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Association of circulating growth differentiation factor-15, Krüppel-like factor 4 and growth arrest-specific 6 with coronary artery disease

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ABSTRACT

Background: Current assessment tools for patients with acute chest pain are either traumatic (coronary angiography) or unreliable (measurement of cardiac troponin concentrations). We investigated whether the novel cardiovascular stress markers, serum growth differentiation factor-15 (GDF-15), Krüppel-like factor 4 (KLF4) and growth arrest-specific 6 (gas6) may be useful biomarkers of coronary artery disease (CAD).

Methods: A total of 350 male patients were enrolled, 198 with CAD and 152 controls, based on coronary angiography. GDF-15, KLF4 and gas6 concentrations were measured using commercial enzyme-linked immunosorbent assay kits. Multivariate logistic regression and multivariate linear regression were performed to evaluate potential associations of GDF-15, KLF4 and gas6 with risk of CAD or CAD severity.

Results: Serum GDF-15, KLF4 and gas6 concentrations were significantly higher in male patients with CAD than in control subjects ($P < .05$), and they correlated significantly with involvement of coronary vessels ($P < .05$). After adjusting for confounding factors, we found that circulating GDF-15 concentrations remained positively associated with the presence of CAD (odds ratio [OR] per 1-standard deviation [SD] increase, 3.182; 95% confidence interval [CI] 1.586 to 6.382; $P = .001$), as did KLF4 concentrations (OR per 1-SD increase, 13.05; 95% CI 2.940 to 57.921, $P = .001$). Moreover, circulating GDF-15 concentrations were positively associated with the Gensini score (estimated SD change per 1-SD increase, 22.091; 95% CI 9.147 to 35.035, $P = .001$), as were KLF4 concentrations (estimated SD change per 1-SD increase, 27.996; 95% CI 10.082 to 45.910, $P = .002$). Gas6, in contrast, showed no relationship to presence of CAD or Gensini score.,

Conclusions: In this case-control study, increased concentrations of circulating GDF-15 and KLF4 were significantly associated with the presence and severity of CAD.

1. Introduction

Coronary artery disease (CAD) is a major cause of death and long-term disability, and it places a considerable economic burden on society [1]. Standard methods for CAD diagnosis and risk assessment include coronary angiography and measurement of circulating concentrations of high-sensitivity troponin, high-sensitivity C-reactive protein (hs-CRP) and Cystatin C [2,3]. However, these tools present several limitations, such as lack of cardiovascular specificity (for example, hs-CRP may reflect inflammation from a variety of causes), invasiveness (in the case of coronary angiography), and low sensitivity [4]. Moreover, some assays are specific but relatively insensitive. These limitations may compromise timely diagnosis and treatment of the disease, which may lead to worse prognosis. Therefore, novel biomarkers are urgently needed that can identify patients at greater risk of future cardiac events.

Growth differentiation factor-15 (GDF-15), Krüppel-like factor 4 (KLF4), and growth arrest-specific 6 (gas6) are biomarkers of cardiovascular stress that have been associated with cardiovascular disease [5–7]. GDF-15 is a member of the transforming growth factor-β superfamily widely expressed in many cell types, including endothelial cells, cardiomyocytes, and adipocytes [8]. It has been associated with ventricular remodeling and reduced ejection fraction [9,10]. It is up-regulated by various types of cardiac stress as well as inflammation due to paracrine/autocrine signaling [5,11]. GDF-15 deficiency was shown in vivo to protect against atherosclerosis by weakening macrophage chemotaxis [12]. KLF4 is a member of the Krüppel-like family, which consists of zinc-finger DNA-binding domains, and it plays important roles in cardiovascular pathophysiology [13]. KLF4 modulates the

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phenotype of smooth muscle cells to contribute to atherosclerotic plaque pathogenesis, and KLF4 regulates angiogenesis through the Notch signaling pathway [14,15]. Gas6 was discovered as a homologue of the anticoagulant protein S and acted as a negative coregulator in the blood coagulation cascade [16]. It is expressed in various cell types, such as leukocytes and endothelial cells [17,18]. It binds to receptor tyrosine kinases of the TAM (Tyro-3, Axl, Mer) family to be involved in the regulation of the process of apoptosis, proliferation, and inflammation [19]. Increased plasma gas6 concentrations have been associated with venous thromboembolic disease [20], as well as cardiac remodeling indices such as left ventricular hypertrophy [6]. In cultured vascular smooth muscle cells, gas6 signaling through Axl can inhibit mineral deposition [21]. The present study examined whether these novel biomarkers of cardiovascular stress may be associated with onset or severity of CAD, which might make them useful biomarkers to improve the early diagnosis and treatment of this condition.

