

Tenascin C: A Potential Biomarker for Predicting the Severity of Coronary Atherosclerosis

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Aims: Coronary artery disease (CAD) is the leading cause of mortality and morbidity worldwide and one of the greatest threats to public health. Tenascin C (TNC) is an extracellular matrix glycoprotein that is found in low concentrations in normal tissues and is enhanced by a range of cardiovascular pathologies. This study aimed to evaluate the value of TNC in assessing the severity of atherosclerosis measured by the Gensini score.

Methods: A total of 157 patients with chest pains who underwent selective coronary angiography for suspected coronary atherosclerosis were enrolled. The patients were divided into the CAD group and non-CAD group according to symptoms and angiography. Demographic data and laboratory analyses were collected.

Results: The mean TNC level was significantly higher in the CAD group than in the non-CAD group ($p < 0.001$). A significant positive correlation between TNC levels and Gensini score ($p < 0.01$, $r = 0.672$) was found. ROC curve analysis demonstrated that the cutoff value for TNC at 89.48 ng/mL was well differentiated in the CAD and non-CAD groups. Furthermore, TNC was also a good predictor for a higher Gensini score (the third tertile) in the ROC curve analysis. When the cutoff was accepted as 100.91 ng/mL, the sensitivity and specificity were 82.7% and 79%, respectively.

Conclusion: A significant relationship was found between the Gensini score and serum TNC level. TNC levels can be considered in risk assessments for CAD before angiography.

Key words: Atherosclerosis, Coronary artery disease, Tenascin C, Gensini score

Introduction

Coronary artery disease (CAD) is the most common cardiovascular disease and the leading cause of mortality and morbidity worldwide¹. According to the official report on cardiovascular diseases in China, more than 3.7 million people in China died from CAD in 2014². Although biomarkers such as creatine kinase-MB and troponin have improved the diagnosis of acute heart attack and survival rate, few markers are available in the risk stratification of angina patients before the onset of acute coronary heart disease. The Gensini score is a well-recognized scoring system for determin-

ing the severity of CAD, but it depends on coronary angiography, thus limiting its applications for the rapid screening of high-risk patients.

Tenascin C (TNC) is a type of extracellular matrix (ECM) glycoprotein that is transiently expressed during embryo genesis. Only low levels of TNC are found in developed organs. Pathological conditions such as inflammation, infection, tumorigenesis, remodeling, injury, and neoplasia could upregulate the expression of TNC³. TNC is strongly expressed in atherosclerotic plaques and is preferentially enriched around the lipid core, shoulder regions, and rupture areas⁴. Several studies using animal models have shown the cor-

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Received: October 13, 2017 Accepted for publication: April 4, 2018

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relation of TNC and atherosclerosis^{5, 6}. TNC level was also found to be significantly correlated with coronary artery calcium score⁷. Therefore, this marker may be applied to reflect the severity of CAD in clinical settings. This study aimed to evaluate the value of TNC in assessing the severity of atherosclerosis measured by the Gensini score.

Methods

Study Population

This cross-sectional study complied with the Declaration of Helsinki and was approved by the ethics committee. Informed consent was obtained from all patients enrolled in this study. From August 2016 to February 2017, 157 patients aged between 18 and 85 years old with chest pain who underwent selective coronary angiography for suspected coronary atherosclerosis were enrolled in the Department of Cardiology of Huashan Hospital. Patients with acute coronary syndrome or who have histories of coronary revascularization, myocardial infarction, all forms of cardiomyopathy, valvular disease, inflammatory and connective tissue diseases, malignancy, serum creatinine >1.2 mg/dL (106 μ mol/L), recent trauma, or surgical intervention (within the last three months) were excluded.

The patients were asked for their detailed medical histories and underwent physical examination, 12-lead electrocardiography, echocardiography, and biochemical analysis including hemoglobin A1C (HbA1C), lipid profile, renal function, neutrophil percentage (NEU), high-sensitivity C-reactive protein (hs-CRP), and serum TNC level. The other risk factors for CAD including smoking, hypertension, diabetes, hyperlipemia, medical history, and family history were also evaluated.

