Association of serum Cyr61 levels with peripheral arterial disease in patients with type 2 diabetes

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Abstract

Background: The prevalence of peripheral artery disease (PAD) is obviously increased in diabetic patients. Existing evidences show that cysteine-rich angiogenic inducer 61 (Cyr61), a 40 kD secreted protein, plays important roles in regulating cellular physiological processes. Recent studies have demonstrated a significant correlation between serum Cyr61 levels and atherosclerosis. However, the relationship between Cyr61 levels and PAD in type 2 diabetic patients remains obscure.

Methods:. A total of 306 subjects with type 2 diabetes were recruited. The extent of PAD was determined by using the Fontaine classification, which defines four stages. We analyzed Cyr61 serum levels by ELISA in patients with and without PAD at Fontaine's stage II, III, or IV. Logistic regression models were used to examine the independent association of Cyr61 with PAD.

Results: Out of the 306 patients enrolled in this study, 150 patients were free from PAD, while 156 had clinically significant PAD. In PAD patients, the prevalences of Fontaine classification stage II, III and IV were 48.7%, 32.1%, and 19.2%, respectively. Patients with more advanced PAD had significantly higher Cyr61 (*P* for trend <0.001). The prevalence of PAD on the basis of severity increased with ascending Cyr61 quartiles (all *P* for trend <0.001), and the severity of PAD was positively correlated with Cyr61 quartiles (r=0.227, *P*=0.006). The association of Cyr61 levels with PAD remained after adjusting for major risk factors in a logistic regression analysis.

Conclusions: Our results demonstrated that Cyr61 was significantly increased in type 2 diabetic patients with PAD and that Cyr61 levels were positively associated with disease severity. It could be a promising biomarker and further studies are needed to assess its clinical utility.

Background

Peripheral artery disease (PAD) is a common manifestation of atherosclerotic disease, which is related to considerable disability and mortality. Currently, approximately 202 million people worldwide are suffering from lower extremity arterial disease, giving rise to a major public health problem and a heavy economic burden [1, 2]. Type 2 diabetes (T2DM) is one of the major risk factors of atherosclerosis, and the prevalence of PAD increases with the prevalence of T2DM. Moreover, compared with non-diabetic patients, PAD has a poorer prognosis diabetic patients. Therefore, early diagnosis and intervention of PAD in T2DM patients are essential to reduce the risk of major adverse limb events (MALE) [3]. At present, various international guidelines recommend the ankle brachial index (ABI) as the preferred screening tool for PAD in diabetic patients [4]. Due to the low sensitivity of ABI for the detection of early stage of PAD, there is an urgent need to find novel biomarkers that can identify PAD among diabetic patients in the initial stage.

Previous studies have found that some members of the CCN family are highly expressed in atherosclerotic plaques, contributing to the development of cardiovascular and cerebrovascular diseases and peripheral arterial diseases [5, 6]. Cysteine-rich angiogenic inducer 61 (Cyr61), belongs to CCN family,

is a 40 kD secreted extracellular matrix (ECM)- related signaling protein that can regulate cell proliferation, adhesion, differentiation and extracellular matrix production [7–9]. Cyr61 is maintained at a low level under normal conditions, but is usually elevated in various status of disease, such as colitis, rheumatoid arthritis, Graves' orbitopathy, diabetic retinopathy and atherosclerosis [10–15]. Moreover, inhibition of Cyr61 expression in carotid balloon injury rats can mitigated the proliferation of vascular smooth muscle cells, thereby attenuating vascular intimal hyperplasia [16]. Additionally, recent studies demonstrated that the Cyr61 levels were independently associated with the 30-day mortality in patients with acute heart failure (AHF) and coronary heart disease (CAD) [17], and could be a potential marker of myocardial ischemic injury and prognosis in patients with acute coronary syndrome (ACS) [9, 18]. Till now, however, the link between circulating Cyr61 and PAD in diabetic patients has not yet been established.