2. Materials and methods

2.1. Study population

A total of 350 male patients (mean age 59.4 ± 10.3 y) were recruited recruited from July 2016 to May 2017 in the Department of Cardiology at Renmin Hospital of Wuhan University (Wuhan, China). CAD was defined as ≥50% stenotic lesions in at least one major coronary vessel, as determined by coronary angiography [22]. The results of coronary angiography were evaluated by 2 expert investigators according to Gensini scoring [23]. The left anterior descending artery, left circumflex artery, and right coronary artery were tested to evaluate the number of stenotic coronary arteries, and vessel disease was defined from 0 to 3 [22,24]. If the left main trunk was involved, this was evaluated as a 2-vessel disease by itself [3]. Patients were excluded if they had other severe illnesses, such as infectious disease, pulmonary edema, chronic renal function, or acute kidney injury; or if they were receiving thrombolysis treatment. Finally, 198 patients were recruited as CAD, and 152 patients were enrolled as controls. The study was approved by the Medical Ethics Review Committee of Renmin Hospital, all participants provided written informed consent.

2.2. Definition of risk factors

Diagnosis of hypertension was based on World Health Organization (WHO) criteria: average systolic blood pressure higher than 140 mmHg, diastolic blood pressure > 90 mmHg, or use of antihypertensive medication [25]. Diabetes was defined according to published criteria [26]: a fasting glucose concentration 126 mg/dl (7.0 mmol/l), non-fasting glucose 200 mg/dl (11.1 mmol/l), or self-reported diabetes coupled with the use of any glucose-lowering medication. Hyperlipidemia was defined as a low-density lipoprotein cholesterol (LDL-c) concentration of > 140 mg/dl or use of any lipid-lowering drugs [22].

2.3. Sample preparation

Plasma samples were obtained in the morning after overnight fasting within 24 h after hospital admission [27]. Venous blood samples were collected into tubes and centrifuged at 3500 rpm/min for 15 min at 25 °C. Plasma was separated and stored at −80 °C until analysis.

2.4. Laboratory analyses

Serum concentrations of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and serum creatinine (sCr) were measured using the enzymatic methods with the Siemens Advia 2400 chemistry analyzer. Plasma hs-CRP concentrations were measured using a polyethylene glycol-enhanced immunoturbidimetric assay by Advia 2400.

To quantify the soluble form of human GDF-15 in crude serum, we used 2 Quantikine enzyme-linked immunosorbent assay (ELISA) kits (R &D Systems), with an intra-assay CVs of < 6% and an inter-assay CV of 2.8%. Samples were diluted 10-fold before assay, and the values obtained ranged from 7.8 to 500 pg/ml. KLF4 was also assayed using a commercial ELISA kit (Cusabio) with an intra-assay CV of 8% and inter-assay CV of 10%. Samples were assayed undiluted, and the measured values ranged from 18.75 to 1200 pg/ml. Gas6 were also assayed by ELISA kits (catalog no. DY885B and DY008, R&D Systems, USA), with an intra-assay coefficient of 6.5% and inter-assay coefficient of 8.5% [28]. Samples were diluted 100-fold before assay, and the values obtained ranged from 15.6 to 1000 pg/ml.

2.5. Statistical analysis

Statistical analysis was performed using SPSS 20.0 (IBM, Armonk, NY, USA) and Graphpad Prism 6.0. Results for continuous variables were expressed as mean value ± SD or median (interquartile range, IQR). In comparisons between two groups, the statistical significance of differences in continuous variables was assessed using the independent-samples *t*-test for normally distributed data or the Mann-Whitney *U* test for skewed data. In comparisons among three or more groups, significance of differences was assessed using ANOVA or the Kruskal-Wallis test. Categorical variables were expressed as percentage (%) and compared using the χ^2 test. Spearman's correlation analysis was performed to evaluate the association of GDF-15, KLF4, and gas6 concentrations with baseline patient characteristics and Gensini score. Potential associations of circulating concentrations of GDF-15, KLF4, and gas6 with CAD were assessed using multivariate logistic regression. Potential associations of circulating concentrations of GDF-15, KLF4, and gas6 with Gensini score were assessed using multivariate liner regression. In these regression analyses, concentrations of GDF-15, KLF4, and gas6 were $\log_{10}$-transformed. Results associated with a two-tailed $P < .05$ were considered significant.

