn, were discarded. Serum and EDTA-plasma were aliquoted and stored at –80℃ until time of analysis. Plasma SDF-1α levels were measured by enzyme-linked immunosorbent assay using a commercial kit (R & D Systems, Minneapolis, MN, USA). In our laboratory, the intra- and inter-assay coefficients of variation were 1.9% and 3.5%, respectively. The minimal detection limit of this assay was 18 pg/mL. Serum C-reactive protein (CRP) levels were assayed by rate nephelometry (Dade Behring, Tokyo, Japan). Plasma levels of brain natriuretic peptide (BNP) were measured by immunoradiometric assay (Shionogi Pharmaceutical, Osaka, Japan). Estimated glomerular filtration rate (eGFR) was calculated by the Modification of Diet in Renal Disease study equation. Echocardiographic LVEF was calculated by the motion-mode method using the Teichholz formula [13].

Statistical analysis

All descriptive data were expressed as mean ± SD, median, or frequency (%). The Shapiro–Wilk test showed that age, body mass index (BMI), heart rate, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), hemoglobin A1c (HbA1c), LVEF, SDF-1α, BNP, and CRP were not normally distributed, hence, these variables were expressed as the median and inter-quartile ranges (25th and 75th percentiles). Non-normally distributed variables were log-transformed before the analysis. Continuous variables were compared between the two groups using the unpaired t-test or the Mann–Whitney U-test, as appropriate. Frequencies were compared using the chi-square test. For comparisons of the mean value of ≥3 groups, one-way analysis of variance (ANOVA) followed by a Scheffe test for post hoc comparisons was employed. The correlation between

the two groups was examined using univariate and multivariate linear regression analyses. Kaplan–Meier analysis was performed on the 2 groups and was based on a cut-off SDF-1α level. The cut-off level of SDF-1α (2162 pg/mL) was determined by receiver-operating characteristic (ROC) analyses of SDF-1α in the study patients with a history of MI (Fig. 1). The predictive values were assessed by univariate or forward stepwise multivariate Cox proportional hazards analysis. For a forward stepwise multivariate Cox hazard analysis, the significance level was set at 0.1. In the univariate and multivariate Cox proportional hazards analyses, continuous variables were estimated for 1-SD change. Dichotomous variables were coded as 1 for the presence of, and 0 for the absence of, each factor. The following factors were included as dichotomous variables: systemic hypertension (≥140/90 mmHg or use of an antihypertensive medication) [14], diabetes mellitus (DM) (as defined by the American Diabetes Association or use of an antidiabetic medication) [15,16], smoking history (defined as smoking ≥10 cigarettes/day for ≥10 years), atrial fibrillation (including paroxysmal atrial fibrillation recorded with an ECG), and multivessel disease. The Cox models included only variables that showed proportionality in a Schoenfeld residuals test. The category-free net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) were used to examine the additive effects of SDF-1α and BNP levels on the predictive value of the baseline model that consisted of conventional cardiovascular risk factors. Conventional risk factors included age, male gender, DM, hypertension, smoking, levels of LDL-C, HDL-C, CRP, LVEF, and multivessel disease. All probability values were presented as 2-tailed with statistical significance inferred at $p < 0.05$. All confidence intervals were computed at the 95% level. Statistical analyses were performed using STATA 10.0 (StataCorp, College Station, TX, USA).

Power analysis

Our previous study showed that the composite endpoints occurred in approximately 52% of stable coronary artery disease (CAD) patients with high coronary risk factors and higher macrophage inhibitory factor (MIF) levels as an inflammatory marker, and in 18% of patients with lower MIF levels during 5 years of follow-up [17]. On the basis of that study, a total of 72 patients were required for a two-sided statistical analysis with sufficient statistical power of 0.90 (b = 0.10 and a = 0.05). This justified the number of patients (n = 220) included in the current prospective study.

Results

Over the course of the study, 7 patients were lost to follow up, and 11 patients were excluded due to non-cardiac related death during follow-up. A total of 192 patients completed the follow-up study (2–90 months, mean = 77 ± 26 months). During the follow-up period, 30 (15.6%) cardiac events occurred, including 5 cardiac deaths, 1 non-fatal MI, 12 uAPs requiring unplanned coronary revascularization, and 12 episodes of worsening heart failure. Patient characteristics are shown in Table 1.

Patients and SDF-1α levels

The time between blood sampling for SDF-1α level and the previous MI ranged from 3 to 26 months, with a mean of 7.7 ± 2.1 months. The time between onset of previous MI and blood sampling did not correlate with SDF-1α levels (r = –0.08, p = 0.28). As shown in Table 2, SDF-1α levels were positively correlated with age, BNP, and CRP but inversely correlated with eGFR in the univariate linear regression analysis (Table 2). SDF-1α levels did not significantly correlate with the frequency of any cardiovascular medication (Table 2).

Prevalence of PCI-related variables was similar between patients with high and low SDF-1α levels (Supplementary Table 1).

Prospective study

Patients (n = 82) with higher SDF-1α levels (≥2162 pg/mL, a cut-off value determined by ROC analysis) had 26 events during follow-up, whereas patients (n = 110) with lower SDF-1α levels (<2162 mg/dL) had 4 events ($p < 0.01$). The cut-off value of SDF-1α levels as 2162 pg/mL, determined by ROC analysis, provided sensitivity of 86.7%, specificity of 65.4%, and accuracy of 68.8% for prediction of future events (Fig. 1). Kaplan–Meier analysis demonstrated a significantly higher probability of a cardiac event in patients with higher SDF-1α levels compared with those with lower SDF-1α levels (Fig. 2). A univariate Cox proportional hazards analysis revealed that SDF-1α levels (HR 1.87; 95% CI 1.35–2.60), age (HR 1.83; 95% CI 1.21–2.78), DM (HR 2.73; 95% CI 1.30–5.75), atrial fibrillation (HR 2.93; 95% CI 1.02–8.42), multivessel disease (HR 2.50; 95% CI 1.02–6.12), LVEF (HR 0.66; 95% CI 0.47–0.94), eGFR (HR 0.60; 95% CI 0.40–0.88), and BNP levels (HR 1.37; 95% CI 1.17–1.60) were significantly associated with future cardiac events, as shown in Table 3. A forward stepwise multivariate Cox proportional hazards analysis demonstrated that SDF-1α levels remained significantly associated with future events after adjustment for potential confounding variables (HR 1.98; 95% CI 1.38–2.85).

Incremental effects of SDF-1α levels on the predictive value of traditional risk factors

Category-free NRI and IDI demonstrated the additive value of SDF-1α to the baseline conventional risk factors consisting of age, male gender, DM, hypertension, smoking, levels of LDL-C, HDL-C, and CRP, LVEF, and multivessel disease (NRI 0.77, $p = 0.0001$;

IDI 0.07, p = 0.001) (Table 4). When BNP was added to the baseline model of risk factors, SDF-1α had significant additive effect on the predictive value of the model (NRI 0.90, p < 0.0001; IDI 0.05, p = 0.002) (Table 4). In contrast, BNP had no significant additive effect on the predictive value of a risk factor model with SDF-1α (Table 4).

Discussion

The present study showed that high SDF-1α levels are a predictor of adverse outcomes in patients with a history of MI. Moreover, high SDF-1α levels had incremental effects on prognostic value of traditional risk factors in this patient population. Thus, SDF-1α levels may aid in risk assessment in stable patients with a history of MI. We have previously shown that SDF-1α is released from the infarcted myocardium into the coronary circulation in both the early (2 weeks after MI) and chronic phases (6 months after MI) following AMI [7]. The myocardial production and release of SDF-1α in the chronic phase of MI, rather than in the early phase, was associated with post-MI LV adverse remodeling and progressive dysfunction [7]. These results support the present finding that SDF-1α levels can predict secondary cardiac events in patients with a history of MI. Animal experiments have demonstrated that local infusion or over-expression of SDF-1α attenuates myocardial ischemic injury following LV dysfunction [18–22]. In contrast, other experimental studies have shown that SDF-1α/CXCR4 signaling worsens post-MI LV function due to increased inflammatory injury and apoptotic mechanisms [23–26]. Thus, the effect of SDF-1α on post-MI LV function in animal models remains unclear. In animal models, the effects of endogenous SDF-1α on post-MI cardiac tissue may be balanced between angiogenic/ reparative and proinflammatory actions of SDF-1α/CXCR4 signaling [21]. In humans, our previous [7] and present studies suggested that endogenous SDF-1α in the infarcted

myocardium and in the peripheral vein might exert detrimental effects on post-MI cardiac tissue.

