

# The Relationship Between the Serum NLRP3 and Adiponectin Levels and Coronary Lesions in Patients with Unstable Angina with Type 2 Diabetes

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**Objective:** To investigate the Levels of Nucleotide-binding, leucine-rich repeat and pyrin domain-containing protein 3 (NLRP3) and Adiponectin (APN) and their relationship with the severity of coronary artery disease in patients with Unstable Angina (UA) and Type 2 Diabetes (T2D).

**Methods:** Two hundred and thirty-one patients with UA were diagnosed by CAG in the Department of Cardiology of the Affiliated Hospital of Xuzhou Medical University from July 2022 to May 2023 were included, and 74 healthy subjects were included as the control group. The levels of NLRP3 and APN in each group were detected by ELISA and the Gensini score in each patient according to the results of CAG. The correlations between NLRP3, APN, and Gensini score were analyzed. According to whether complicated with T2D or not, we further analyze the effect of NLRP3 and APN levels of patients with UA and T2D on the severity of coronary artery stenosis.

**Results:** The levels of NLRP3 in UA with T2D group were the highest, followed by simple UA group, and the lowest in the control group, and the level of APN was the opposite. Spearman Correlation analysis showed that the level of NLRP3 was positively correlated with Gensini score ( $\rho_1=0.688$ ,  $P<0.05$ ) and the level of APN was negatively associated with Gensini score ( $\rho_2=-0.515$ ,  $P<0.05$ ). There was a negative correlation between NLRP3 and the level of APN ( $\rho_3=-0.366$ ,  $P<0.05$ ). High NLRP3 and low APN levels are the risk factors for atherosclerosis.

**Conclusion:** The NLRP3 and APN were abnormally expressed in patients with UA complicated with T2D. With the aggravation of atherosclerosis, the level of NLRP3 increased and the level of APN decreased.

**Keywords:** NLRP3, adiponectin, gensini score, diabetes

## Introduction

Cardiovascular disease is the major cause of death in the world, and atherosclerosis is one of its underlying causes. With the improvement of economic level, the incidence of Type 2 Diabetes (T2D) is increasing year by year, becoming the main burden of national medical expenditure, but also an important risk factor in the process of atherosclerosis. The nature of both atherosclerosis and T2D is a chronic inflammatory reaction.<sup>1</sup> The activation of the Nucleotide-binding, leucine-rich repeat, and pyrin domain-containing protein 3 (NLRP3) inflammasome plays an important role in the development of the disease. The complex composed of NLRP3, apoptosis-associated particulate protein (ASC) and pro-Caspase-1 can promote the release of inflammatory factors such as IL-1 $\beta$  and IL-18, and accelerate the inflammatory response.<sup>2</sup> CANTOS studies have shown that Canakinumab can exert anti-inflammatory effects by targeting inhibition of IL-1 $\beta$ , slow down the progression of cardiovascular disease, and reduce the occurrence of major cardiovascular events, which further proves that NLRP3 is involved in the atherosclerosis process.<sup>3</sup> Adiponectin (APN) can inhibit the inflammatory response by inhibiting the expression of NLRP3 inflammasome, alleviating target organ damage, and

promoting functional repair.<sup>4,5</sup> The correlation between both and the degree of coronary artery lesions and their correlation in patients with UA and T2D have not been studied. The purpose of this study was to explore the relationship between NLRP3 and APN and the severity of coronary lesions (Gensini score) in patients suffering from both UA and T2D.

## Materials and Methods

### Study Subjects

A total of 231 patients with UA diagnosed by coronary angiography in the affiliated Hospital of Xuzhou Medical University, with unexplained chest pain from July 1, 2022, to May 31, 2023, including 135 males, 96 females, aged 65 (5672) years. All subjects completed blood routine, liver and kidney function, coagulation function, and ECG before surgery. Patients with UA were divided into subgroup of UA with T2D and simple UA subgroup according to whether complicated with T2D. At the same time, 74 healthy subjects were included in the same period as the control group as the object of this study. There was no significant difference in age or gender in each group ( $P > 0.05$ ).

### Diagnostic Criteria

Diagnostic criteria for coronary heart disease:<sup>6</sup> coronary CTA or coronary angiography examination indicates that at least one major vessel or branch vessel has stenosis diameter  $\geq 50\%$ ;

Diagnostic criteria for Type 2 Diabetes:<sup>7</sup> 1) Fasting blood glucose  $\geq 7$  mmol/L or 126 mg/dL (after 8 h of fasting), 2) after fasting for 8 hours, OGTT test showed that blood glucose  $\geq 11.1$  mmol/L or 200 mg/dL, 3) hyperglycemia symptoms such as excessive drinking, eating, and polyuria and random blood glucose  $\geq 11.1$  mmol/L, 4) HbA1c level  $\geq 6.5\%$ .