Patients with CAD had exertional chest pressure due to $\geq 50\%$ narrowing in the left main coronary artery or $\geq 70\%$ in at least one of the major coronary arteries⁸. Patients whose coronary angiogram did not achieve the diagnostic criteria of CAD or showed no evidence of CAD or CAD equivalents with respect to their symptoms were considered eligible for the non-CAD group. The diagnosis of diabetes was made according to the Standards of Medical Care in Diabetes enacted by the American Diabetes Association⁹. A diagnosis of hypertension was made according to the JNC8 guidelines for hypertension¹⁰. A diagnosis of metabolic syndrome was made by considering the presence of three or more of the following criteria¹¹: triglycerides ≥ 150 mg/dL or HDL cholesterol ≤ 40 mg/dL, fasting glucose ≥ 110 mg/dL or diagnosed diabetes, systolic/diastolic blood pressure $\geq 140/90$ mmHg or diagnosed hypertension, and overweight or BMI ≥ 25 for age and sex.

Laboratory Measurements

Peripheral venous blood samples were collected from the patients at baseline upon admission. Serum samples reserved for TNC analysis were stored at -80°C , and the TNC levels were measured using the enzyme-linked immunosorbent assay kit for Human Tenascin from IBL (Immuno-Biological Laboratories, #27751). TNC levels were measured by the large subunit containing the C dominant of FN III repeats by using double antibody sandwich enzyme immunoassay kits with detection wavelengths between 450 and 630 nm. The typical calibration curves are shown in the Supplementary Appendix.

Lipid profile, HbA1C, hs-CRP, and creatinine level were measured by standard hematological and biochemical tests. The estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation¹².

Severity of Coronary Atherosclerosis

The Gensini score was calculated according to the degree of luminal narrowing and the geographic importance of its location¹³. The lumen diameter was reduced by 25%, 50%, 75%, 90%, and 99%, and complete occlusion were evaluated as 1, 2, 4, 8, 16, and 32. A multiplier factor such as $\times 1$, $\times 2.5$, and so on was assigned to each segment on the basis of the functional significance of the myocardial area supplied by that segment.

Statistical Analysis

Values were expressed as mean \pm SEM or median for the continuous variables and percentage of categorical variables. The fit normal distribution of continuous variables was analyzed by Student's *t*-tests. Categorical variables were analyzed by the chi-squared statistical test. The paired group comparisons of independent numerical variables were performed by Student's *t*-tests or the Mann-Whitney *U* test. The correlation between numerical variables was assessed by Pearson's correlation analysis or Spearman's rho test.

The area under the receiver operating characteristic (ROC) curve was used to calculate the discriminative ability of TNC to determine the Gensini score). On the basis of the tertiles of the Gensini score, the enrolled patients were classified into three groups (low group 0–5 points, $n=53$; intermediate group, 5–24 points, $n=52$; high group >24 point, $n=52$). The predictive ability of TNC for high Gensini score (over than 24 point) and CAD was calculated according to the ROC curves for TNC assessed by sensitivity and specificity. A *p*-value < 0.05 was considered statistically significant.

Table 1. Clinical characteristics of the study groups

	non-CAD (<i>n</i> =76)	CAD (<i>n</i> =81)	<i>P</i>
Age, year	68.22 ± 1.27	64.95 ± 1.09	0.051
Male gender, <i>n</i> (%)	38 (50.00)	62 (76.54)	0.001
Hypertension, <i>n</i> (%)	50 (65.79)	53 (65.43)	0.962
Diabetes mellitus, <i>n</i> (%)	8 (10.52)	33 (40.74)	<0.001
Metabolic syndrome, <i>n</i> (%)	13 (17.11)	25 (30.86)	0.044
HbA1C (%)	5.95 ± 0.11	6.70 ± 0.17	<0.001
Smoking	20 (26.32)	34 (41.98)	0.039
Creatinine, μmol/L	75.59 ± 1.79	73.60 ± 1.99	0.460
eGFR (mL/min/1.73 m ²)	80.84 ± 1.82	88.29 ± 1.58	0.002
TC, mmol/L	4.03 ± 0.12	3.96 ± 0.11	0.667
TG, mmol/L	1.61 ± 0.10	1.68 ± 0.11	0.653
HDL-C, mmol/L	1.13 ± 0.03	0.99 ± 0.03	<0.001
LDL-C, mmol/L	2.29 ± 0.10	2.39 ± 0.09	0.471
NT-pro BNP, pg/mL	322.60 ± 56.23	441.70 ± 59.73	0.150
TNC	67.87 ± 5.14	117.40 ± 9.32	<0.001
NEU (%)	60.52 ± 1.00	62.79 ± 1.00	0.109
ACEI or ARB, <i>n</i> (%)	45 (59.21)	50 (61.73)	0.747
CCB, <i>n</i> (%)	26 (34.21)	19 (23.46)	0.136
Statin or other lipid-lowering drugs, <i>n</i> (%)	70 (92.11)	80 (98.76)	0.043
β-blocker, <i>n</i> (%)	47 (61.84)	60 (74.07)	0.100
Antidiabetic drug, <i>n</i> (%)	8 (10.53)	25 (30.86)	0.002