3. Results

3.1. Characteristics of the study population

The clinical and demographic characteristics of the individuals included in the study are shown in Table 1. There were 198 male patients with CAD and 152 male patients without CAD, according to coronary angiograph results. The two groups showed no significant differences in the majority of cardiovascular risk factors, including age, hypertension, diabetes, smoking, TC, TG, HDL-c, TC/HDL-c, and sCr concentrations. Patients with CAD had significantly higher hs-CRP and LDL-c concentrations, and Gensini score, as well as higher circulating concentrations of GDF-15 [804.76 (550.25–1135.29) vs. 666.77 (337.34–953.82) pg/ml, $P = .004$], KLF4 [210.78 (156.60–294.66) vs. 184.70 (134.56–242.30) pg/ml, $P = .001$] and gas6 [13.76 (9.90–17.22) vs. 15.93 (12.28–20.55), $P = .030$]. Furthermore, in CAD patients, circulating concentrations of GDF-15 in acute coronary syndrome patients were higher than patients with stable CAD. However, circulating concentrations of KLF4 and gas6 did not differ significantly between patients with stable CAD or acute coronary syndrome (Supplementary Table 1).

3.2. Correlation of circulating GDF-15, KLF4 and gas6 concentrations with baseline characteristics in the CAD group

Associations between laboratory parameters and serum concentrations of GDF-15, KLF4, and gas6 in patients with CAD were tested using Spearman's correlation analysis. As shown in Table 2, GDF-15 concentrations weakly correlated with concentrations of hs-CRP ($r = 0.0.148$, $P = .048$) and sCr ($r = 0.196$, $P = .006$); similarly, KLF4 concentrations weakly correlated with hs-CRP ($r = 0.212$, $P = .009$)

Table 1
Baseline characteristics of the study subjects.

Variable	Controls	CAD	*P* value
	n = 152	*n* = 198	
Age (y)	60.0 (54.2–67.0)	59.0 (53.0–65.0)	NS
Clinical variables			
Diabetes (%)	7.41	11.55	NS
Hypertension (%)	47.72	41.43	NS
Smoking (%)	33.33	35.15	NS
Hyperlipidemia (%)	36.72	41.74	NS
Gensini score	2.50 (1.50–3.50)	35.50 (18.00–72.00)	**< 0.001**
Biochemical analyses			
TC (mmol/l)	3.87 ± 0.73	4.06 ± 0.87	NS
TG (mmol/l)	1.40 (1.06–1.97)	1.45 (0.99–2.11)	NS
HDL-c (mmol/l)	0.95 (0.79–1.06)	0.90 (0.75–1.10)	NS
LDL-c (mmol/l)	2.07 (1.52–2.38)	2.19 (1.63–2.79)	**0.015**
TC/HDL-c	4.26 ± 1.12	4.70 ± 2.19	NS
sCr (μmol/l)	80.00 (71.00–89.00)	74.00 (66.00–87.50)	NS
hs-CRP (mg/l)	0.44 (0.21–3.12)	2.51 (0.53—12.55)	**< 0.001**
Biomarkers			
GDF-15 (pg/ml)	666.77 (337.34–953.82)	804.76 (550.25–1135.29)	**0.004**
KLF4 (pg/ml)	184.70 (134.56–242.30)	210.78 (156.60—294.66)	**0.001**
gas6 (ng/ml)	13.76 (9.90–17.22)	15.93 (12.28–20.55)	**0.030**

Data are presented as the mean value ± SD and median (interquartile range). Differences between groups were analyzed by the independent Student *t*-test, $\chi 2$ test, or Mann-Whitney-*U* test.
Abbreviations: CAD, coronary artery disease; GDF-15, growth differentiation factor-15; KLF4, Krüppel-Like Factor 4; gas6, growth arrest-specific 6.
Bold signifies P < 0.05.