Previous clinical reports have demonstrated that, when SDF-1α levels were measured in the acute phase of ischemic events, high SDF-1α levels in the peripheral vein correlated with recurrent cardiac events in patients with AMI and non-ST elevation ACS [8,9]. However, our previous study showed that myocardial production of SDF-1α in the early phase of AMI did not reflect post-MI LV adverse remodeling and dysfunction [7]. These results suggest that SDF-1α in the infarcted myocardium and in the peripheral vein might reflect different tissues of origin. That is, SDF-1α levels in the peripheral vein during the early phase of cardiac ischemic events may not originate from the infarcted myocardium. SDF-1α is produced in the ischemic myocardium through induction of hypoxia-inducible factor [27]. Also, SDF-1α may be induced by proinflammatory stimuli in various systemic tissues [28,29] as systemic activation of proinflammatory responses occurs in the chronic phase of MI [30,31]. However, the precise source of SDF-1α in the peripheral vein remains undefined. A previous report showed that SDF-1α levels in the peripheral vein increased during the acute phase of MI [9]. In the present stable patients with a history of MI, SDF-1α levels were higher in the patients with future secondary cardiac events than in control subjects. The mechanism by which high SDF-1α levels were sustained in the peripheral vein during the chronic phase of MI is unclear. A systemic inflammatory response occurs during the acute phase of MI, but is resolved in the chronic phase [1,3]. The mechanisms leading to resolution, including inhibition of proinflammatory cytokine synthesis [1], may be impaired in the patients who have recurrent cardiac events. For example, post-MI LV dysfunction might induce systemic activation of the renin– angiotensin–aldosterone system, leading to prolonged proinflammatory responses in the chronic phase of MI [32–34]. Thus, sustained elevation of SDF-1α levels may reflect

persistent LV adverse remodeling and dysfunction after MI. In support of this, we observed that SDF-1α levels positively correlated with BNP levels in the present study. In the present study, BNP did not have a significant predictive value in the multivariate Cox proportional hazard analysis. This was explained by the strong correlation of BNP with LVEF which was included in the co-variables of the multivariate Cox hazard analysis. The present study included a relatively small number of patients evaluated at a single center. The present results could not be generalized in other cohorts including patients with acute coronary syndrome or heart failure. A large prospective trial is required to understand the precise role of SDF-1α in the pathogenesis of chronic phase of MI. In conclusion, high levels of SDF-1α in the peripheral vein predicted secondary cardiac events in stable patients with a history of MI. The high SDF-1α levels had incremental effects on the prognostic value of the traditional risk factors in this patient population. Hence, SDF-1α levels may be a useful risk assessment tool in patients with a history of MI.

Funding

This study was supported by JSPS KAKENHI Grant Number B2- 19390209 and B-22390158.

Disclosures

The authors declare that there is no conflict of interest.

Table 1. Characteristics of study patients with a history of MI and control subjects.

	Patients with events (n=30)	Patients without events (n=162)	Control (n=31)
Age (yr)	72 (61-79)*†	65 (56-75)	63 (58-70)
Male gender, *n* (%)	26 (87)†	128 (79)†	15 (48)
Smoking history, *n* (%)	21 (70)†	119 (73)†	11 (35)
Hypertension, *n* (%)	18 (60)	106 (65)	15 (48)
Diabetes mellitus, *n* (%)	19 (63)*†	58 (36)†	1 (3)
Atrial fibrillation, *n* (%)	4 (13)	7 (4)	4 (13)
Multivessel disease, *n* (%)	24 (80)*	95 (59)	-
BMI (kg/m^2)	23 (22-25)	24 (22-26)	24 (21-26)
Systolic BP (mmHg)	131 ± 28	138 ± 25	145 ± 29
Heart Rate (beats/min)	65 (56-71)	65 (60-74)	64 (60-78)
LDL-C (mg/dL)	95 (81-129)†	103 (84-126)	116 (101-135)
HDL-C (mg/dL)	43 (36-55)†	44 (37-50)†	58 (51-67)
HbA1c (%)	6.7 (5.9-7.4)†	6.1 (5.8-6.8)†	5.8 (5.5-6.0)
LVEF (%)	52 (38-61)*†	57 (49-66)†	68 (62-75)
eGFR (mL/min)	58 ± 18*†	67 ± 17	70 ± 15
SDF-1α (pg/mL)	2346 (2221-2504)*†	2030 (1829-2300)	1948 (1715-2086)
BNP (pg/mL)	74 (41-207)*†	38 (20-80)	15 (6-29)
CRP (mg/L)	0.7 (0.4-1.3)*	0.5 (0.2-1.0)	0.3 (0.2-1.1)
Medications, *n* (%)			
Aspirin	30 (100)†	162 (100)†	3 (10)
Thienopyridines	26 (87)†	125 (77)†	0 (0)
β-blocker	8 (27)†	44 (27)†	3 (10)
ACEI / ARB	25 (83)†	119 (73)†	7 (23)
Statin	18 (60)†	117 (72)†	6 (19)

Data are expressed either as the mean value ± SD, median and range (25th and 75th percentile), or number (%) of patients. MI, myocardial infarction; BMI, body mass index; BP, blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; SDF-1α, stromal cell-derived factor-1α; BNP, brain natriuretic peptide; CRP, C-reactive protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

* $p < 0.05$ vs. patients without events.

† $p < 0.05$ vs. control patients.

Table 2. Univariate and multivariate linear regression analysis of the correlations between SDF-1α and baseline clinical parameters in patients with a history of MI.

	Univariate analysis		Multivariate analysis	
	r	p value	β	p value
Age	0.179	0.01	0.043	0.59
Male gender	- 0.004	0.95	-	-
Smoking	0.043	0.56	-	-
Hypertension	0.053	0.46	-	-
Diabetes mellitus	- 0.010	0.16	-	-
Atrial fibrillation	0.138	0.06	-	-
Multivessel disease	0.010	0.89	-	-
BMI	- 0.095	0.19	-	-
Systolic BP	- 0.131	0.08	-	-
Heart rate	0.036	0.62	-	-
LDL-C	- 0.014	0.85	-	-
HDL-C	0.007	0.93	-	-
HbA1c	- 0.142	0.05	-	-
LVEF	0.084	0.25	-	-
eGFR	- 0.214	0.003	- 0.124	0.12
BNP	0.346	< 0.0001	0.295	< 0.0001
CRP	0.149	0.04	0.088	0.20
Medications			-	-
Thienopyridines	0.079	0.27	-	-
β-blocker	0.050	0.49	-	-
ACEI / ARB	0.005	0.94	-	-
Statin	- 0.070	0.34	-	-

The multivariate analysis consisted of covariates that demonstrated a significant correlation in the univariate analysis. r = regression coefficient. β = standardized regression coefficient. Aspirin was not included in this analysis because it was used by all patients. SDF-1α, stromal cell-derived factor-1α; MI, myocardial infarction; BMI, body mass index; BP, blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; BNP, brain natriuretic peptide; CRP, C-reactive protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angio-tensin II receptor blocker.

Table 3. Univariate and stepwise multivariate Cox hazard analysis of the risk factors for future cardiac events.

	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Age	1.83	1.21-2.78	< 0.01	1.83	1.16-2.91	0.01
Male gender	1.57	0.55-4.49	0.40			
Smoking	0.82	0.37-1.79	0.62			
Hypertension	0.82	0.39-1.70	0.59			
Diabetes mellitus	2.73	1.30-5.75	< 0.01	2.79	1.28-6.10	0.01
Atrial fibrillation	2.93	1.02-8.42	0.04			
Multivessel disease	2.50	1.02-6.12	0.04			
BMI	0.84	0.57-1.22	0.35			
Systolic BP	0.78	0.53-1.13	0.19			
Heart Rate	0.77	0.52-1.15	0.20			
LDL-C	0.85	0.58-1.23	0.38			
HDL-C	1.03	0.73-1.47	0.86			
HbA1c	1.14	0.84-1.53	0.41			
LVEF	0.66	0.47-0.94	0.02	0.69	0.50-0.97	0.03
eGFR	0.60	0.40-0.88	0.01			
SDF-1α	1.87	1.35-2.60	< 0.001	1.98	1.38-2.85	< 0.001
BNP	1.37	1.17-1.60	< 0.001			
CRP	1.25	0.96-1.62	0.10			
Medications						
Thienopyridines	1.78	0.62-5.10	0.28			
β-blocker	0.99	0.44-2.23	0.98			
ACEI / ARB	1.55	0.59-4.04	0.37			
Statin	0.66	0.32-1.37	0.27			

The hazard ratios and 95% CI for continuous variables were estimated by a 1-SD increase. Dichotomous variables were coded as 1 for the presence of, and 0 for the absence of, each factor. BMI, body mass index; BP, blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; SDF-1α, stromal cell-derived factor-1α; BNP, brain natriuretic peptide; CRP, C-reactive protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

Table 4. Incremental effects of SDF-1α levels on the predictive value of traditional risk factors.

	Category-free NRI		IDI	
	NRI value	*p* value	IDI value	*p* value
Traditional risk factors				
+ BNP	0.44	0.03	0.03	0.31
+ SDF-1α	0.77	0.0001	0.07	0.001
Traditional risks + BNP				
+ SDF-1α	0.90	< 0.0001	0.05	0.002
Traditional risks + SDF-1α				
+ BNP	0.10	0.62	0.004	0.69

NRI, net reclassification improvement; IDI, integrated discrimination improve-ment; BNP, brain natriuretic peptide; SDF-1α, stromal cell-derived factor-1α. The traditional risk factors consisted of age, male gender, diabetes mellitus, hypertension, smoking, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, C-reactive protein, left ventricular ejection fraction, and multivessel disease.

Supplementary Table 1. Comparison of clinical variables between patients with high and low SDF-1α.

	High SDF-1α (≥ 2162 pg/mL) (n=82)	Low SDF-1α (< 2162 pg/mL) (n=110)
Age (years)	70 (60-77) *	63 (54-73)
Male gender, *n* (%)	65 (79)	89 (81)
Smoking history, *n* (%)	59 (72)	81 (74)
Hypertension, *n* (%)	55 (67)	69 (63)
Diabetes mellitus, *n* (%)	34 (41)	43 (39)
Atrial fibrillation, *n* (%)	8 (10)	3 (3)
Multivessel disease, *n* (%)	51 (62)	68 (62)
BMI (kg/m^2)	23 (22-25)	24 (22-26)
Systolic BP (mmHg)	134 ± 27	139 ± 24
Heart Rate (beats/min)	65 (60-74)	65 (59-73)
LDL-C (mg/dL)	97 (78-123)	106 (87-129)
HDL-C (mg/dL)	44 (38-52)	44 (37-50)
HbA1c (%)	6.1 (5.8-6.8)	6.2 (5.8-7.0)
LVEF (%)	57 (45-64)	56 (49-66)
eGFR (mL/min)	60 ± 18 *	69 ± 17
BNP (pg/mL)	66 (33-105) *	32 (17-72)
CRP (mg/L)	0.6 (0.3-1.4)	0.5 (0.2-1.0)
Medications, *n* (%)		
Aspirin	82 (100)	110 (100)
Thienopyridines	66 (80)	85 (77)
β-Blocker	25 (30)	27 (25)
ACEI / ARB	64 (78)	80 (73)
Statin	52 (63)	83 (75)
PCI-related variables		
Pre-dilatation, *n* (%)	37 (45)	49 (45)
Post-dilatation, *n* (%)	12 (15)	15 (14)
BMS use, *n* (%)	42 (51)	51 (46)
DES use, *n* (%)	16 (20)	23 (21)
Total stent length (mm)	20 (16-25)	18 (16-24)
Thrombus aspiration, *n* (%)	41 (50)	64 (58)

Data are expressed either as the mean value ± SD, median and range (25th and 75th percentile), or number (%) of patients. The cut-off value of SDF-1α levels as 2162 pg/mL

was determined by ROC analysis. SDF-1α, stromal cell-derived factor-1α; BMI, body mass index; BP, blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; BNP, brain natriuretic peptide; CRP, C-reactive protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; PCI, percutaneous coronary intervention that was performed in the culprit coronary lesion at the occurrence of MI; BMS, bare metal stent; DES, drug-eluting stent.
* $p<0.05$ vs. low SDF-1α patients.