Exclusion criteria: ① absence of data, ② patients with heart valve disease, ③ patients with cardiomyopathy, ④ patients with malignancy, blood system disease, immune system disease, ⑤ patients with previous PCI, CABG, ⑥ severe hepatic and renal insufficiency.

Degree of coronary stenosis and the Gensini score:<sup>8</sup> According to the Gensini score system, two experienced surgeons calculated the score of coronary artery disease degree of each subject in the observation group through the results of coronary angiography. The flowchart for the study is displayed in Figure 1.

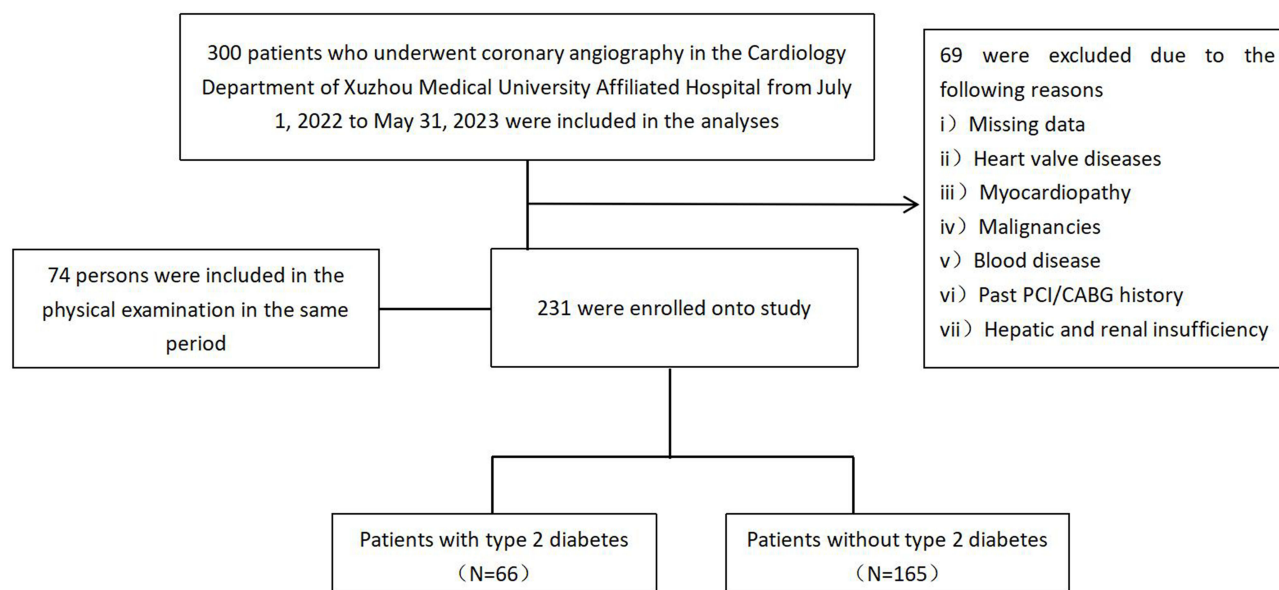


Figure 1 Flowchart of study.

## Methods

Clinical data Collection included general data of patients (age, sex, body mass index (BMI), smoking history, alcohol history, other medical history (hypertension, cerebral infarction, hyperlipidemia, etc.), medication history (aspirin, clopidogrel, statins, etc.) and laboratory indicators (white blood cells, glycosylated hemoglobin, triglycerides, total cholesterol, low density lipoprotein, high density lipoprotein, hsCRP, etc).

Detection of serum NLRP3 and APN levels hospitalized patients collected 5 mL of fasting morning venous blood after admission, centrifuged at 2500 r/min, centrifuged for 15 min, the upper serum was taken, and the samples were stored at  $-80^{\circ}\text{C}$ . The levels of NLRP3 and APN in serum were detected by ELISA, all test procedures were carried out in strict accordance with the instructions, and the kit was purchased from Wuhan YunClone Technology Co., Ltd.

## Statistical Analysis

SPSS26.0 was used to analyze the data. The measurement data in accordance with the normal distribution were expressed as  $\bar{x} \pm s$ , the data between the two groups were compared by independent sample *t*-test, the data in accordance with skewness distribution were expressed by *M* (*P*25, *P*75), and the data between the two groups were compared by Mann–Whitney *U*-test. The counting data were expressed as an example (%) and chi-square test was used for comparison between two groups. Logistic regression was used to analyze the factors affecting the degree of coronary artery disease in patients with coronary heart disease.  $P < 0.05$  is considered to be statistically significant.