CAD: coronary artery disease; HbA1C: hemoglobin A1C; eGFR: estimate glomerular filtration rate; TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TNC: tenascin-C; NEU: neutrophil percentage; ACEI: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blocker; CCB: calcium channel blocker.

Results

Baseline Characteristics

The baseline characteristics of the study patients are shown in **Table 1**. Patients were divided into two groups, namely, the CAD group (*n*=81) and the non-CAD group (*n*=76), on the basis of the criterion of the methods. There were no significant differences between the two groups in terms of age, hypertension, creatinine level, NT-pro BNP level, NEU, and lipid profile except for the HDL cholesterol. The CAD group had a higher proportion of male patients, diabetic and metabolic syndrome patients, smokers, patients with low HDL cholesterol levels, and patients with high TNC. The CAD group had higher use of statin and antidiabetic drugs. The mean TNC level was significantly higher in the CAD group than in the non-CAD group ($p < 0.001$, **Fig. 1A**).

Correlation between TNC Levels and Gensini Score

The correlation between TNC levels and other variables were assessed by Pearson's correlation analysis. The results showed a significant positive correlation between TNC levels and Gensini score in all enrolled patients ($p < 0.01$, $r = 0.672$, **Fig. 1B**). Subanal-

ysis showed a significant positive correlation between TNC level and Gensini score in CAD patients ($p < 0.01$, $r = 0.557$, **Fig. 1C**) and non-CAD patients ($p < 0.01$, $r = 0.515$, **Fig. 1D**). Furthermore, TNC levels had a positive correlation with NT-pro BNP ($p < 0.01$, $r = 0.211$), hs-CRP ($p < 0.01$, $r = 0.252$), and HbA1C ($p < 0.05$, $r = 0.163$). We did not find correlations between TNC and age, triglycerides, total cholesterol, LDL cholesterol, and hypertension.

To further explore the relationship of Gensini score and TNC levels, as well as other major cardiovascular risk factors in this study population, a multivariable linear regression analysis using stepwise selection process was performed. The variables included in the model are age, sex, NT-pro BNP, eGFR, hypertension, HbA1C, smoking, HDL and LDL cholesterol, triglycerides, total cholesterol, NEU, and TNC level. The results demonstrated an association between the Gensini score and TNC that was modified by HDL and eGFR (**Table 2**).

Clinical Predictors of CAD and High Gensini Score

ROC curve analysis was used to evaluate the ability of TNC to predict CAD and the high Gensini score. As shown by **Fig. 1E**, the sensitivity and speci-