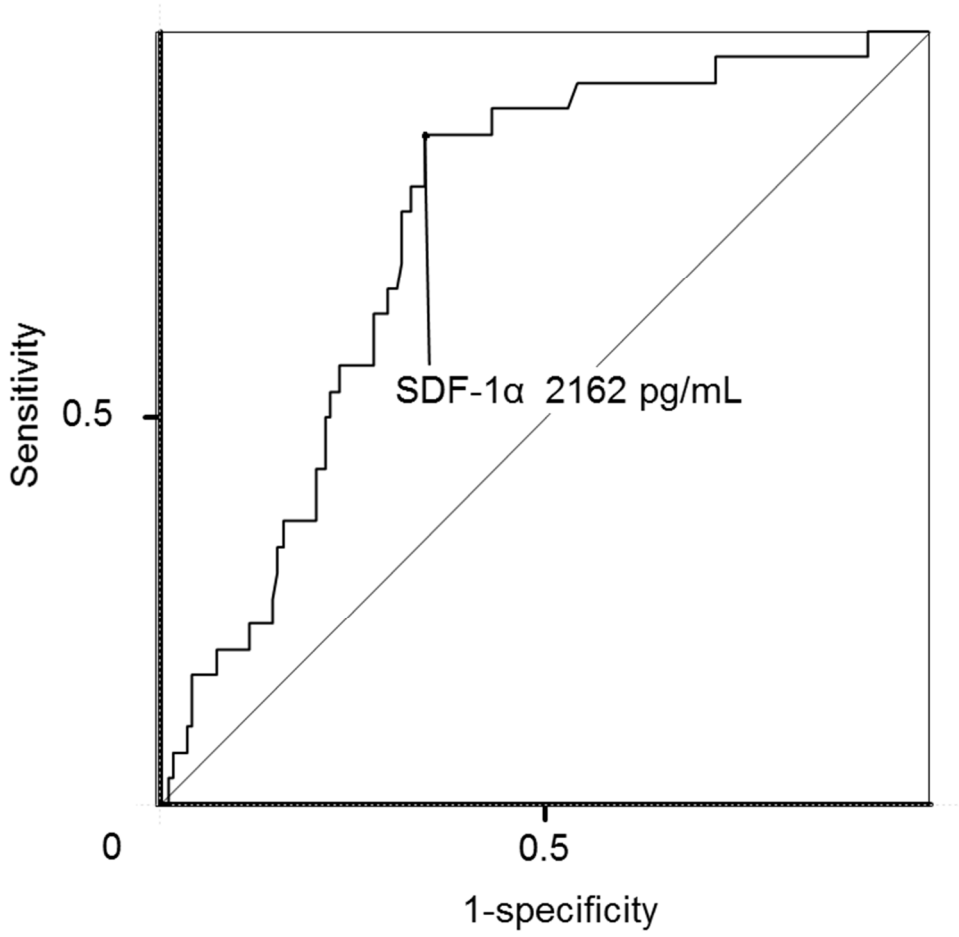

Fig. 1. Receiver operator characteristic curve to obtain optimal cut-off level of stromal cell-derived factor-1α (SDF-1α) for the prediction of cardiac events.

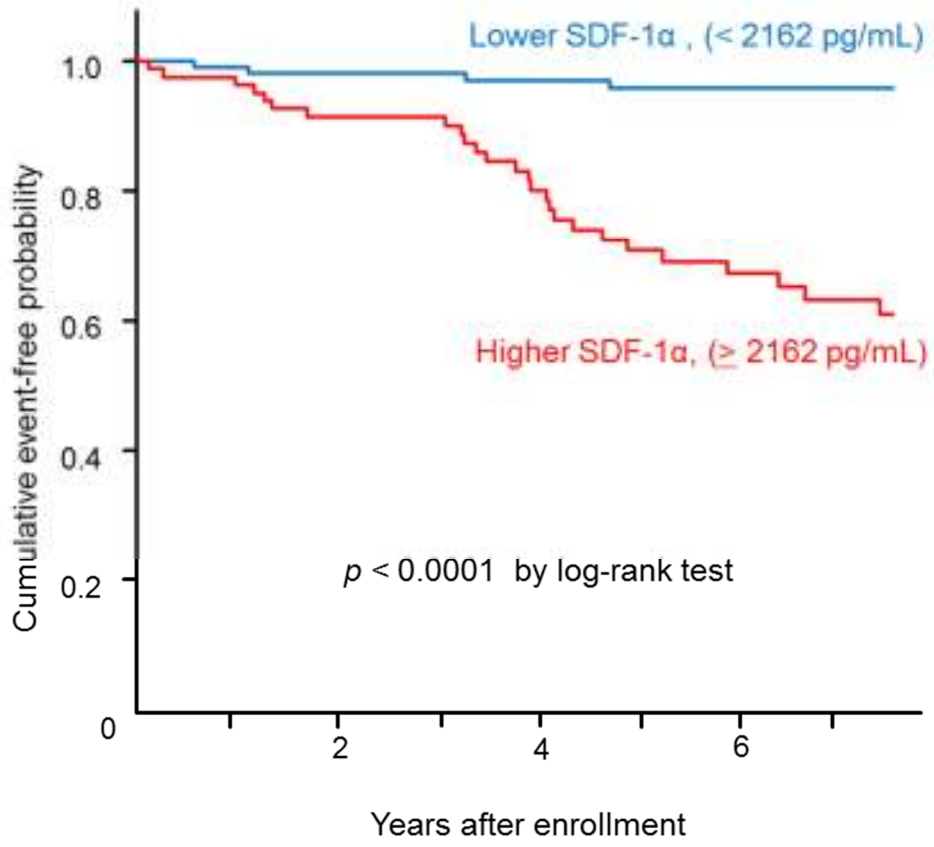

Fig. 2. Kaplan–Meier analysis according to stromal cell-derived factor-1α (SDF-1α) levels. The cut-off value of SDF-1α (2162 pg/mL) was determined by receiver-operating characteristic analysis in the study patients. High levels of SDF-1α (≥2162 pg/mL, n = 82) resulted in higher probability of future cardiac events than the lower one (<2162 pg/mL, n = 110) ($p < 0.0001$).

References

[1] Frangogiannis NG. Regulation of the inflammatory response in cardiac repair. Circ Res 2012;110:159–73.

[2] Nahrendorf M, Pittet MJ, Swirski FK. Monocytes: protagonists of infarct inflammation and repair after myocardial infarction. Circulation 2010;121: 2437–45.

[3] Nikolaos G, Frangogiannis Dewald O, Ren G, Duerr GD, Zoerlein M, Klemm C, Gersch C, Tincey S, Michael LH, Entman ML, Frangogiannis NG. Of mice and dogs: species-specific differences in the inflammatory response following myocardial infarction. Am J Pathol 2004;164:665–77.

[4] Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. Circulation 1990;81: 1161–72.

[5] White HD, Norris RM, Brown MA, Brandt PW, Whitlock RM, Wild CJ. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. Circulation 1987;76:44–51.

[6] Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, Nagler A, Ben-Hur H, Many A, Shultz L, Lider O, Alon R, Zipori D, Lapidot T. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science 1999;283:845–8.

[7] Uematsu M, Yoshizaki T, Shimizu T, Obata JE, Nakamura T, Fujioka D, Watanabe K, Watanabe Y, Kugiyama K. Sustained myocardial production of stromal cell-derived factor-1α was associated with left ventricular adverse remodelling in patients with myocardial infarction. Am J Physiol Heart Circ Physiol 2015;309:H1764–71.

[8] Fortunato O, Spinetti G, Specchia C, Cangiano E, Valgimigli M, Madeddu P. Migratory activity of circulating progenitor cells and serum SDF-1α predict adverse events in patients with myocardial infarction. Cardiovasc Res 2013;100:192–200.

[9] Tong G, Wang N, Zhou Y, Leng J, Gao W, Tong X, Shen Y, Yang J, Ye X, Zhou L, Gao Y. Role of stromal cell-derived factor-1 in patients with non-ST elevation acute coronary syndrome. Int Heart J 2014;55:219–27.

[10] Kugiyama K, Motoyama T, Hirashima O, Ohgushi M, Soejima H, Misumi K, Kawano H, Miyao Y, Yoshimura M, Ogawa H, Matsumura T, Sugiyama S, Yasue H. Vitamin C attenuates abnormal vasomotor reactivity in spasm coronary arteries in patients with coronary spastic angina. J Am Coll Cardiol 1998;32: 103–9.