Since numerous studies have demonstrated links between Cyr61 and various aspects of atherosclerosis [11, 19] and diabetic microvascular complications [10], it is reasonable to hypothesize a correlation between Cyr61 and PAD in the diabetic condition. Therefore, we sought to investigate the potential role of Cyr61 as a marker of endothelial dysfunction and PAD in patients with T2DM.

Methods

Study population and design

Individuals with type 2 diabetes were consecutively recruited from the Department of Endocrinology and Metabolism of the First Affiliated Hospital of Soochow University and the Department of Cardiology of the Affiliated Suzhou Hospital of Nanjing Medical University, Jiangsu, China, from 1 July 2018 to 31 March 2020. The diagnosis of type 2 diabetes is based on the 1999 World Health Organization (WHO) criteria. Inclusion criteria were age ≥ 18 years with the presence of type 2 diabetes. Exclusion criteria included renal failure with estimated glomerular filtration rate (eGFR) < 30 ml/min; Acute infectious disease at the time of evaluation; a history of malignancy, mental disorders, or severe heart or liver dysfunction; History of solid or hematological neoplasia or active neoplasia. Diabetic patients with or without PAD were enrolled. Each patient enrolled was assessed by a history of PAD symptoms or a confirmed PAD diagnosis. Ankle-brachial index (ABI) measurement was performed on patients with clinical manifestations consistent with PAD, and according to the judgment of the attending physician, both lower limbs were further assessed using arterial Doppler ultrasound-enhanced ultrasonography, computed tomography (CT) angiography, or lower limb angiography. Patients with an ABI > 0.90 and no symptoms of PAD were deemed to be without PAD and did not undergo further testing.

The patients were considered to have PAD if: they had a previous history of lower limb percutaneous transluminal angioplasty, with or without stenting, or they met at least one instrumental and one clinical criterion listed in Table 1.

Table 1

Criteria for PAD definition in patients without a history of lower limb amputation, PTA or by-pass surgery.

PTA, percutaneous transluminal coronary angioplasty.

Clinical Criteria	Instrumental criteria
Presence of Intermittent claudication	ABI < 0.90
Rest pain	$TcPO_2$ < 30 mmHg
Non-healing distal ulcer	Ultrasound or angiography found atherosclerosis with stenosis, with a reduction at least 50% of the lumen diameter, consistent with clinical symptoms
Gangrene	Ultrasonographic finding of post-stenotic blood flow profile, consistent with symptoms

The extent of PAD was determined by using the Fontaine classification, which defines four stages: stage I, asymptomatic; stage II, intermittent claudication; stage III, rest pain; stage IV, ischemic ulcers or gangrene [20].

The study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University and the Affiliated Suzhou Hospital of Nanjing Medical University in accordance with the principles of the Helsinki Declaration. Written informed consent was obtained from each participant.

Anthropometric and biochemical measurements

Anthropometric measurements included height, weight and blood pressure. Body mass index (BMI) was defined as the patient's weight in kilograms divided by their height (in meters) squared. Blood samples were collected afterAfter a 10 h overnight fast. HbA1c total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) were estimated on a biochemical analyser (HITACHI 7450, Tokyo, Japan). eGFR was calculated using the simplified Modification of Diet in Renal Disease (MDRD) formula. Blood samples were stored at − 80 ^{o}C before every measurement. Serum Cyr61 was determined by a commercially available ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. For each patient, the serum sample was measured twice, and the results were averaged.

Statistical analysis

Demographic and clinical data of the groups were compared using the χ^2 test and a t-test. For non-normally distributed variables, a logarithmic transformation was carried out before further analysis, The trends of continuous variables across the various groups were assessed with the use of linear polynomial contrasts in ANOVA for normally distributed variables and the Jonckheere-Terpstra test for non–normally distributed data. The Cochran-Armitage trend test was used to examine trends of rates across groups. Status by severity of PAD was treated as an ordinal categorical variable (0 = non-PAD, 1 = Fontaine stage II, 2 = Fontaine stage III, 3 = Fontaine stage IV). Binary logistic regression analysis was performed to

investigate the effect of serum Cyr61 on risk of PAD. The area under the receiver operating characteristics (ROC) curve was calculated to test the predictive discrimination of PAD. Graphs were created using Prism 8.0 (GraphPad Software), and statistical analysis was performed with GraphPad Prism. A *P* value < 0.05 was considered to be statistically significant.