Table 2
Correlation of circulating GDF-15, KLF4, and gas6 concentrations with baseline characteristics in male patients with CAD.

Variable	GDF-15		KLF4		gas6	
	r	*P*	*r*	*P*	*r*	*P value*
Age	0.285	**< 0.001**	0.001	NS	0.020	NS
TC	0.007	NS	−0.031	NS	−0.023	NS
TG	0.003	NS	−0.061	NS	0.020	NS
HDL-c	−0.166	**0.027**	−0.048	NS	−0.059	NS
LDL-c	0.093	NS	0.038	NS	−0.022	NS
TC/HDL-c	0.162	**0.032**	0.013	NS	0.014	NS
sCr	0.196	**0.006**	0.203	**0.005**	0.139	NS
hs-CRP	0.148	**0.048**	0.212	**0.009**	0.300	**< 0.001**

Abbreviations: GDF-15, growth differentiation factor-15; KLF4, Krüppel-Like Factor 4; gas6, growth arrest-specific.
Bold signifies P < 0.05.

and sCr ($r = 0.203$, $P = .005$). Gas6 also correlated positively with hs-CRP ($r = 0.300$, $P < .001$). Moreover, GDF-15 showed a weak positive correlation with TC/HDLc ($r = 0.162$, $P = .032$), and a weak negative correlation with HDL-c ($r = -0.166$, $P = .027$), which may suggest that GDF-15 concentrations are associated with the deposition of serum lipids. GDF-15 also showed a weak positive correlation with age ($r = 0.285$, $P < .001$).

In CAD patients, GDF-15 concentrations were significantly higher in the presence of hypertension than in its absence [881.94 (582.15—1252.75) vs. 720.64 (451.51–1002.43) pg/ml, $P = .009$; Fig. 1A). On the other hand, KLF4 and gas6 concentrations showed no relationship with hypertension [220.14 (145.98—301.42) pg/ml vs. 206.33 (156.60—316.04) pg/ml, $P = .773$; 15.84 (12.01–20.95) vs. 14.75 (9.61–19.00) ng/ml, $P = .122$, Figs. 1B and C]. GDF-15 concentrations were significantly higher in the presence of diabetes than in its absence [929.38 (619.54–1358.04) vs. 804.16 (556.50–1119.87) pg/ml, $P = .044$], as were KLF4 and gas6 concentrations [247.76 (184.49–467.21) vs. 205.97 (149.80–288.11) pg/ml, $P = .011$; 17.61 (13.30–20.87) vs. 15.01 (10.59–19.11) ng/ml, $P = .026$], Figs. 1D, E and F].

3.3. Associations of circulating concentrations of GDF-15, KLF4, and gas6 with angiographic findings

Among the 198 CAD patients, 67 had 1-vessel, 85 had 2-vessel, and 46 had 3-vessel disease. An increase in the number of stenotic coronary vessels was associated with significant increases in GDF-15, KLF4, and gas6 concentrations ($P < .05$, Kruskal-Wallis test; Table 3). GDF-15, KLF4, and gas6 concentrations were significantly higher in patients with 3-vessel disease than in controls ($P < .05$, Mann-Whitney *U* test). GDF-15 concentrations also showed a weak positive correlation with stenosis severity as defined by Gensini score ($r = 0.192$, $P = .004$; Fig. 2A); the same was observed for KLF4 concentrations ($r = 0.211$, $P = .002$; Fig. 2B). In contrast, no correlation was found between gas6 concentrations and Gensini score ($r = 0.121$, $P = .101$, Fig. 2C).

3.4. Association of GDF-15, KLF4, and gas6 concentrations with presence of CAD

Associations between CAD and circulating concentrations of GDF-15, KLF4 and gas6 concentrations were tested in different logistic regression models. In unadjusted analyses, GDF-15 concentrations were associated with the presence of CAD: the OR per SD increase in $\log_{10}$-transformed concentration was 2.780 (95% CI 1.606 to 4.814, $P < .001$). A similar result was observed for KLF4: the OR per SD increase was 10.054 (95% CI 3.120 to 32.394, $P < .001$). These associations persisted after adjustment for conventional CAD risk factors, including age, hypertension, diabetes, smoking status, TG, TC, LDL-c, HDL-c, TC/HDL-c, and sCr. After adjustment, the OR per SD increase was 3.182 (95% CI 1.586 to 6.382, $P = .001$) for GDF-15 and 13.050 (95% CI 2.940 to 57.921, $P < .001$) for KLF4 (Table 4). In contrast, no association was found between gas6 concentrations and the presence of CAD.