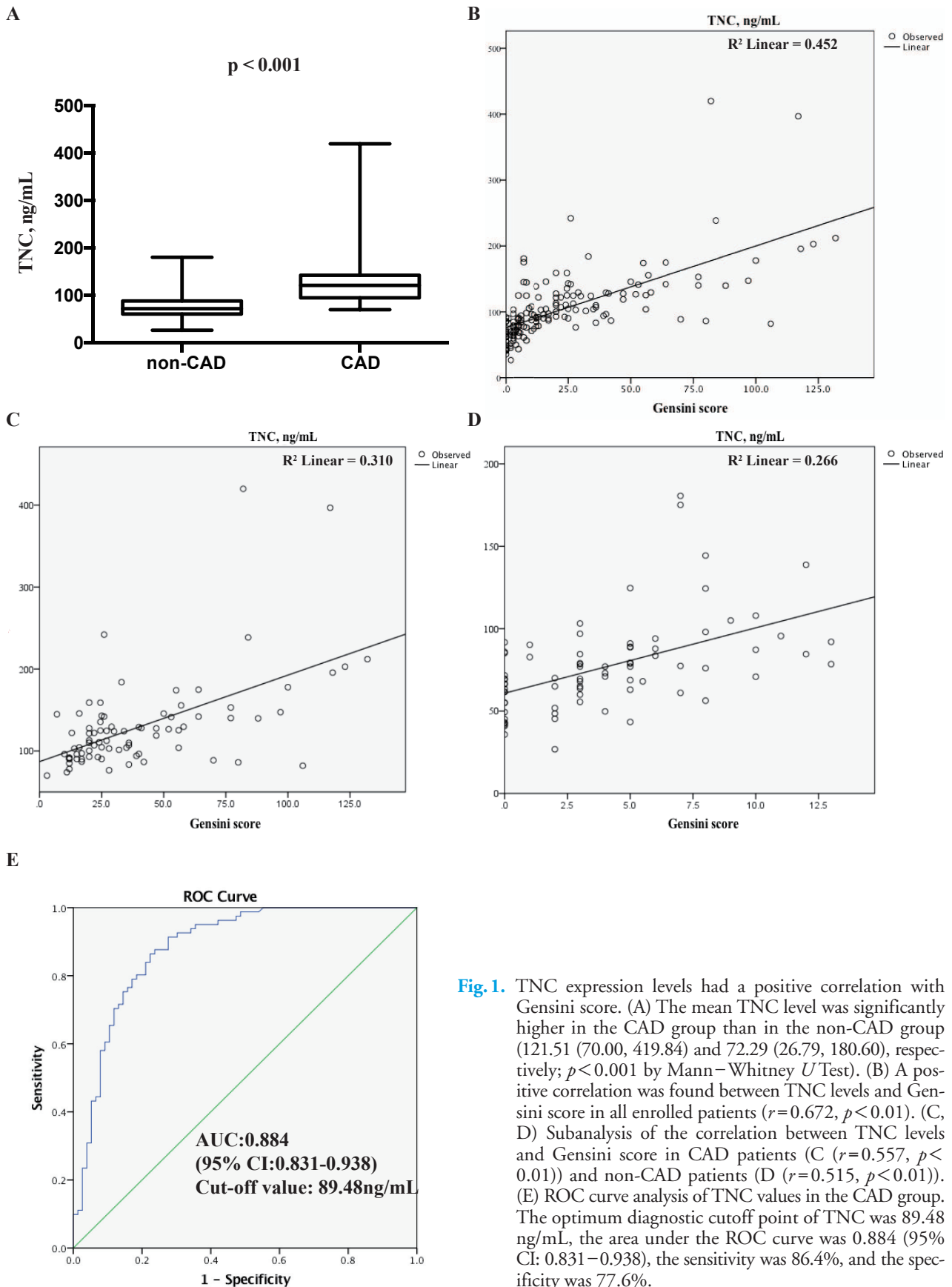


Fig. 1. TNC expression levels had a positive correlation with Gensini score. (A) The mean TNC level was significantly higher in the CAD group than in the non-CAD group (121.51 (70.00, 419.84) and 72.29 (26.79, 180.60), respectively; $p < 0.001$ by Mann-Whitney U Test). (B) A positive correlation was found between TNC levels and Gensini score in all enrolled patients ($r = 0.672$, $p < 0.01$). (C, D) Subanalysis of the correlation between TNC levels and Gensini score in CAD patients (C ($r = 0.557$, $p < 0.01$)) and non-CAD patients (D ($r = 0.515$, $p < 0.01$)). (E) ROC curve analysis of TNC values in the CAD group. The optimum diagnostic cutoff point of TNC was 89.48 ng/mL, the area under the ROC curve was 0.884 (95% CI: 0.831–0.938), the sensitivity was 86.4%, and the specificity was 77.6%.