Results

The clinical characteristics of the patients are shown in Table 2. The mean ± SD age of the enrolled participants was 61.2 ± 11.4 years; they had a mean ± SD diabetes duration of 8.6 ± 5.6 years, fasting glucose of 7.4 ± 1.6 mmol/L and HbA1c of 8.8 ± 1.7%. There were no significant differences between groups regarding BMI (*P* = 0.640), smokers (*P* = 0.953), blood pressure (*P* = 0.344), TC (*P* = 0.172), LDL-C (*P* = 0.557) and TG (*P* = 0.692). In PAD patients, the prevalences of Fontaine classification stageII, III and IV were 48.7%, 32.1%, and 19.2%, respectively. Patients with more severe PAD had longer diabetes duration; higher HDL-C, fasting glucose and HbA1c; and lower eGFR. They also were more likely to be treated with insulin and had a higher propensity to receive renin-angiotensin-aldosterone system (RASS) inhibitors (Table 2).

Table 2

Demographic and clinical data of diabetic subjects by the presence and severity of PAD. Data are mean ± SD or percentage unless otherwise indicated. BMI, body mass index; RAAS, renin-angiotensin-aldosterone system.

Variables	All subjects (n = 306)	No PAD (n = 150)	Fontaine classification			*P* value for trend
			II (n = 76)	III (n = 50)	IV (n = 30)	
Male	52.9	45.3	47.4	44.0	53.3	0.606
Age (years)	61.2 ± 11.4	60.4 ± 12.0	59.2 ± 11.0	61.2 ± 11.0	62.6 ± 10.8	0.233
BMI (kg/m^2)	25.2 ± 3.3	25.1 ± 3.4	25.1 ± 3.4	25.0 ± 3.5	24.8 ± 3.3	0.640
Current smoker	26.1	25.3	28.9	24.0	26.7	0.953
Hypertension	51.0	48.0	52.6	56.0	53.3	0.344
Diabetes duration (years)	8.6 ± 5.6	7.3 ± 6.1	9.6 ± 6.4	10.7 ± 6.0	12.8 ± 5.8	< 0.001
Total cholesterol (mmol/L)	4.5 ± 1.3	4.6 ± 1.3	4.5 ± 1.3	4.7 ± 1.3	4.9 ± 1.1	0.172
TG (mmol/L)	1.8 ± 1.5	1.9 ± 1.7	1.8 ± 1.5	1.7 ± 1.4	1.8 ± 1.7	0.692
HDL-C (mmol/L)	1.2 ± 0.4	1.1 ± 0.4	1.2 ± 0.4	1.2 ± 0.4	1.3 ± 0.3	0.015
LDL-C (mmol/L)	3.1 ± 0.7	3.1 ± 0.9	3.1 ± 0.7	3.1 ± 0.7	3.2 ± 0.8	0.557
HbA_{1c}	8.8 ± 1.7	8.2 ± 1.5	8.9 ± 1.6	8.9 ± 1.5	9.1 ± 1.7	0.006
Fasting glucose (mmol/L)	7.4 ± 1.6	7.1 ± 1.5	7.9 ± 1.7	7.7 ± 1.5	8.1 ± 1.5	0.004
eGFR ($ml/min/1.73\ m^2$)	79.4 ± 8.4	82.3 ± 8.7	79.5 ± 8.9	78.5 ± 8.8	76.5 ± 8.4	< 0.001
Serum Cyr61 (pg/ml)	219.1 ± 13.5	195.4 ± 11.9	221.0 ± 14.4	247.6 ± 11.8	275.5 ± 12.3	< 0.001
Use antidiabetes agents						
Oral drugs	58.2	61.3	60.5	48.0	55.3	0.225
Insulin	64.7	54.7	68.4	76.0	86.7	< 0.001
Use antihypertension agents						
β-Blockers	13.1	10.7	13.2	16.0	20.0	0.123