To assess the incremental power of these biomarkers, receiver operating characteristic curve analysis was performed. The biomarkers together gave areas under the curve of only approximately 0.6 (data not shown). Thus, these markers should be combined with other characteristics to help identify patients at higher risk of CAD.

3.5. Association of GDF-15, KLF4 and gas6 concentrations with CAD severity

Results from linear regression models for the relationship between biomarkers and Gensini scores are presented in Table 5. GDF-15 concentrations remained significantly associated with Gensini score (estimated SD change per 1-SD increase, 22.091, 95% CI 9.147 to 35.035, $P = .001$) after adjustment for age, hypertension, diabetes, smoking status, TG, TC, LDL-c, HDL-c, TC/HDL-c and sCr. The same was observed for KLF4 concentrations (estimated SD change per 1-SD increase, 27.996, 95% CI 10.082 to 45.910, $P = .002$). In contrast, no adjusted association was found between gas6 concentrations and Gensini score.

4. Discussion

The present study showed that concentrations of novel biomarkers of cardiovascular stress, GDF-15 and KLF4 were associated with the presence and severity of CAD in a cohort of male Chinese patients. In contrast, gsa6 concentrations were not associated with the presence or severity of CAD. Circulating GDF-15, KLF4 and gas6 concentrations were higher in patients with CAD, especially in those with 3-vessel disease. Higher concentrations of GDF-15, KLF4 and gas6 also moderately correlated with the presence of hypertension, diabetes and concentrations of hs-CRP, an important inflammatory factor. Furthermore,