ficity of TNC in predicting CAD were 86.4% and 77.6%, respectively; when the cutoff value of TNC was settled at 89.48 ng/mL, the area under the curve was 0.884. After adjusting for the risk factors associated with the Gensini score analyzed in this study, including gender, eGFR, HbA1C, NT-pro BNP, smoking, and NEU, TNC remained significantly associated with the increased risk of high Gensini score (OR=1.076, 95% CI, 1.049–1.105, $p < 0.001$)

The patients were then classified into three groups on the basis of the tertiles of Gensini score, as described in the method. As shown by Fig. 2A, the mean TNC level was significantly higher in the high Gensini score group (68.00 (26.79–124.64)) than in both the low (94.74 (56.30–180.60)) and middle groups (127.82 (76.48–419.84)) ($p < 0.001$). ROC curve analysis showed that the cutoff point of TNC at 100.91 ng/mL also predicted a high Gensini score with a sensitivity of 82.7% and a specificity of 79% (the area under the curve was 0.870, Fig. 2B).

Discussion

CAD is a sequence of increasing disease progression including endothelial injury and dysfunction, lipid deposition, and atherosclerotic plaque formation. TNC, as an extraordinarily pleiotropic molecule, is involved in the entire process of CAD¹⁴. The persistent expression of TNC is associated with arterial pathology, and atherosclerosis is the best studied example. Immunohistochemical staining studies has demonstrated a correlation between TNC and ruptured plaques^{15, 16}, TNC level was significantly higher in the ruptured acute coronary syndrome group than in the nonruptured group¹⁷. However, the relation between the expression of TNC in serum and the severity of CAD has not been investigated.

In this study, we found that TNC level is a useful tool for assessing the severity of atherosclerosis. The TNC level in serum was significantly higher in the CAD group than in the non-CAD group ($p < 0.001$). Given that the Gensini score is calculated according to the degree of luminal narrowing, as well as the diseased vessel number and the geographic importance of the vessel location, it could reflect the ischemic extent of the heart and correlates with the outcomes closely. TNC levels were significantly positively correlated with the Gensini score in the patients enrolled in this study ($r = 0.672$, $p < 0.01$). Several factors have been shown to induce TNC expression, including inflammatory cytokines, oxidative stress, and mechanical stress¹⁸, which are also mediators of CAD progress. Hs-CRP was an important marker of inflammation and a predictor of cardiovascular events. The results showed a

Table 2. Multivariable linear regression analysis of Gensini score.

Variables	β	95%CI	p Value
HDL	-0.121	[-27.601, -0.436]	0.043
eGFR	0.136	[0.035, 0.466]	0.023
TNC	0.643	[0.285, 0.411]	<0.001

Multivariable linear regression analysis using stepwise selection process was performed. Variables included in the model are age, sex, NT-pro BNP, eGFR, hypertension, HbA1C, smoking, HDL- and LDL-cholesterol, triglycerides, total-cholesterol, neutrophil percentage and TNC level. eGFR: estimate glomerular filtration rate; TNC: tenascin-C.

significant positive correlation between TNC levels and hs-CRP ($p < 0.01$, $r = 0.252$), which is consistent with a previous report¹⁹) and demonstrates the role of inflammation in the expression of TNC. The mechanisms of TNC in CAD progression can be listed as follows: (1) TNC expression drives smooth muscle cell changes from a nonproliferative phenotype to a migratory, synthetic state, thus resulting in plaque development²⁰; (2) TNC can form a positive feedforward loop with matrix metalloproteinases and drive plaque progression, destabilization, and plaque rupture²¹; (3) TNC provides adhesions for glycoprotein receptors on platelet to promote thrombus formation during late atherosclerosis²². These are all important factors in the progress of CAD, but more laboratory experiments are needed to determine whether the high TNC is the cause or consequence of atherosclerosis. Moreover, circulating TNC levels have also been shown to be correlated with poor prognosis and mortality in myocardial infarction²³.

Multivariable linear regression analysis demonstrated that the association between Gensini score and TNC was modified by HDL and eGFR (Table 2). TNC has emerged as one of the most significant ECM components in the kidney. It was reported to play important roles not only in nephrogenesis but also in pathological processes in the glomerulus and renal interstitium^{24, 25}. Therefore, it is understandable that TNC level was affected by eGFR. It was reported that TNC binds to glycosphingolipids^{26, 27}. Many receptor proteins for TNC have properties that are common in lipid raft-associated molecules, and these receptor proteins seem to form a competition between the two cargos. Given that HDL was a protective factor of atherosclerosis, the relationship of TNC and HDL deserve further investigation.