Variables	All subjects (n = 306)	No PAD (n = 150)	Fontaine classification			*P* value for trend
			II (n = 76)	III (n = 50)	IV (n = 30)	
Calcium-channel blockers	24.2	24.0	23.7	24.0	26.7	0.826
RAAS inhibitors	47.1	40.0	47.4	56.0	66.7	0.003
Diuretics	6.5	5.3	7.9	8.0	6.7	0.551
Use lipid-lowering agents						
Statins	24.8	3221.3	2026.3	1428.0	1033.3	0.119
Fibrates	7.2	85.3	810.5	48.0	26.7	0.530

Next, all of the participants were stratified according to quartiles of serum Cyr61 level (quartile 1 [Q1]: ≤169.7 pg/ml; quartile 2 [Q2]: 169.7-206.6 pg/ml; quartile 3 [Q3]: 206.6-282.3 pg/ml; quartile 4 [Q4]: ≥282.3 pg/ml). Table 3 presents the characteristics of subjects by serum Cyr61 quartiles. In general, the prevalence of PAD by severity increased with ascending quartiles of Cyr61 (all *P* for trend < 0.001) (Fig. 1). A significant association between Cyr61 quartile and the severity of PAD was observed after spearman correlation analysis (r = 0.227; *P* = 0.006).

Table 3

Characteristics of study participants by quartiles of serum Cyr61. Data are mean ± SD or percentage unless otherwise indicated. BMI, body mass index; RAAS, renin-angiotensin-aldosterone system.

Variables	Serum Cyr61 quartiles				
	Q1 (n = 77)	Q2 (n = 89)	Q3 (n = 69)	Q4 (n = 71)	*P* value for trend
Serum Cyr61 (pg/ml)	≤ 169.7	169.7-206.6	206.6-282.3	≥ 282.3	
Male	49.4	46.1	44.9	45.1	0.589
Age (years)	61.4 ± 12.0	62.6 ± 11.1	61.2 ± 11.1	59.6 ± 11.3	0.251
BMI (kg/m^2)	25.0 ± 3.0	25.0 ± 3.3	25.3 ± 3.3	25.8 ± 3.2	0.106
Current smoker	23.4	25.8	27.5	28.2	0.481
Hypertension	51.9	49.4	52.2	50.7	0.974
Diabetes duration (years)	7.7 ± 6.2	8.6 ± 6.2	10.0 ± 7.3	12.1 ± 7.3	< 0.001
Total cholesterol (mmol/L)	4.6 ± 1.1	4.6 ± 1.1	4.7 ± 1.0	4.9 ± 1.2	0.117
TG (mmol/L)	1.7 ± 1.4	1.7 ± 1.4	1.8 ± 1.5	1.9 ± 1.5	0.352
HDL-C (mmol/L)	1.2 ± 0.3	1.2 ± 0.3	1.1 ± 0.2	1.1 ± 0.2	0.003
LDL-C (mmol/L)	3.0 ± 0.3	3.1 ± 0.3	3.1 ± 0.4	3.2 ± 0.7	0.009
HbA_{1c}	7.7 ± 1.1	8.2 ± 1.3	8.9 ± 1.5	9.4 ± 1.5	< 0.001
Fasting glucose (mmol/L)	7.2 ± 1.2	7.7 ± 1.5	7.8 ± 1.6	8.0 ± 1.5	0.001
eGFR (ml/min/1.73 m^2)	81.9 ± 8.4	79.8 ± 8.6	78.6 ± 8.4	76.5 ± 8.2	< 0.001
Use antidiabetes agents					
Oral drugs	62.3	58.4	57.9	53.5	0.295
Insulin	49.4	58.4	72.4	81.7	< 0.001
Use antihypertension agents					
β-Blockers	11.7	13.5	14.5	12.7	0.819
Calcium-channel blockers	23.4	23.6	23.2	26.8	0.663
RAAS inhibitors	37.7	43.8	52.2	56.3	0.013
Diuretics	3.9	5.6	7.2	5.6	0.563
Use lipid-lowering agents					