[11] Braunwald E, Antman EM, Beasley JW, Califf RM, Cheitlin MD, Hochman JS, Jones RH, Kereiakes D, Kupersmith J, Levin TN, Pepine CJ, Schaeffer JW, Smith 3rd EE, Steward DE, Theroux P, et al. ACC/AHA guidelines for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction: executive summary and recommendations. A report of the American College of Cardiology/American Heart Association task force on practice guidelines (committee on the management of patients with unstable angina). Circulation 2000;102:1193–209.

[12] Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143–421.

[13] Teichholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of asynergy. Am J Cardiol 1976;37:7–11.

[14] Norimatsu K, Miura S, Suematsu Y, Shiga Y, Miyase Y, Nakamura A, Yamada M, Matsunaga A, Saku K. Associations between glycated albumin or hemo-globin A1c and the presence of coronary artery disease. Int J Cardiol 2015;65:487–93.

[15] American Diabetes Association. Standards of medical care in diabetes – 2006. Diabetes

Care 2006;29(Suppl. 1):S4–2.

[16] Ikeda N, Hara H, Hiroi Y. 1,5-Anhydro-D-glucitol predicts coronary artery disease prevalence and complexity. Int J Cardiol 2014;64:297–301.

[17] Makino A, Nakamura T, Hirano M, Kitta Y, Sano K, Kobayashi T, Fujioka D, Saito Y, Watanabe K, Watanabe Y, Kawabata K, Obata JE, Kugiyama K. High plasma levels of macrophage migration inhibitory factor are associated with adverse long-term outcome in patients with stable coronary artery disease and impaired glucose tolerance or type 2 diabetes mellitus. Atherosclerosis 2010;213:573–8.

[18] Abbott JD, Huang Y, Liu D, Hickey R, Krause DS, Giordano FJ. Stromal cellderived factor-1α plays a critical role in stem cell recruitment to the heart after myocardial infarction but is not sufficient to induce homing in the absence of injury. Circulation 2004;110:3300–5.

[19] Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, Rovner A, Ellis SG, Thomas JD, DiCorleto PE, Topol EJ, Penn MS. Effect of stromal-cellderived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 2003;362:697–703.

[20] Elmadbouh I, Haider HKh, Jiang S, Idris NM, Lu G, Ashraf M. Ex vivo delivered stromal cell-derived factor-1α promotes stem cell homing and induces angiomyogenesis in the infarcted myocardium. J Mol Cell Cardiol 2007;42: 792–803.

[21] Frangogiannis NG. The stromal cell-derived factor-1/CXCR4 axis in cardiac injury and repair. J Am Coll Cardiol 2011;58:2424–6.

[22] Saxena A, Fish JE, White MD, Yu S, Smyth JW, Shaw RM, DiMaio JM, Srivastava D. Stromal cell-derived factor-1α is cardioprotective after myocardial infarction. Circulation 2008;117:2224–31.

[23] Chen J, Chemaly E, Liang L, Kho C, Lee A, Park J, Altman P, Schecter AD, Hajjar RJ,

Tarzami ST. Effects of CXCR4 gene transfer on cardiac function after ischemiareperfusion injury. Am J Pathol 2010;176:1705–15.

[24] Jujo K, Hamada H, Iwakura A, Thorne T, Sekiguchi H, Clarke T, Ito A, Misener S, Tanaka T, Klyachko E, Kobayashi K, Tongers J, Roncalli J, Tsurumi Y, Hagiwara N, et al. CXCR4 blockade augments bone marrow progenitor cell recruitment to the neovasculature and reduces mortality after myocardial infarction. Proc Natl Acad Sci U S A 2010;107:11008–13.

[25] Liehn EA, Tuchscheerer N, Kanzler I, Drechsler M, Fraemohs L, Schuh A, Koenen RR, Zander S, Soehnlein O, Hristov M, Grigorescu G, Urs AO, Leabu M, Bucur I, Merx MW, et al. Double-edged role of the CXCL12/CXCR4 axis in experimental myocardial infarction. J Am Coll Cardiol 2011;58:2415–23.

[26] Proulx C, El-Helou V, Gosselin H, Clement R, Gillis MA, Villeneuve L, Calderone A. Antagonism of stromal cell-derived factor-1α reduces infarct size and improves ventricular function after myocardial infarction. Pflugers Arch 2007;455:241–50.

[27] Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, Capla JM, Galiano RD, Levine JP, Gurtner GC. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004;10:858–64.

[28] Jin C, Fu WX, Xie LP, Qian XP, Chen WF. SDF-1α production is negatively regulated by mouse estrogen enhanced transcript in a mouse thymus epithelial cell line. Cell Immunol 2003;223:26–34.

[29] Hohensinner PJ, Kaun C, Rychli K, Niessner A, Pfaffenberger S, Rega G, Furnkranz A, Uhrin P, Zaujec J, Afonyushkin T, Bochkov VN, Maurer G, Huber K, Wojta J. The inflammatory mediator oncostatin M induces stromal derived factor-1 in human adult cardiac cells. FASEB J 2009;23:774–82.

[30] Fang L, Moore XL, Dart AM, Wang LM. Systemic inflammatory response following acute

myocardial infarction. J Geriatr Cardiol 2015;12:305–12.

[31] Libby P, Nahrendorf M, Swirski FK. Leukocytes link local and systemic inflammation in ischemic cardiovascular disease: an expanded cardiovascular continuum. J Am Coll Cardiol 2016;67:1091–103.

[32] Rouleau JL, de Champlain J, Klein M, Bichet D, Moye´ L, Packer M, Dagenais GR, Sussex B, Arnold JM, Sestier F, et al. Activation of neurohumoral systems in postinfarction left ventricular dysfunction. J Am Coll Cardiol 1993;22:390–8.

[33] Sigurdsson A, Held P, Swedberg K. Short- and long-term neurohormonal activation following acute myocardial infarction. Am Heart J 1993;126: 1068–76.

[34] Nabah YN, Mateo T, Estelle´ s R, Mata M, Zagorski J, Sarau H, Cortijo J, Morcillo EJ, Jose PJ, Sanz MJ. Angiotensin II induces neutrophil accumulation in vivo through generation and release of CXC chemokines. Circulation 2004;110: 3581–6.

High levels of stromal cell-derived factor-1α predict secondary cardiac events in stable patients with a history of myocardial infarction

Satoshi Matsuoka (MD)[a], Manabu Uematsu (MD)[a], Takamitsu Nakamura (MD, PhD)[a], Takuya Shimizu (MD)[a], Mika Futamata (MD)[a], Jun-ei Obata (MD, PhD)[a], Daisuke Fujioka (MD, PhD)[a], Kazuto Nakamura (MD, PhD)[a], Toru Yoshizaki (MD)[a], Kiyotaka Kugiyama (MD, PhD, FJCC)[a]

[a] *Department of Internal Medicine II, University of Yamanashi, Faculty of Medicine, Chuo, Yamanashi, Japan*

Key words: stromal cell-derived factor-1α, myocardial infarction, prognosis, cardiac events, inflammatory marker.

This paper was published in the following journal: Journal of Cardiology, 69(1), 320-325. doi.org/10.1016/j.jjcc.2016.06.011

Abstract

Background: We recently showed that stromal cell-derived factor (SDF)-1α, a proinflammatory mediator, is produced in infarcted myocardium and is associated with left ventricular (LV) adverse remodeling and progressive dysfunction following acute myocardial infarction (AMI). The current study examined whether SDF-1α levels in the peripheral vein can provide prognostic information of outcomes in stable patients with a history of MI.

Methods: Plasma levels of SDF-1α in the peripheral vein were measured by enzyme-linked immunosorbent assay in 192 stable patients with a history of MI. All patients were followed prospectively for a period of 90 months or until occurrence of one of the following cardiac events: cardiac death, non-fatal myocardial infarction, unstable angina requiring unplanned coronary revascularization, or worsening heart failure requiring hospital admission.

Results: During the follow-up period (77 ± 26 months), 30 patients had cardiac events. Multivariate Cox analysis revealed that high levels of SDF-1α (≥2162 pg/mL; a cut-off value determined by receiver-operating characteristic analysis) were a significant predictor of cardiac events, independent of traditional risk factors (HR: 1.98; 95% CI: 1.38–2.85; $p < 0.001$). The addition of high levels of SDF-1α to conventional risk factors including brain natriuretic peptide improved net reclassification improvement (NRI) and integrated discrimination improvement (IDI) (NRI 0.90, $p < 0.000$-1αnd IDI 0.05, $p = 0.002$).

Conclusions: High levels of SDF-1α predicted secondary cardiac events in stable patients with a history of MI. SDF-1α levels may be a useful risk assessment tool in patients with a history of MI.

Introduction

Acute myocardial infarction (AMI) induces the production of chemokines and growth factors that recruit neutrophils and phagocytes to the ischemic cardiac tissue, leading to clearance of dead cells and matrix debris [1,2]. This acute, localized inflammatory response is transient, and is followed by resolution of the inflammation [1,3]. However, prolonged inflammation leads to dilative remodeling and progressive dysfunction of the infarcted myocardium through matrix degradation and cardiomyocyte apoptosis [1,2]. Left ventricle (LV) adverse remodeling and dysfunction after MI are predictors of poor outcome following MI, and are associated with ventricular arrhythmias, heart failure, and increased mortality [4,5]. Stromal cell-derived factor (SDF)-1α/CXCL12 is a CXC chemokine with chemotactic effects on CXCR4-expressing progenitor cells [6]. We have recently shown that SDF-1α, a proinflammatory mediator, is produced in the infarcted myocardial lesion and released into the circulation in patients with AMI [7]. In addition, we showed that the myocardial production of SDF-1α in the chronic phase (6 months after MI) but not in the early phase (2 weeks after MI) was associated with LV adverse remodeling and progressive dysfunction in patients with AMI [7]. Thus, prolonged production of SDF-1α in the infarcted myocardium may exert detrimental effects on post-MI LV function in the chronic phase following MI. The prognostic value of SDF-1α levels in the peripheral vein was previously evaluated in patients with AMI/ acute coronary syndrome (ACS) [8,9]. However, it remains unclear whether SDF-1α levels can provide prognostic information in patients in the chronic phase of MI. The current study examined the prognostic value of SDF-1α levels in the peripheral vein in stable patients with a history of MI.