In this study, ROC curve analysis was performed to evaluate the ability of TNC in the prediction of CAD and high Gensini score. The cutoff value was 89.48 ng/mL for CAD diagnosis and 100.91 ng/mL for the high Gensini score subgroup in the present

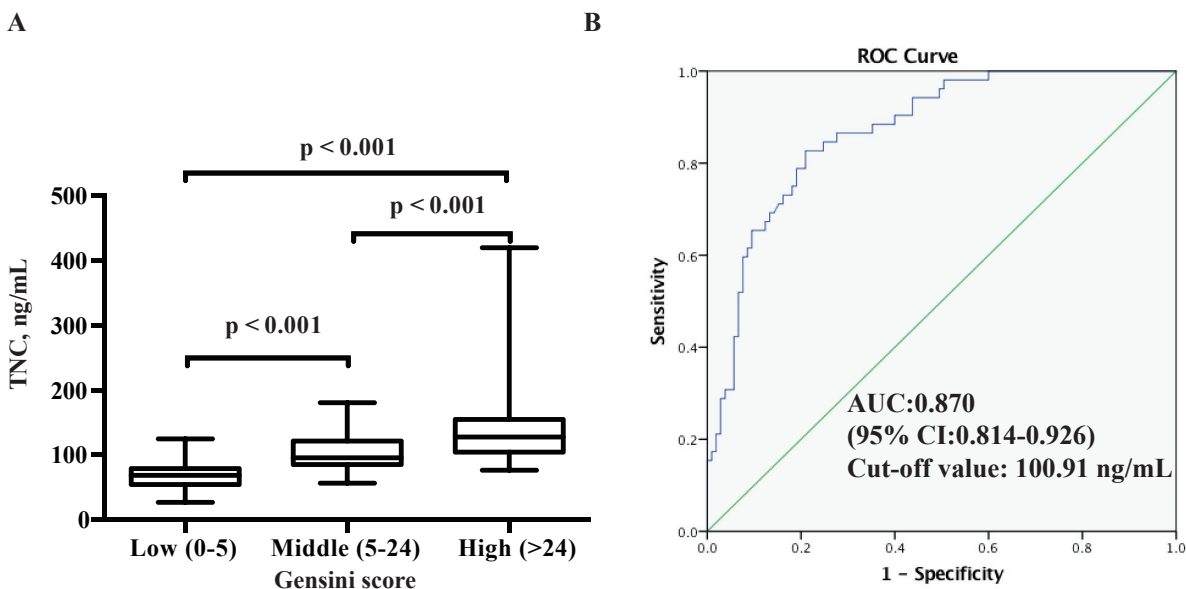


Fig. 2. ROC curve analysis of TNC values in the high Gensini score group. A. The patients were classified into three groups on the basis of the tertiles of the Gensini score. TNC expression levels between the different Gensini score groups. The mean TNC level was significantly higher in the high Gensini score group (68.00 (26.79–124.64)) than in both the low (94.74 (56.30–180.60)) and middle groups (127.82 (76.48–419.84)) ($p < 0.001$ by Mann–Whitney U Test). B. The optimum diagnostic cutoff point of TNC was 100.91 ng/mL, the area under the ROC curve was 0.870 (95% CI: 0.814–0.926), the sensitivity was 82.7%, and the specificity was 79%.

study. Logistic analysis showed that TNC was an independent predictor for CAD diagnosis after adjusting for the risk factors correlated with the Gensini score. These results demonstrated that TNC is a valid marker to prejudge the severity of atherosclerosis before coronary angiography. However, given that TNC was also related to NT-pro BNP and other biochemistry indexes such as HbA1C and renal function, synthetic judgment involving the history, symptoms, risk factors, and examinations should be made cautiously.

Several limitations in this study should be acknowledged. First, this study is a cross-sectional study, thus limiting our ability to infer a causal relationship between TNC and cardiovascular events. Second, the number of patients enrolled was small, thus limiting the statistical power of the study. A prospective study with a larger sample size is needed to better evaluate the value of TNC in CAD.