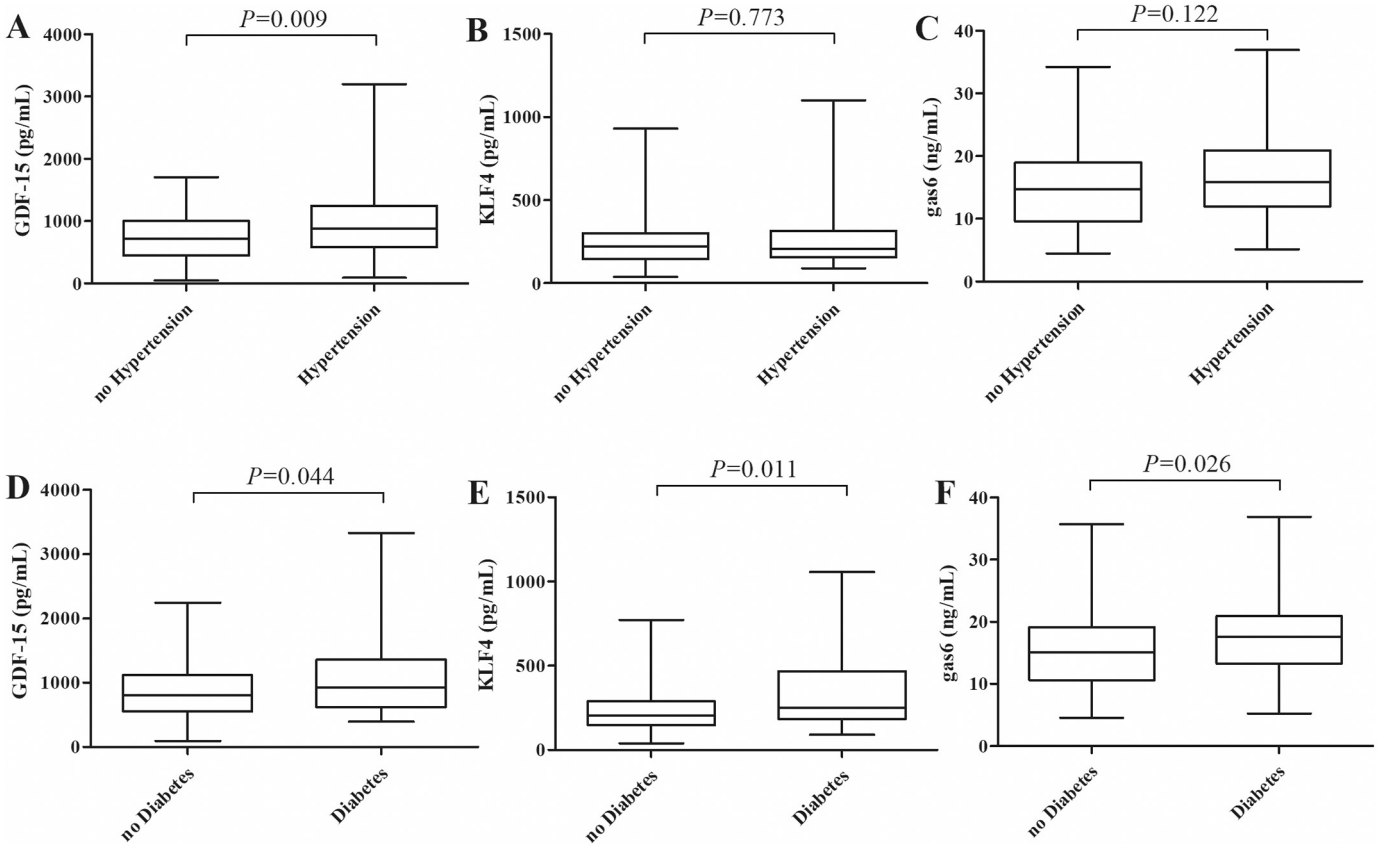

Fig. 1. Association of circulating concentrations of GDF-15, KLF4, and gas6 concentrations with clinical hypertension and diabetes in male patients with CAD.
Abbreviations: GDF-15, growth differentiation factor-15; KLF4, Krüppel-like factor 4; gas6, growth arrest-specific 6.

Table 3
Soluble GDF-15, KLF4, and gas6 concentrations in patients with and without CAD.

Variable	Controls	Patients with CAD			*P* value
	n = 152	1-vessel	2-veseel	3-vessel	
		n = 85	*n* = 67	*n* = 125	
GDF-15 (pg/ml)	666.77 (337.34—953.82)	717.09 (451.51—1070.84)	816.85 (544.92—1144.30)	942.33 (681.84—1243.41)	0.004
KLF4 (pg/ml)	184.71 (134.56–243.30)	180.28 (136.99–245.53)	212.35 (157.54–327.65)	223.37 (184.68–398.50)	0.001
Gas6 (ng/ml)	13.76 (9.90–17.22)	13.27 (9.74–17.38)	16.04 (11.96–21.11)	16.39 (12.26–20.28)	0.022

Data are presented as median (interquartile range). Differences among groups were analyzed by the Kruskal–Wallis test.
Abbreviations: CAD, coronary artery disease; GDF-15, growth differentiation factor-15; KLF4, Krüppel-like factor 4; gas6, growth arrest-specific 6.

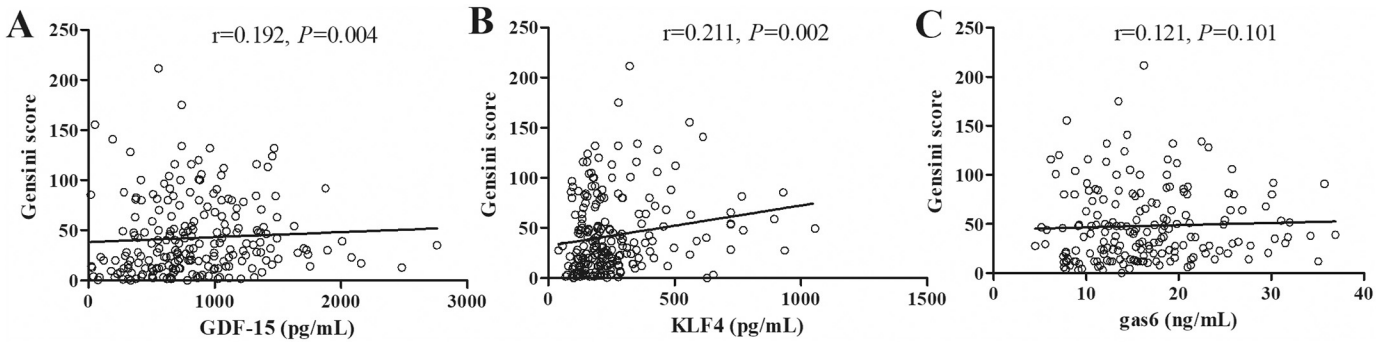

Fig. 2. Correlations of plasma concentrations of GDF-15 (A), KLF4 (B) or gas6 (C) with severity of coronary stenosis in male patients with CAD.
Abbreviations: GDF-15, growth differentiation factor-15; KLF4, Krüppel-like factor 4; gas6, growth arrest-specific 6.