Variables	Serum Cyr61 quartiles				
	Q1 (n = 77)	Q2 (n = 89)	Q3 (n = 69)	Q4 (n = 71)	*P* value for trend
Statins	22.1	25.8	26.1	25.4	0.655
Fibrates	3.9	8.9	7.2	8.5	0.380

Logistic regression analysis revealed that serum Cyr61 was significantly associated with risk of PAD both in the crude and adjusted models including age, sex, BMI, diabetes duration, fasting glucose, hypertension, TG, HDL, LDL, HbA1c, eGFR, smoking status, use of RAAS inhibitors, statins and fibrates as covariates (all P < 0.005, Table 4).

Table 4
Association of serum Cyr61 with PAD by logistic regression analyses. Model 1 is adjusted for age, sex and BMI; Model 2 includes all variables in Model 1 plus diabetes duration, fasting glucose, hypertension, TG, HDL-C and LDL-C; Model 3 includes all variables in Model 2 plus HbA1c, eGFR, smoking status, use of RAAS inhibitors, statins and fibrates.

Modes	OR	95% confidence interval	*P* value
Crude	0.915	0.854–0.949	< 0.001
Mode1	0.923	0.867–0.964	0.005
Mode2	0.936	0.872–0.981	0.008
Mode3	0.947	0.879–0.993	0.048

The ability of the area under the ROC curve based on Cyr61 levels to predict the presence of PAD in type 2 diabetic patients was 0.859 (Fig. 2) and the best cut-off value of Cyr61 for prediction of the occurrence of PAD in our study population was > 233.7 ng/ml (Sensitivity 82.7%, Specificity 72.3%).

Discussion

Peripheral arterial disease usually seriously affects the daily life quality of patients and imposes huge personal and societal healthcare burdens [21, 22]. Considering the certain relevance of the disease, effective earlier screening and diagnosis of PAD for diabetic patients has become increasingly important. However, there is still a lack of effective biomarkers with high sensitivity and specificity for early diagnosis, and often a patient consults the specialist when the disease is already significantly worsened. To the best of our knowledge, this is the first study to evaluate the association between serum Cyr61 level

and the risk of PAD. Among a population of 306 patients with type 2 diabetes, we observed a positive correlation of serum Cyr61 with the prevalence of all stages of PAD.

ABI is a commonly used clinical examination tool, simple to operate and it can identify PAD very effectively. However, in most cases, when ABI is indicative of PAD, the patient is usually already at an advanced stage [23]. As PAD in diabetic patients can be more aggressive and may manifest more severe symptoms later, the research for early diagnosis tools for PAD is essential [3]. Recent years, several potential biomarkers were found to identify PAD in diabetic patients. For instance, serum high mobility group box 1 (HMGB1), fibroblast growth factor (FGF) 23 and osteopontin (OPN) have been correlated with the presence of PAD in diabetic patients [24, 25]. In addition, thrombospondin-4 (TSP-4) levels were significantly increased with PAD severity in patients with concomitant diabetes and could be a novel marker of atherosclerotic activity [26]. Lately, the association between circulating serum growth differentiation factor 15 (GDF15) and lower extremity atherosclerotic disease (LEAD) has been investigated in Chinese diabetes subjects [27]. Furthermore, Hayashi, A. et al. reported a method for accurate skin temperature measurement using noncontact, handheld infrared skin thermometer, which could serve as a new, cost-effective screening strategy for earlier diagnosis of PAD [28]. In the present study, Cyr61 has potential clinical significance as a simple, easy-to-measure biochemical marker of atherosclerosis.