Methods

Study patients

The study screened 386 patients with a history of MI, who underwent cardiac catheterization in Yamanashi University Hospital between February 2003 and March 2009. All patients underwent routine blood testing at the time of discharge. The inclusion criteria were: (1) stable previous MI; (2) no episode of angina at rest and no changes in the frequency of angina in response to sublingual nitroglycerin in the previous 2 months. Exclusion criteria included: (1) ACS, stroke, cardiogenic shock, pulmonary edema, major surgery, trauma or serious infectious disease within 4 weeks prior to enrollment; (2) neoplasm, significant hepatic or inflammatory disease; (3) chronic renal failure or serum creatinine >2.5 mg/dL, congestive heart failure, or left main coronary artery disease; (4) other serious diseases. Finally, a total of 210 patients were enrolled in the study according to these inclusion and exclusion criteria. The study also included 31 control patients without echocardiographic findings of significant valvular disease (moderate or more regurgitation, stenosis), cardiomyopathy [chamber size dilatation, reduced left ventricular ejection fraction (LVEF), ventricular asynergy, ventricular hypertrophy, aneurysm], and pulmonary hypertension, chosen from among the 6-1αngiographically normal patients [without percutaneous coronary intervention (PCI) history] who were evaluated during the study period. Control patients served as a reference group for plasma SDF-1α concentrations. Each of the control patients underwent diagnostic coronary angiography for atypical chest pain at rest at Yamanashi University Hospital during the study period. Control patients fulfilled all of the following inclusion criteria: (1) no significant ST segment changes on 12-lead electrocardiogram (ECG) while having chest pain or on ambulatory ECG; (2) neither chest pain nor ST segment changes during the treadmill test; (3) no coronary artery spasm during provocation with intra-

coronary infusion of acetylcholine [10]. Clinical characteristics of patients with a history of MI and control patients are shown in Table -1αll study participants were ethnic Japanese. All patients gave written, informed consent at the time of enrollment. The study was approved by the ethics committee of Yamanashi University Hospital. The investigation conformed to the principles outlined in the 1975 Declaration of Helsinki.

Prospective study

Patients were followed every month in the hospital or with a clinic visit for 90 months, or until the occurrence of cardiac death, non-fatal MI, refractory unstable angina pectoris (uAP) requiring unplanned coronary revascularization, or decompensated heart failure. The time to the first event was evaluated prospectively. Cardiac death was confirmed by hospital records. Acute MI and uAP were diagnosed by the presence of acute ischemic symptoms lasting $\geq$20 min within 48 h prior to hospital admission, and ECG changes. Acute MI was diagnosed when creatine kinase-MB levels increased to at least 2 times the upper limit of normal or when troponin T levels were >0.1 ng/mL [11]. The diagnoses of MI and uAP were confirmed by coronary angiography. Decompensated heart failure was defined as resting dyspnea with progressive fluid retention requiring hospitalization and treatment with an intravenous diuretic. Follow-up data were collected from the patients' primary physicians every 3 months by two blinded investigators (T.N., J.O.). During the follow-up period, standard medications were prescribed to all patients according to the guidelines of the American Heart Association [12], as shown in Table 1. Diet and lifestyle recommendations were continued throughout the follow-up period.

Laboratory measurements

Venous blood was obtained from all patients on the morning of discharge after a 12-h overnight fast. The initial volumes of each blood sample, including those forcibly drawn, were discarded. Serum and EDTA-plasma were aliquoted and stored at –80℃ until time of analysis. Plasma SDF-1α levels were measured by enzyme-linked immunosorbent assay using a commercial kit (R & D Systems, Minneapolis, MN, USA). In our laboratory, the intra- and inter-assay coefficients of variation were 1.9% and 3.5%, respectively. The minimal detection limit of this assay was 18 pg/mL. Serum C-reactive protein (CRP) levels were assayed by rate nephelometry (Dade Behring, Tokyo, Japan). Plasma levels of brain natriuretic peptide (BNP) were measured by immunoradiometric assay (Shionogi Pharmaceutical, Osaka, Japan). Estimated glomerular filtration rate (eGFR) was calculated by the Modification of Diet in Renal Disease study equation. Echocardiographic LVEF was calculated by the motion-mode method using the Teichholz formula [13].

Statistical analysis

All descriptive data were expressed as mean ± SD, median, or frequency (%). The Shapiro–Wilk test showed that age, body mass index (BMI), heart rate, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), hemoglobin A1c (HbA1c), LVEF, SDF-1α, BNP, and CRP were not normally distributed, hence, these variables were expressed as the median and inter-quartile ranges (25th and 75th percentiles). Non-normally distributed variables were log-transformed before the analysis. Continuous variables were compared between the two groups using the unpaired t-test or the Mann–Whitney U-test, as appropriate. Frequencies were compared using the chi-square test. For comparisons of the mean value of ≥3 groups, one-way analysis of variance (ANOVA) followed by a Scheffe test for post hoc comparisons was employed. The correlation between

the two groups was examined using univariate and multivariate linear regression analyses. Kaplan–Meier analysis was performed on the 2 groups and was based on a cut-off SDF-1α level. The cut-off level of SDF-1α (2162 pg/mL) was determined by receiver-operating characteristic (ROC) analyses of SDF-1α in the study patients with a history of MI (Fig. 1). The predictive values were assessed by univariate or forward stepwise multivariate Cox proportional hazards analysis. For a forward stepwise multivariate Cox hazard analysis, the significance level was set at 0.1. In the univariate and multivariate Cox proportional hazards analyses, continuous variables were estimated for 1-SD change. Dichotomous variables were coded as 1 for the presence of, and 0 for the absence of, each factor. The following factors were included as dichotomous variables: systemic hypertension (≥140/90 mmHg or use of an antihypertensive medication) [14], diabetes mellitus (DM) (as defined by the American Diabetes Association or use of an antidiabetic medication) [15,16], smoking history (defined as smoking ≥10 cigarettes/day for ≥10 years), atrial fibrillation (including paroxysmal atrial fibrillation recorded with an ECG), and multivessel disease. The Cox models included only variables that showed proportionality in a Schoenfeld residuals test. The category-free net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) were used to examine the additive effects of SDF-1α and BNP levels on the predictive value of the baseline model that consisted of conventional cardiovascular risk factors. Conventional risk factors included age, male gender, DM, hypertension, smoking, levels of LDL-C, HDL-C, CRP, LVEF, and multivessel disease. All probability values were presented as 2-tailed with statistical significance inferred at $p < 0.05$. All confidence intervals were computed at the 95% level. Statistical analyses were performed using STATA 10.0 (StataCorp, College Station, TX, USA).

Power analysis

Our previous study showed that the composite endpoints occurred in approximately 52% of stable coronary artery disease (CAD) patients with high coronary risk factors and higher macrophage inhibitory factor (MIF) levels as an inflammatory marker, and in 18% of patients with lower MIF levels during 5 years of follow-up [17]. On the basis of that study, a total of 72 patients were required for a two-sided statistical analysis with sufficient statistical power of 0.90 (b = 0.10 and a = 0.05). This justified the number of patients (n = 220) included in the current prospective study.

Results

Over the course of the study, 7 patients were lost to follow up, and 11 patients were excluded due to non-cardiac related death during follow-up. A total of 192 patients completed the follow-up study (2–90 months, mean = 77 ± 26 months). During the follow-up period, 30 (15.6%) cardiac events occurred, including 5 cardiac deaths, 1 non-fatal MI, 12 uAPs requiring unplanned coronary revascularization, and 12 episodes of worsening heart failure. Patient characteristics are shown in Table 1.

Patients and SDF-1α levels

The time between blood sampling for SDF-1α level and the previous MI ranged from 3 to 26 months, with a mean of 7.7 ± 2.1 months. The time between onset of previous MI and blood sampling did not correlate with SDF-1α levels (r = –0.08, p = 0.28). As shown in Table 2, SDF-1α levels were positively correlated with age, BNP, and CRP but inversely correlated with eGFR in the univariate linear regression analysis (Table 2). SDF-1α levels did not significantly correlate with the frequency of any cardiovascular medication (Table 2).

Prevalence of PCI-related variables was similar between patients with high and low SDF-1α levels (Supplementary Table 1).

Prospective study

Patients (n = 82) with higher SDF-1α levels (≥2162 pg/mL, a cut-off value determined by ROC analysis) had 26 events during follow-up, whereas patients (n = 110) with lower SDF-1α levels (<2162 mg/dL) had 4 events ($p < 0.01$). The cut-off value of SDF-1α levels as 2162 pg/mL, determined by ROC analysis, provided sensitivity of 86.7%, specificity of 65.4%, and accuracy of 68.8% for prediction of future events (Fig. 1). Kaplan–Meier analysis demonstrated a significantly higher probability of a cardiac event in patients with higher SDF-1α levels compared with those with lower SDF-1α levels (Fig. 2). A univariate Cox proportional hazards analysis revealed that SDF-1α levels (HR 1.87; 95% CI 1.35–2.60), age (HR 1.83; 95% CI 1.21–2.78), DM (HR 2.73; 95% CI 1.30–5.75), atrial fibrillation (HR 2.93; 95% CI 1.02–8.42), multivessel disease (HR 2.50; 95% CI 1.02–6.12), LVEF (HR 0.66; 95% CI 0.47–0.94), eGFR (HR 0.60; 95% CI 0.40–0.88), and BNP levels (HR 1.37; 95% CI 1.17–1.60) were significantly associated with future cardiac events, as shown in Table 3. A forward stepwise multivariate Cox proportional hazards analysis demonstrated that SDF-1α levels remained significantly associated with future events after adjustment for potential confounding variables (HR 1.98; 95% CI 1.38–2.85).