Table 4
Multivariate logistic regression to examine associations of circulating GDF-15, KLF4, and gas6 concentrations with risk of CAD.

Variable	Unadjusted		Model 1		Model 2		Model 3	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
GDF-15	2.780 (1.606—4.814)	< 0.001	3.344 (1.876—5.962)	< 0.001	3.959 (2.067—7.586)	< 0.001	3.182 (1.586—6.382)	0.001
KLF4	10.054 (3.120—32.394)	< 0.001	8.224 (2.473—27.343)	0.001	8.673 (2.149—35.004)	0.002	13.050 (2.940—57.921)	0.001
gas6	0.055 (0.994–1.123)	NS	0.051 (0.988–1.121)	NS	0.062 (0.989–1.146)	NS	0.058 (0.984–1.141)	NS

Model 1: adjusted for age, hypertension, diabetes, and smoking status.
Model 2: adjusted as in Model 1 + TG, TC, LDL-c, HDL-c, and TC/HDL-c.
Model 3: adjusted for Model 2 + sCr.
Abbreviations: CAD, coronary artery disease; GDF-15, growth differentiation factor-15; KLF4, Krüppel-like factor 4; gas6, growth arrest-specific 6; TC, total cholesterol; TG, triglycerides; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol;sCr, serum creatinine.

circulating GDF-15 and KLF4 concentrations were positively associated with Gensini score even after adjustment for confounding factors.

GDF-15 is a lesion-induced factor implicated in a number of pathophysiological processes, including atherosclerosis, inflammation, cancer, chronic vascular diseases, and ischemia [29–31]. GDF-15 is secreted as a 25-kDa disulfide-linked dimeric protein. Previous work suggested that GDF-15 contributes to atherosclerotic lesion progression by regulating apoptotic cell death and interleukin (IL)-6-dependent inflammatory responses to vascular injury [32]. GDF-15 has also been proposed to exert proatherogenic effects by regulating C—C motif chemokine receptor 2 (CCR2)-mediated macrophage chemotaxis [12]. GDF-15 may be associated with impaired endothelial function and higher arterial stiffness in central, medium-sized arteries [33].

In our study, high concentrations of GDF-15 were associated with the severity of CAD and several risk factors at baseline, such as hypertension and diabetes, older age and higher concentrations of hs-CRP, and sCr. Higher concentrations of GDF-15 were also associated with increased Gensini score, which supports the pan-vascular functions of GDF-15 noted in the literature [33]. In a previous study, increased circulating concentrations of GDF-15 were associated with reduced endothelium-dependent vasodilation in resistant vessels, lower plaque burden, and smaller left ventricle ejection fraction [34]. These prior observations support our findings since carotid plaque burden correlates positively with Gensini score [35].

KLF4 was originally identified as a transcription factor required for establishing the barrier function of the skin [36]. It is widely expressed in a number of cells and tissues and plays an important role in inflammation, differentiation, development, proliferation, and cell death. It is reported to be a pleiotropic cytokine with various cellular effects [13,37]. KLF4 may help mediate proinflammatory signaling in macrophages during atherosclerosis, and it may accelerate the development of a variety of vascular inflammatory pathologies [38]. In activated macrophages, KLF4 can induce high-mobility group box 1 (HMGB1) expression by binding to its promoter. HMGB1 exerts proatherogenic effects and contributes to lesion development by regulating macrophage migration, proinflammatory mediators, and the accumulation of immune and smooth muscle cells [39].