## Results

Comparison of clinical data between the two groups A total of 305 subjects were included in this study, including 231 in the observation group and 74 in the control group. There was no significant difference in sex, age, BMI, drinking history and hypertension between the two groups ( $P > 0.05$ ), but there were significant differences in smoking, WBC, hsCRP, NLRP3, APN, FPG, and HbA1c between the two groups ( $P < 0.05$ , Table 1).

**Table 1** Comparison of Basic Data Between Patients with UA and Non-UA

Variable	Observation (n=231)	Control (n=74)	P value
Age (>60, n,%)	148 (64.1%)	38 (51.4%)	$P > 0.05$
Sex (male, n,%)	135 (58.4%)	37 (50.0%)	$P > 0.05$
BMI (>24kg/□, n,%)	158 (68.4%)	46 (62.2%)	$P > 0.05$
Tobacco use (yes, n, %)	107 (46.3%)	24 (32.4%)	$P < 0.05$
Alcohol consumption (yes, n, %)	44 (19.0%)	20 (27.0%)	$P > 0.05$
Hypertension (yes, n, %)	147 (63.6%)	38 (51.4%)	$P > 0.05$
WBC (*10 <sup>9</sup> /L)	6.1 (5.0,7.4)	5.6 (4.6,6.6)	$P < 0.05$
HGB (g/L)	140 (128,149)	136 (127,150)	$P > 0.05$
PLT (*10 <sup>9</sup> /L)	217 (184,258)	215 (186,248)	$P > 0.05$
hsCRP (mg/L)	1.7 (0.6,4.3)	0.8 (0.5,2.3)	$P < 0.05$
AST (U/L)	18 (16,25)	18 (15,21)	$P < 0.05$
ALT (U/L)	18 (13,25)	18 (15,23)	$P > 0.05$
GGT (U/L)	23 (16,33)	20 (15,27)	$P > 0.05$
ALB (g/L)	43.3±3.5	43.5±3.4	$P > 0.05$
TC (mmol/L)	4.2 (3.6,5.1)	4.6 (4.0,5.2)	$P > 0.05$
TG (mmol/L)	1.4 (1.1,2.0)	1.3 (1.0,1.9)	$P > 0.05$
HDL-C (mmol/L)	1.0 (0.9,1.2)	1.1 (1.0,1.4)	$P > 0.05$
LDL-C (mmol/L)	2.5 (1.9,3.1)	2.6 (2.1,3.1)	$P > 0.05$
sd-LDL (mmol/L)	0.7 (0.5,1.1)	0.7 (0.5,1.0)	$P > 0.05$
LP (a) (mg/L)	246.0 (136.0,390.0)	180 (91.8,312.8)	$P > 0.05$
UA (umol/L)	302.3±84.4	299.4±83.1	$P > 0.05$

(Continued)

Table 1 (Continued).

Variable	Observation (n=231)	Control (n=74)	P value
Scr (umol/L)	61.4±13.4	58.7±11.7	P>0.05
PT (sec)	10.3 (10.0,10.9)	10.4 (10.0,10.8)	P>0.05
APTT (sec)	26.8 (25.2,28.2)	26.6 (25.1,28.8)	P>0.05
FPG (mmol/L)	5.6 (5.1,6.8)	5.1 (4.6,5.6)	P<0.05
HbA1c (%)	5.9 (5.6,6.5)	5.7 (5.4,6.0)	P<0.05
NLRP3 (ng/mL)	5.2 (3.7,7.4)	3.1 (2.3,3.9)	P<0.05
APN (ng/mL)	4.9 (3.9,6.7)	8.1 (7.0,9.0)	P<0.05

Logistic regression analysis of UA Compared with FPG, HbA1c can be used as a risk factor for the occurrence of cardiovascular events in patients suffering from both CHD and diabetes.<sup>9,10</sup> Multivariate Logistic regression analysis was performed with UA as the dependent variable and smoking history, WBC, hsCRP, NLRP3, APN, and HbA1c as independent variables. The results showed that high NLRP3 level, low APN level, and high HbA1c level were independent risk factors for UA ( $P < 0.05$ , Figure 2).