Incremental effects of SDF-1α levels on the predictive value of traditional risk factors

Category-free NRI and IDI demonstrated the additive value of SDF-1α to the baseline conventional risk factors consisting of age, male gender, DM, hypertension, smoking, levels of LDL-C, HDL-C, and CRP, LVEF, and multivessel disease (NRI 0.77, $p = 0.0001$;

IDI 0.07, p = 0.001) (Table 4). When BNP was added to the baseline model of risk factors, SDF-1α had significant additive effect on the predictive value of the model (NRI 0.90, p < 0.0001; IDI 0.05, p = 0.002) (Table 4). In contrast, BNP had no significant additive effect on the predictive value of a risk factor model with SDF-1α (Table 4).

Discussion

The present study showed that high SDF-1α levels are a predictor of adverse outcomes in patients with a history of MI. Moreover, high SDF-1α levels had incremental effects on prognostic value of traditional risk factors in this patient population. Thus, SDF-1α levels may aid in risk assessment in stable patients with a history of MI. We have previously shown that SDF-1α is released from the infarcted myocardium into the coronary circulation in both the early (2 weeks after MI) and chronic phases (6 months after MI) following AMI [7]. The myocardial production and release of SDF-1α in the chronic phase of MI, rather than in the early phase, was associated with post-MI LV adverse remodeling and progressive dysfunction [7]. These results support the present finding that SDF-1α levels can predict secondary cardiac events in patients with a history of MI. Animal experiments have demonstrated that local infusion or over-expression of SDF-1α attenuates myocardial ischemic injury following LV dysfunction [18–22]. In contrast, other experimental studies have shown that SDF-1α/CXCR4 signaling worsens post-MI LV function due to increased inflammatory injury and apoptotic mechanisms [23–26]. Thus, the effect of SDF-1α on post-MI LV function in animal models remains unclear. In animal models, the effects of endogenous SDF-1α on post-MI cardiac tissue may be balanced between angiogenic/ reparative and proinflammatory actions of SDF-1α/CXCR4 signaling [21]. In humans, our previous [7] and present studies suggested that endogenous SDF-1α in the infarcted

myocardium and in the peripheral vein might exert detrimental effects on post-MI cardiac tissue.

Previous clinical reports have demonstrated that, when SDF-1α levels were measured in the acute phase of ischemic events, high SDF-1α levels in the peripheral vein correlated with recurrent cardiac events in patients with AMI and non-ST elevation ACS [8,9]. However, our previous study showed that myocardial production of SDF-1α in the early phase of AMI did not reflect post-MI LV adverse remodeling and dysfunction [7]. These results suggest that SDF-1α in the infarcted myocardium and in the peripheral vein might reflect different tissues of origin. That is, SDF-1α levels in the peripheral vein during the early phase of cardiac ischemic events may not originate from the infarcted myocardium. SDF-1α is produced in the ischemic myocardium through induction of hypoxia-inducible factor [27]. Also, SDF-1α may be induced by proinflammatory stimuli in various systemic tissues [28,29] as systemic activation of proinflammatory responses occurs in the chronic phase of MI [30,31]. However, the precise source of SDF-1α in the peripheral vein remains undefined. A previous report showed that SDF-1α levels in the peripheral vein increased during the acute phase of MI [9]. In the present stable patients with a history of MI, SDF-1α levels were higher in the patients with future secondary cardiac events than in control subjects. The mechanism by which high SDF-1α levels were sustained in the peripheral vein during the chronic phase of MI is unclear. A systemic inflammatory response occurs during the acute phase of MI, but is resolved in the chronic phase [1,3]. The mechanisms leading to resolution, including inhibition of proinflammatory cytokine synthesis [1], may be impaired in the patients who have recurrent cardiac events. For example, post-MI LV dysfunction might induce systemic activation of the renin– angiotensin–aldosterone system, leading to prolonged proinflammatory responses in the chronic phase of MI [32–34]. Thus, sustained elevation of SDF-1α levels may reflect

persistent LV adverse remodeling and dysfunction after MI. In support of this, we observed that SDF-1α levels positively correlated with BNP levels in the present study. In the present study, BNP did not have a significant predictive value in the multivariate Cox proportional hazard analysis. This was explained by the strong correlation of BNP with LVEF which was included in the co-variables of the multivariate Cox hazard analysis. The present study included a relatively small number of patients evaluated at a single center. The present results could not be generalized in other cohorts including patients with acute coronary syndrome or heart failure. A large prospective trial is required to understand the precise role of SDF-1α in the pathogenesis of chronic phase of MI. In conclusion, high levels of SDF-1α in the peripheral vein predicted secondary cardiac events in stable patients with a history of MI. The high SDF-1α levels had incremental effects on the prognostic value of the traditional risk factors in this patient population. Hence, SDF-1α levels may be a useful risk assessment tool in patients with a history of MI.

Funding

This study was supported by JSPS KAKENHI Grant Number B2- 19390209 and B-22390158.

Disclosures

The authors declare that there is no conflict of interest.

Table 1. Characteristics of study patients with a history of MI and control subjects.

	Patients with events (n=30)	Patients without events (n=162)	Control (n=31)
Age (yr)	72 (61-79)*†	65 (56-75)	63 (58-70)
Male gender, *n* (%)	26 (87)†	128 (79)†	15 (48)
Smoking history, *n* (%)	21 (70)†	119 (73)†	11 (35)
Hypertension, *n* (%)	18 (60)	106 (65)	15 (48)
Diabetes mellitus, *n* (%)	19 (63)*†	58 (36)†	1 (3)
Atrial fibrillation, *n* (%)	4 (13)	7 (4)	4 (13)
Multivessel disease, *n* (%)	24 (80)*	95 (59)	-
BMI (kg/m^2)	23 (22-25)	24 (22-26)	24 (21-26)
Systolic BP (mmHg)	131 ± 28	138 ± 25	145 ± 29
Heart Rate (beats/min)	65 (56-71)	65 (60-74)	64 (60-78)
LDL-C (mg/dL)	95 (81-129)†	103 (84-126)	116 (101-135)
HDL-C (mg/dL)	43 (36-55)†	44 (37-50)†	58 (51-67)
HbA1c (%)	6.7 (5.9-7.4)†	6.1 (5.8-6.8)†	5.8 (5.5-6.0)
LVEF (%)	52 (38-61)*†	57 (49-66)†	68 (62-75)
eGFR (mL/min)	58 ± 18*†	67 ± 17	70 ± 15
SDF-1α (pg/mL)	2346 (2221-2504)*†	2030 (1829-2300)	1948 (1715-2086)
BNP (pg/mL)	74 (41-207)*†	38 (20-80)	15 (6-29)
CRP (mg/L)	0.7 (0.4-1.3)*	0.5 (0.2-1.0)	0.3 (0.2-1.1)
Medications, *n* (%)			
Aspirin	30 (100)†	162 (100)†	3 (10)
Thienopyridines	26 (87)†	125 (77)†	0 (0)
β-blocker	8 (27)†	44 (27)†	3 (10)
ACEI / ARB	25 (83)†	119 (73)†	7 (23)
Statin	18 (60)†	117 (72)†	6 (19)

Data are expressed either as the mean value ± SD, median and range (25th and 75th percentile), or number (%) of patients. MI, myocardial infarction; BMI, body mass index; BP, blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; SDF-1α, stromal cell-derived factor-1α; BNP, brain natriuretic peptide; CRP, C-reactive protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

* $p < 0.05$ vs. patients without events.

† $p < 0.05$ vs. control patients.

Table 2. Univariate and multivariate linear regression analysis of the correlations between SDF-1α and baseline clinical parameters in patients with a history of MI.

	Univariate analysis		Multivariate analysis	
	r	p value	β	p value
Age	0.179	0.01	0.043	0.59
Male gender	- 0.004	0.95	-	-
Smoking	0.043	0.56	-	-
Hypertension	0.053	0.46	-	-
Diabetes mellitus	- 0.010	0.16	-	-
Atrial fibrillation	0.138	0.06	-	-
Multivessel disease	0.010	0.89	-	-
BMI	- 0.095	0.19	-	-
Systolic BP	- 0.131	0.08	-	-
Heart rate	0.036	0.62	-	-
LDL-C	- 0.014	0.85	-	-
HDL-C	0.007	0.93	-	-
HbA1c	- 0.142	0.05	-	-
LVEF	0.084	0.25	-	-
eGFR	- 0.214	0.003	- 0.124	0.12
BNP	0.346	< 0.0001	0.295	< 0.0001
CRP	0.149	0.04	0.088	0.20
Medications			-	-
Thienopyridines	0.079	0.27	-	-
β-blocker	0.050	0.49	-	-
ACEI / ARB	0.005	0.94	-	-
Statin	- 0.070	0.34	-	-

The multivariate analysis consisted of covariates that demonstrated a significant correlation in the univariate analysis. r = regression coefficient. β = standardized regression coefficient. Aspirin was not included in this analysis because it was used by all patients. SDF-1α, stromal cell-derived factor-1α; MI, myocardial infarction; BMI, body mass index; BP, blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; BNP, brain natriuretic peptide; CRP, C-reactive protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angio-tensin II receptor blocker.

Table 3. Univariate and stepwise multivariate Cox hazard analysis of the risk factors for future cardiac events.