Previous studies have demonstrated that CyrR61 is overexpressed in vascular smooth muscle cells of atherosclerotic lesions both in humans and in animal models [19, 29, 30], and could stimulate adhesion of vascular smooth muscle cells in a dose-dependent manner [11]. In particular, it has been evidenced that inhibition of Cyr61 in rat models of carotid balloon injury decreased proliferation of vascular smooth muscle cells and in turn intimal hyperplasia [16]. Therefore, we speculated that in the diabetic population of this study, higher Cyr61 levels are involved, at least in part, in the occurrence of atherosclerotic lesions of the lower limbs. To our knowledge, this is the first clinical study that serum Cyr61 levels were assessed as a potential biomarker for PAD in a diabetic population. Of particular interest, we also found that serum Cyr61 levels increase according to severity of PAD, as if there were a dose-dependent relationship. Therefore, values of Cyr61 may help to stratify patients to facilitate a more accurate diagnosis and individualized treatment. In addition, we still demonstrated that the relationship between higher Cyr61 levels and PAD in T2DM remains significant also after adjustment for potential confounding variables such as age, BMI, smoking status, diabetes duration, hypertension, eGFR and serum lipid profile. The ROC curve confirms that Cyr61 levels are able to predict the presence of PAD in our participants. If the results are confirmed, serum Cyr61 concentration could become a new biomarker with excellent sensitivity and specificity for early diagnosis and effective follow-up of PAD in diabetic subjects.

The study has several major limitations that should be noted. First, due to the cross sectional design of the study, we could not establish a causal relationship between serum Cyr61 and the development of PAD. Even so, our results support the hypothesis that Cyr61 is important in the peripheral atherosclerosis physiopathology. Second, the cross-sectional measurements may not represent the participant’s stable level of serum Cyr61. Therefore the results of this study should be interpreted with caution. Third, the

enrolled individuals were hospitalized diabetic patients in Suzhou area (Jiangsu, China), the generalizability of the results to other diabetic populations needs to be tested. A further limitation is that we need prospective data to confirm whether higher Cyr61 levels may suffice as an effective biomarker for PAD in type 2 diabetes patients. In addition, we did not use healthy controls to determine normal level of Cyr61. In fact, there is no unequivocal report regarding the normal level of serum Cyr61 in humans, and we believe that our results can provide a relative reference for future studies on a large scale.

Conclusion

In summary, we provided evidence that circulating Cyr61 levels are significantly associated with the prevalence of PAD and correlate with disease severity in T2DM population. Therefore, Cyr61 is a promising marker that may help clinicians to better stratify atherosclerotic risk in diabetic states. Further prospective studies are warranted to obtain a definitive picture of the role of Cyr61 in the onset and progression of PAD in T2DM patients.

Abbreviations

PAD, peripheral artery disease; T2DM, Type 2 diabetes; MALE, major adverse limb events; AHF, acute heart failure; CAD, coronary heart disease; ACS, acute coronary syndrome; eGFR, estimated glomerular filtration rate; ABI, ankle-brachial index; CT, computed tomography; BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; RAAS, renin-angiotensin-aldosterone system; HMGB1, high mobility group box 1; FGF, fibroblast growth factor; OPN, osteopontin; TSP-4, thrombospondin-4; GDF15, growth differentiation factor 15; LEAD, lower extremity atherosclerotic disease

Declarations

Ethics approval and consent to participate

The study was approved by local ethics committees and adhered to the principles of the Declaration of Helsinki.All patients agreed to participate in the study and gave informed consent.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed for this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

BF and NNZ conceived the study, GDX and KYS performed data analysis and reviewed the manuscript. NNZ and BMS participated in its design and coordination and BF wrote the manuscript. All authors read and approved the final manuscript.