In conclusion, TNC had a significant association with the severity of atherosclerosis; the TNC level significantly increased as the risk of CAD increased (OR = 1.076, 95% CI, 1.049–1.105, $p < 0.001$). TNC levels can be considered in the risk assessment for CAD before angiography.

Acknowledgments

This work was partially supported by the National

Natural Science Foundation of China (No. 81270278; No. 81770348) and the Shanghai Science and Technology Fund (No. 16411965600).

Conflict of Interest

The authors report no relationships that could be construed as a conflict of interest.

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Supplementary Appendix

TNC was measured using the ELISA kit for Human Tenascin from IBL (Immuno-Biological Laboratories, #27751). The calibration curve for these kits using representative samples are listed as follows.

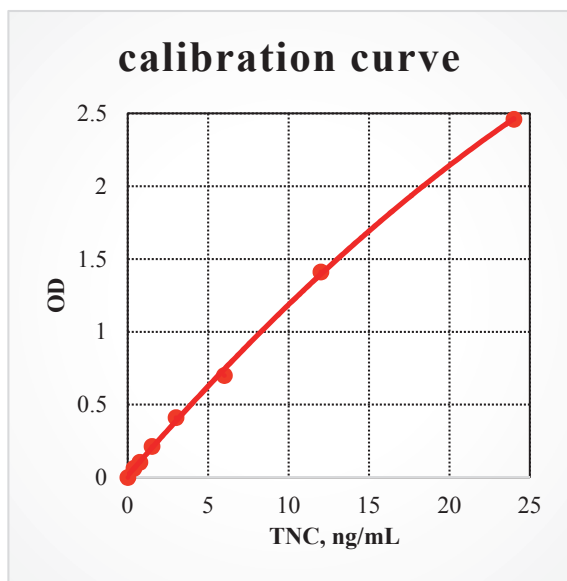
Sample 1

TNC, ng/mL	Measured OD	Actual OD (background subtraction)
24	2.667	2.461
12	1.618	1.412
6	0.907	0.701
3	0.617	0.411
1.5	0.42	0.214
0.75	0.311	0.105
0.38	0.269	0.063
0	0.206	0

Sample 2

TNC, ng/mL	Measured OD	Actual OD (background subtraction)
24	2.701	2.445
12	1.718	1.462
6	0.977	0.721
3	0.667	0.411
1.5	0.46	0.204
0.75	0.351	0.095
0.38	0.301	0.045
0	0.256	0

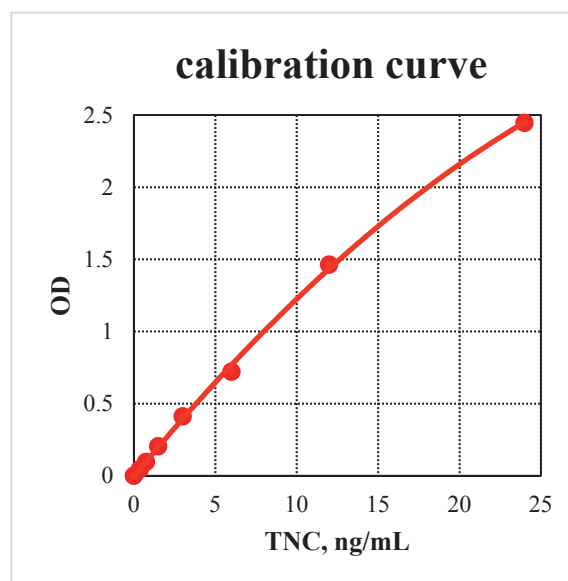
The calibration curve was traced according to the actual OD and the concentration of TNC.



The quartic curve-fitting equation is $y = (A - D) / [1 + (x/C)^B] + D$

$A = 10.82507$, $B = -1.00548$, $C = 81.21054$, $D = 0.01036$, $r^2 = 0.99939$.

The sum of squared residuals is 0.00314.



The quartic curve-fitting equation is $y = (A - D) / [1 + (x/C)^B] + D$

$A = 6.43011$, $B = -1.09728$, $C = 37.47286$, $D = 0.00785$, $r^2 = 0.99933$.

The sum of squared residuals is 0.00352.