	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Age	1.83	1.21-2.78	< 0.01	1.83	1.16-2.91	0.01
Male gender	1.57	0.55-4.49	0.40			
Smoking	0.82	0.37-1.79	0.62			
Hypertension	0.82	0.39-1.70	0.59			
Diabetes mellitus	2.73	1.30-5.75	< 0.01	2.79	1.28-6.10	0.01
Atrial fibrillation	2.93	1.02-8.42	0.04			
Multivessel disease	2.50	1.02-6.12	0.04			
BMI	0.84	0.57-1.22	0.35			
Systolic BP	0.78	0.53-1.13	0.19			
Heart Rate	0.77	0.52-1.15	0.20			
LDL-C	0.85	0.58-1.23	0.38			
HDL-C	1.03	0.73-1.47	0.86			
HbA1c	1.14	0.84-1.53	0.41			
LVEF	0.66	0.47-0.94	0.02	0.69	0.50-0.97	0.03
eGFR	0.60	0.40-0.88	0.01			
SDF-1α	1.87	1.35-2.60	< 0.001	1.98	1.38-2.85	< 0.001
BNP	1.37	1.17-1.60	< 0.001			
CRP	1.25	0.96-1.62	0.10			
Medications						
Thienopyridines	1.78	0.62-5.10	0.28			
β-blocker	0.99	0.44-2.23	0.98			
ACEI / ARB	1.55	0.59-4.04	0.37			
Statin	0.66	0.32-1.37	0.27			

The hazard ratios and 95% CI for continuous variables were estimated by a 1-SD increase. Dichotomous variables were coded as 1 for the presence of, and 0 for the absence of, each factor. BMI, body mass index; BP, blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; SDF-1α, stromal cell-derived factor-1α; BNP, brain natriuretic peptide; CRP, C-reactive protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

Table 4. Incremental effects of SDF-1α levels on the predictive value of traditional risk factors.

	Category-free NRI		IDI	
	NRI value	*p* value	IDI value	*p* value
Traditional risk factors				
+ BNP	0.44	0.03	0.03	0.31
+ SDF-1α	0.77	0.0001	0.07	0.001
Traditional risks + BNP				
+ SDF-1α	0.90	< 0.0001	0.05	0.002
Traditional risks + SDF-1α				
+ BNP	0.10	0.62	0.004	0.69

NRI, net reclassification improvement; IDI, integrated discrimination improve-ment; BNP, brain natriuretic peptide; SDF-1α, stromal cell-derived factor-1α. The traditional risk factors consisted of age, male gender, diabetes mellitus, hypertension, smoking, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, C-reactive protein, left ventricular ejection fraction, and multivessel disease.

Supplementary Table 1. Comparison of clinical variables between patients with high and low SDF-1α.

	High SDF-1α (≥ 2162 pg/mL) (n=82)	Low SDF-1α (< 2162 pg/mL) (n=110)
Age (years)	70 (60-77) *	63 (54-73)
Male gender, *n* (%)	65 (79)	89 (81)
Smoking history, *n* (%)	59 (72)	81 (74)
Hypertension, *n* (%)	55 (67)	69 (63)
Diabetes mellitus, *n* (%)	34 (41)	43 (39)
Atrial fibrillation, *n* (%)	8 (10)	3 (3)
Multivessel disease, *n* (%)	51 (62)	68 (62)
BMI (kg/m^2)	23 (22-25)	24 (22-26)
Systolic BP (mmHg)	134 ± 27	139 ± 24
Heart Rate (beats/min)	65 (60-74)	65 (59-73)
LDL-C (mg/dL)	97 (78-123)	106 (87-129)
HDL-C (mg/dL)	44 (38-52)	44 (37-50)
HbA1c (%)	6.1 (5.8-6.8)	6.2 (5.8-7.0)
LVEF (%)	57 (45-64)	56 (49-66)
eGFR (mL/min)	60 ± 18 *	69 ± 17
BNP (pg/mL)	66 (33-105) *	32 (17-72)
CRP (mg/L)	0.6 (0.3-1.4)	0.5 (0.2-1.0)
Medications, *n* (%)		
Aspirin	82 (100)	110 (100)
Thienopyridines	66 (80)	85 (77)
β-Blocker	25 (30)	27 (25)
ACEI / ARB	64 (78)	80 (73)
Statin	52 (63)	83 (75)
PCI-related variables		
Pre-dilatation, *n* (%)	37 (45)	49 (45)
Post-dilatation, *n* (%)	12 (15)	15 (14)
BMS use, *n* (%)	42 (51)	51 (46)
DES use, *n* (%)	16 (20)	23 (21)
Total stent length (mm)	20 (16-25)	18 (16-24)
Thrombus aspiration, *n* (%)	41 (50)	64 (58)

Data are expressed either as the mean value ± SD, median and range (25th and 75th percentile), or number (%) of patients. The cut-off value of SDF-1α levels as 2162 pg/mL

was determined by ROC analysis. SDF-1α, stromal cell-derived factor-1α; BMI, body mass index; BP, blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction; eGFR, estimated glomerular filtration rate; BNP, brain natriuretic peptide; CRP, C-reactive protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; PCI, percutaneous coronary intervention that was performed in the culprit coronary lesion at the occurrence of MI; BMS, bare metal stent; DES, drug-eluting stent.
* $p<0.05$ vs. low SDF-1α patients.

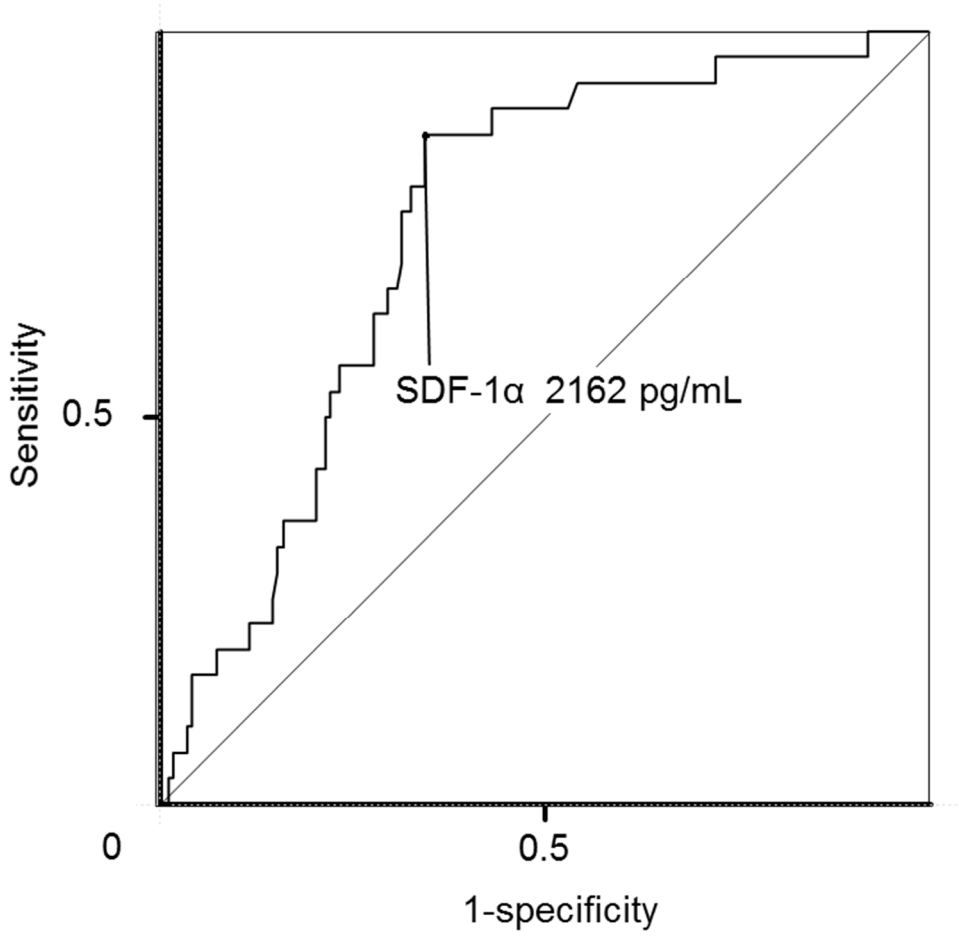

Fig. 1. Receiver operator characteristic curve to obtain optimal cut-off level of stromal cell-derived factor-1α (SDF-1α) for the prediction of cardiac events.

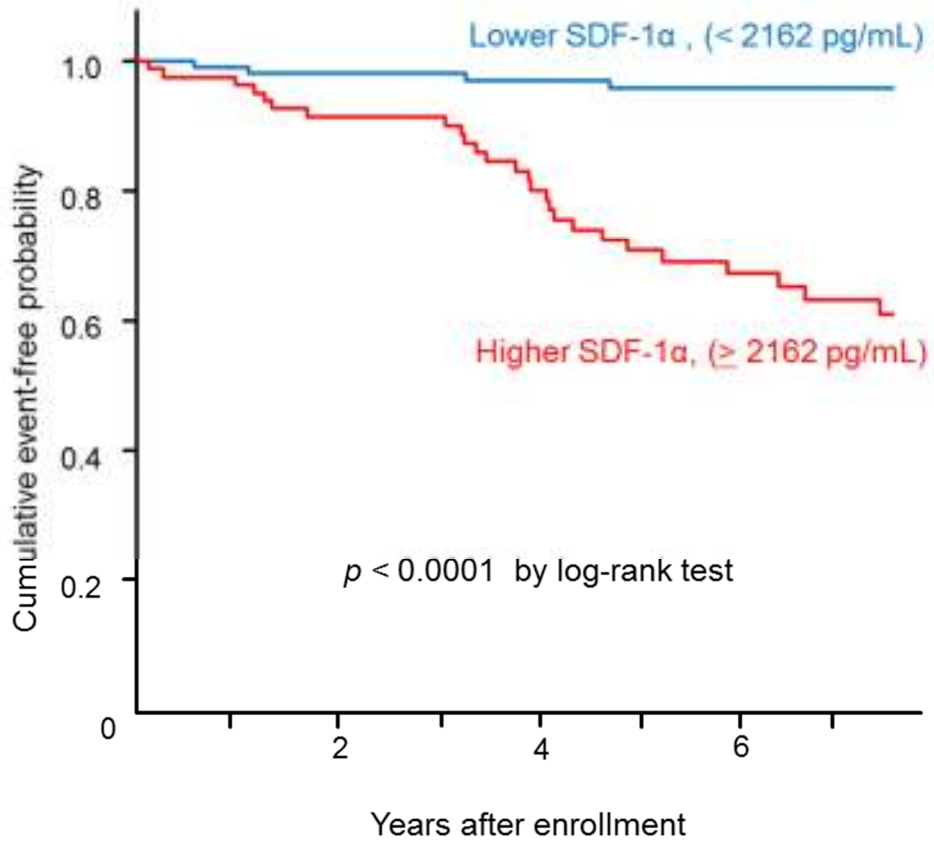

Fig. 2. Kaplan–Meier analysis according to stromal cell-derived factor-1α (SDF-1α) levels. The cut-off value of SDF-1α (2162 pg/mL) was determined by receiver-operating characteristic analysis in the study patients. High levels of SDF-1α (≥2162 pg/mL, n = 82) resulted in higher probability of future cardiac events than the lower one (<2162 pg/mL, n = 110) ($p < 0.0001$).