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Figures

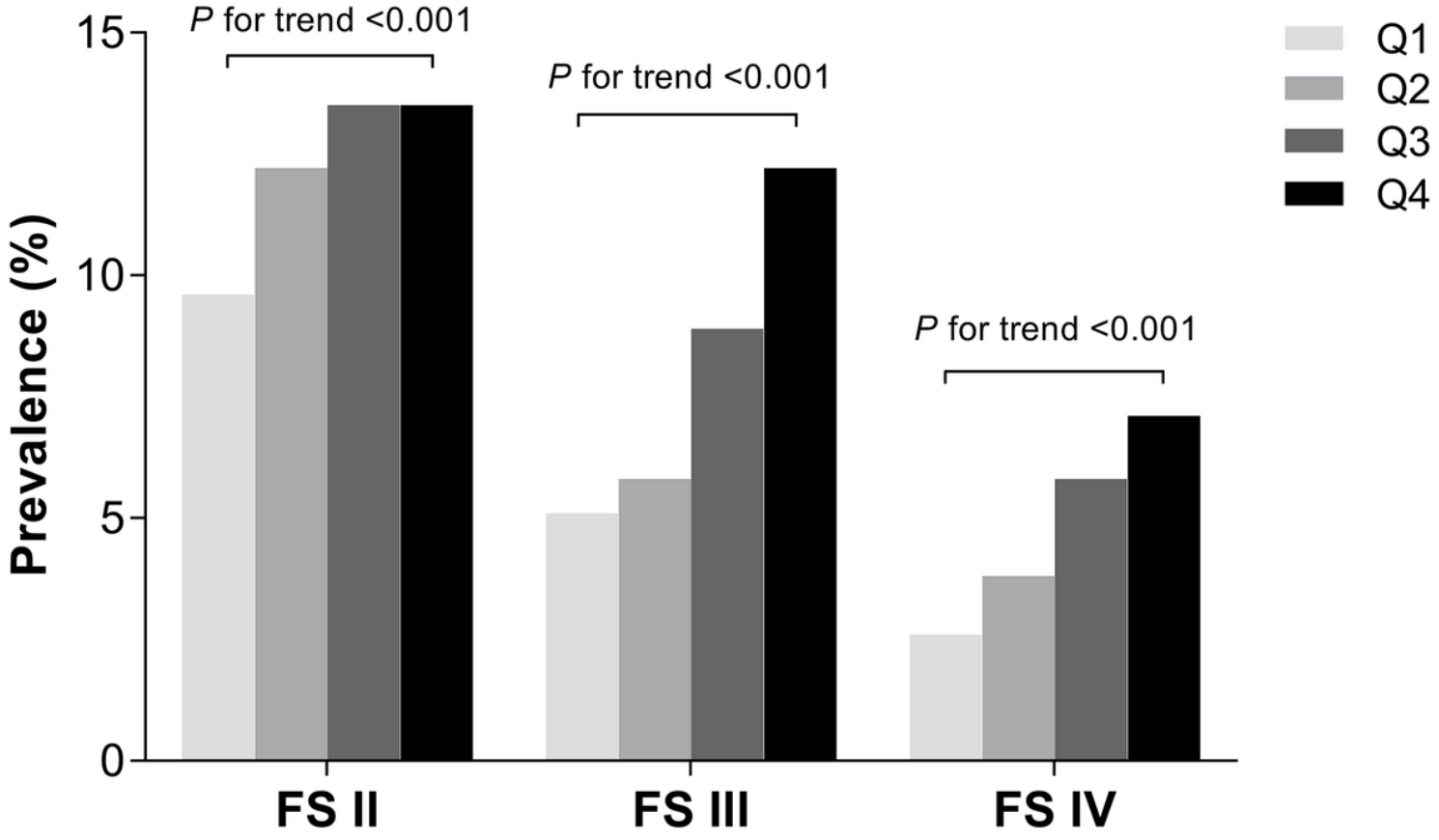

Figure 1

Prevalence of PAD by severity, as a function of Cyr61 quartile. FS, Fontaine Stage; Cyr61 quartile: Q1: ≤169.7 pg/ml, Q2: 169.7-206.6 pg/ml, Q3: 206.6-282.3 pg/ml, Q4: ≥282.3 pg/ml.