KLF4 exerts multiple effects on the phenotypic modulation of smooth muscle cells, and plays a crucial role in lesion development, plaque composition, and stability [14,40]. Loss of KLF4 in smooth muscle cells results in reduced numbers of macrophage-like smooth muscle cells, a marked reduction in atherosclerosis, and increases in multiple indices of plaque stability, including fibrous cap thickness, when compared with wild-type controls [14]. These findings are in line with our results that increasing concentrations of KLF4 are associated with CAD severity and hs-CRP concentration. Other studies demonstrated that KLF4 acts as a transcription factor that controls GDF-15 promoter activity and that thereby plays a crucial role in human and mouse colorectal cancers, and those studies identified the functional KLF4 binding sites in the GDF-15 promoter [41,42]. In our study, we found that GDF-15 and KLF4 positively correlated with hs-CRP, an important molecule in atherosclerosis. This could indicate that GDF-15 and KLF4 may participate in the same atherogenic pathway in the progression of atherosclerosis. Therefore, interaction between GDF-15, and KLF4 should be evaluated in future experiments.

Gas6 was first discovered as a 75-kDa, vitamin K–dependent protein, and it is structurally similar to protein S [16]. Gas6 plays a vital role in cell proliferation, survival, adhesion, and migration [43] by acting via receptors in the Axl subfamily of receptor tyrosine kinases (Axl, Sky, and Mer). Gas6 accelerates the development of pressure overload–induced cardiac hypertrophy, fibrosis, and heart failure, suggesting that it impairs ventricular adaptation to chronic pressure overload [6]. Loss of gas6 reduces plaque inflammation and increases plaque fibrosis, thereby stabilizing atherosclerotic lesions [44]. Recently, several studies demonstrated that circulating concentrations of gas6 were associated with fasting glucose and endothelial dysfunction markers [45,46]. In our study, CAD patients had higher concentrations of gas6 than controls, suggesting gas6 participates in the development of CAD. We found that gas6 positively correlated with hs-CRP, which was also demonstrated in previous work that associated gas6 with systemic inflammation [47]. Nevertheless, our regression analyses showed no association of gas6 with presence or severity of CAD.

This work presents several limitations. In our population, angiography was performed to evaluate coronary atherosclerosis, but

Table 5
Multivariate linear regression to examine association of circulating GDF-15, KLF4 and gas6 concentrations with Gensini score.

Variable	Unadjusted		Model 1		Model 2		Model 3	
	B (95%CI)	*P* value	B (95%CI)	*P* value	B (95%CI)	*P* value	B (95%CI)	*P* value
GDF-15	10.033(−1.188—21.253)	0.080	12.158(0.799—23.517)	0.036	21.199(8.425—33.974)	0.001	22.091 (9.147—35.035)	0.001
KLF4	32.557(16.837—48.278)	< 0.001	31.838(16.040—47.635)	< 0.001	25.259(8.008—42.510)	0.004	27.996(10.082—45.910)	0.002
gas6	0.225 (−0.588–1.037)	NS	0.199 (−0.621–1.018)	NS	0.079 (−0.782–0.941)	NS	0.152 (−0.753–1.056)	NS

Model 1: adjusted for age, hypertension, diabetes, and smoking status.
Model 2: adjusted as in Model 1 + TG, TC, LDL-c, HDLc-, and TC/HDL-c.
Model 3: adjusted as in Model 2 + sCr.
Abbreviations: CAD, coronary artery disease; GDF-15, growth differentiation factor-15; KLF4, Krüppel-like factor 4; gas6, growth arrest-specific 6; TC, total cholesterol; TG, triglycerides; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; sCr, serum creatinine.

angiography cannot visualize plaques and can show only lumen characteristics. Moreover, it is impossible to demonstrate causality or the direction of influence based on our findings. Longitudinal studies and a larger sample are needed to confirm our results.

Despite these limitations, our results suggest that increased circulating concentrations of GDF-15 and KLF4 are associated with the presence and severity of CAD. These biomarkers may also be associated with the presence of hypertension and diabetes, and with concentrations of hs-CRP. At the same time, our analysis of receiver operating characteristic curves suggests that the biomarkers cannot reliably predict CAD on their own. It may be necessary to combine these biomarkers with other factors, or to apply these biomarkers to only certain types of CAD patients. These possibilities should be explored in future work.

5. Conclusions

Increased circulating concentrations of GDF-15, and KLF4 were significantly associated with the presence and severity of CAD in our study. Our findings justify further work to explore whether these 2 biomarkers, combined with other factors, can aid in early diagnosis and treatment of CAD.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.05.029.

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