References

[1] Frangogiannis NG. Regulation of the inflammatory response in cardiac repair. Circ Res 2012;110:159–73.

[2] Nahrendorf M, Pittet MJ, Swirski FK. Monocytes: protagonists of infarct inflammation and repair after myocardial infarction. Circulation 2010;121: 2437–45.

[3] Nikolaos G, Frangogiannis Dewald O, Ren G, Duerr GD, Zoerlein M, Klemm C, Gersch C, Tincey S, Michael LH, Entman ML, Frangogiannis NG. Of mice and dogs: species-specific differences in the inflammatory response following myocardial infarction. Am J Pathol 2004;164:665–77.

[4] Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. Circulation 1990;81: 1161–72.

[5] White HD, Norris RM, Brown MA, Brandt PW, Whitlock RM, Wild CJ. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. Circulation 1987;76:44–51.

[6] Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, Nagler A, Ben-Hur H, Many A, Shultz L, Lider O, Alon R, Zipori D, Lapidot T. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science 1999;283:845–8.

[7] Uematsu M, Yoshizaki T, Shimizu T, Obata JE, Nakamura T, Fujioka D, Watanabe K, Watanabe Y, Kugiyama K. Sustained myocardial production of stromal cell-derived factor-1α was associated with left ventricular adverse remodelling in patients with myocardial infarction. Am J Physiol Heart Circ Physiol 2015;309:H1764–71.

[8] Fortunato O, Spinetti G, Specchia C, Cangiano E, Valgimigli M, Madeddu P. Migratory activity of circulating progenitor cells and serum SDF-1α predict adverse events in patients with myocardial infarction. Cardiovasc Res 2013;100:192–200.

[9] Tong G, Wang N, Zhou Y, Leng J, Gao W, Tong X, Shen Y, Yang J, Ye X, Zhou L, Gao Y. Role of stromal cell-derived factor-1 in patients with non-ST elevation acute coronary syndrome. Int Heart J 2014;55:219–27.

[10] Kugiyama K, Motoyama T, Hirashima O, Ohgushi M, Soejima H, Misumi K, Kawano H, Miyao Y, Yoshimura M, Ogawa H, Matsumura T, Sugiyama S, Yasue H. Vitamin C attenuates abnormal vasomotor reactivity in spasm coronary arteries in patients with coronary spastic angina. J Am Coll Cardiol 1998;32: 103–9.

[11] Braunwald E, Antman EM, Beasley JW, Califf RM, Cheitlin MD, Hochman JS, Jones RH, Kereiakes D, Kupersmith J, Levin TN, Pepine CJ, Schaeffer JW, Smith 3rd EE, Steward DE, Theroux P, et al. ACC/AHA guidelines for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction: executive summary and recommendations. A report of the American College of Cardiology/American Heart Association task force on practice guidelines (committee on the management of patients with unstable angina). Circulation 2000;102:1193–209.

[12] Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143–421.

[13] Teichholz LE, Kreulen T, Herman MV, Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of asynergy. Am J Cardiol 1976;37:7–11.

[14] Norimatsu K, Miura S, Suematsu Y, Shiga Y, Miyase Y, Nakamura A, Yamada M, Matsunaga A, Saku K. Associations between glycated albumin or hemo-globin A1c and the presence of coronary artery disease. Int J Cardiol 2015;65:487–93.

[15] American Diabetes Association. Standards of medical care in diabetes – 2006. Diabetes

Care 2006;29(Suppl. 1):S4–2.

[16] Ikeda N, Hara H, Hiroi Y. 1,5-Anhydro-D-glucitol predicts coronary artery disease prevalence and complexity. Int J Cardiol 2014;64:297–301.

[17] Makino A, Nakamura T, Hirano M, Kitta Y, Sano K, Kobayashi T, Fujioka D, Saito Y, Watanabe K, Watanabe Y, Kawabata K, Obata JE, Kugiyama K. High plasma levels of macrophage migration inhibitory factor are associated with adverse long-term outcome in patients with stable coronary artery disease and impaired glucose tolerance or type 2 diabetes mellitus. Atherosclerosis 2010;213:573–8.

[18] Abbott JD, Huang Y, Liu D, Hickey R, Krause DS, Giordano FJ. Stromal cellderived factor-1α plays a critical role in stem cell recruitment to the heart after myocardial infarction but is not sufficient to induce homing in the absence of injury. Circulation 2004;110:3300–5.

[19] Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, Rovner A, Ellis SG, Thomas JD, DiCorleto PE, Topol EJ, Penn MS. Effect of stromal-cellderived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 2003;362:697–703.

[20] Elmadbouh I, Haider HKh, Jiang S, Idris NM, Lu G, Ashraf M. Ex vivo delivered stromal cell-derived factor-1α promotes stem cell homing and induces angiomyogenesis in the infarcted myocardium. J Mol Cell Cardiol 2007;42: 792–803.

[21] Frangogiannis NG. The stromal cell-derived factor-1/CXCR4 axis in cardiac injury and repair. J Am Coll Cardiol 2011;58:2424–6.

[22] Saxena A, Fish JE, White MD, Yu S, Smyth JW, Shaw RM, DiMaio JM, Srivastava D. Stromal cell-derived factor-1α is cardioprotective after myocardial infarction. Circulation 2008;117:2224–31.

[23] Chen J, Chemaly E, Liang L, Kho C, Lee A, Park J, Altman P, Schecter AD, Hajjar RJ,

Tarzami ST. Effects of CXCR4 gene transfer on cardiac function after ischemiareperfusion injury. Am J Pathol 2010;176:1705–15.

[24] Jujo K, Hamada H, Iwakura A, Thorne T, Sekiguchi H, Clarke T, Ito A, Misener S, Tanaka T, Klyachko E, Kobayashi K, Tongers J, Roncalli J, Tsurumi Y, Hagiwara N, et al. CXCR4 blockade augments bone marrow progenitor cell recruitment to the neovasculature and reduces mortality after myocardial infarction. Proc Natl Acad Sci U S A 2010;107:11008–13.

[25] Liehn EA, Tuchscheerer N, Kanzler I, Drechsler M, Fraemohs L, Schuh A, Koenen RR, Zander S, Soehnlein O, Hristov M, Grigorescu G, Urs AO, Leabu M, Bucur I, Merx MW, et al. Double-edged role of the CXCL12/CXCR4 axis in experimental myocardial infarction. J Am Coll Cardiol 2011;58:2415–23.

[26] Proulx C, El-Helou V, Gosselin H, Clement R, Gillis MA, Villeneuve L, Calderone A. Antagonism of stromal cell-derived factor-1α reduces infarct size and improves ventricular function after myocardial infarction. Pflugers Arch 2007;455:241–50.

[27] Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, Capla JM, Galiano RD, Levine JP, Gurtner GC. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004;10:858–64.

[28] Jin C, Fu WX, Xie LP, Qian XP, Chen WF. SDF-1α production is negatively regulated by mouse estrogen enhanced transcript in a mouse thymus epithelial cell line. Cell Immunol 2003;223:26–34.

[29] Hohensinner PJ, Kaun C, Rychli K, Niessner A, Pfaffenberger S, Rega G, Furnkranz A, Uhrin P, Zaujec J, Afonyushkin T, Bochkov VN, Maurer G, Huber K, Wojta J. The inflammatory mediator oncostatin M induces stromal derived factor-1 in human adult cardiac cells. FASEB J 2009;23:774–82.

[30] Fang L, Moore XL, Dart AM, Wang LM. Systemic inflammatory response following acute

myocardial infarction. J Geriatr Cardiol 2015;12:305–12.

[31] Libby P, Nahrendorf M, Swirski FK. Leukocytes link local and systemic inflammation in ischemic cardiovascular disease: an expanded cardiovascular continuum. J Am Coll Cardiol 2016;67:1091–103.

[32] Rouleau JL, de Champlain J, Klein M, Bichet D, Moye´ L, Packer M, Dagenais GR, Sussex B, Arnold JM, Sestier F, et al. Activation of neurohumoral systems in postinfarction left ventricular dysfunction. J Am Coll Cardiol 1993;22:390–8.

[33] Sigurdsson A, Held P, Swedberg K. Short- and long-term neurohormonal activation following acute myocardial infarction. Am Heart J 1993;126: 1068–76.

[34] Nabah YN, Mateo T, Estelle´ s R, Mata M, Zagorski J, Sarau H, Cortijo J, Morcillo EJ, Jose PJ, Sanz MJ. Angiotensin II induces neutrophil accumulation in vivo through generation and release of CXC chemokines. Circulation 2004;110: 3581–6.

目次