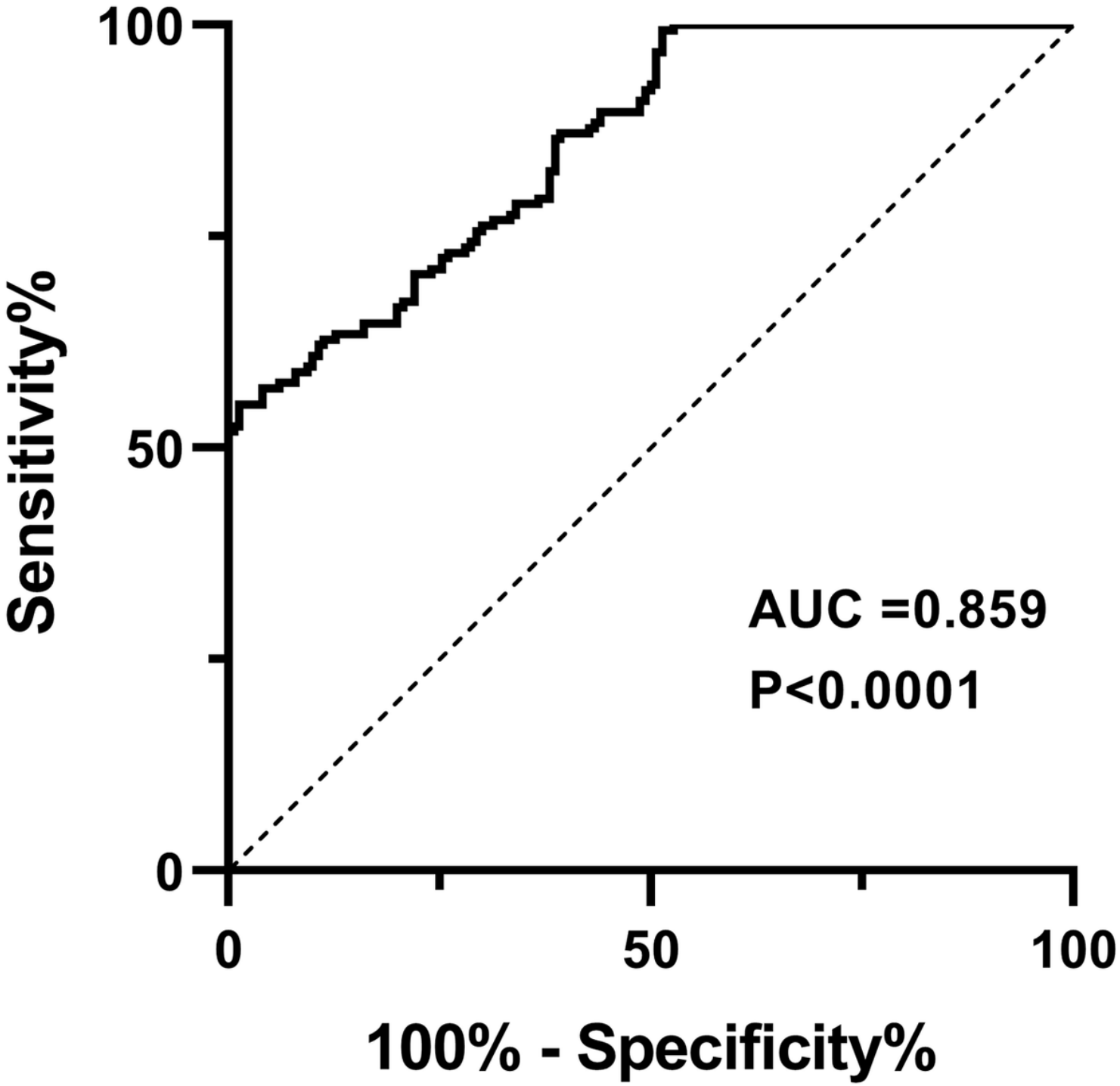

Figure 2

ROC curve analysis of the ability of Cyr61 to predict the presence of PAD in diabetic patients. The ability of the area under the ROC curve was 0.859.