Correlation analysis between NLRP3, APN levels, and Gensini score Spearman correlation analysis showed that there was a correlation between serum NLRP3 and APN levels and Gensini score in patients with UA ( $\rho_1=0.688$ ,  $P<0.05$ ;  $\rho_2=-0.515$ ,  $P < 0.05$ ). Figures 3 and 4 are scatter plots of the correlation between NLRP3 and APN and Gensini score, respectively.

Correlation analysis of NLRP3 and APN Spearman correlation analysis showed that there was a correlation between serum NLRP3 and APN levels in patients with UA ( $\rho_3=-0.366$ ,  $P < 0.05$ ). Figure 5 is the scatter plot of the correlation between NLRP3 and APN.

Comparison of NLRP3 and APN levels between observation group. The level of serum NLRP3 in patients with UA complicated with T2D was higher than that in patients with UA, and the level of APN was lower than that in patients with UA. The difference was statistically significant ( $P < 0.05$ , Figure 6).

The value of NLRP3 and APN in predicting UA+T2D. The ROC curves of NLRP3 and APN were drawn to predict the occurrence of UA complicated with T2D. The results showed that the AUC of NLRP3 was 0.800, with the sensitivity was 63.6%, and the specificity was 84.5%. APN predicted that the AUC of UA complicated with T2D was 0.787, the sensitivity was 78.8%, and the specificity was 70.3% (Figure 7).

## Discussion

With further research on atherosclerosis, chronic inflammation participates in the occurrence and development of atherosclerosis has been widely recognized. The essence of atherosclerosis is vascular endothelial cell injury and lipid deposition.<sup>11</sup>

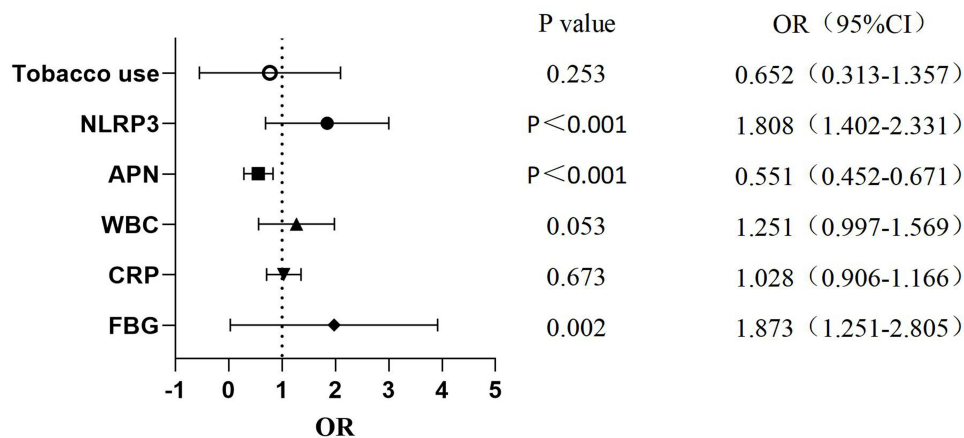


Figure 2 Logistic regression analysis of unstable angina.

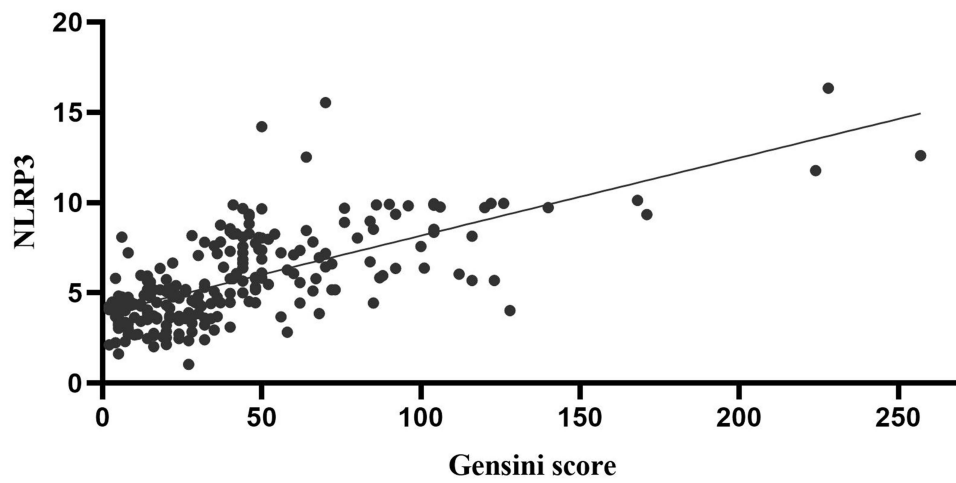


Figure 3 The correlation of serum NLRP3 levels and Gensini score.

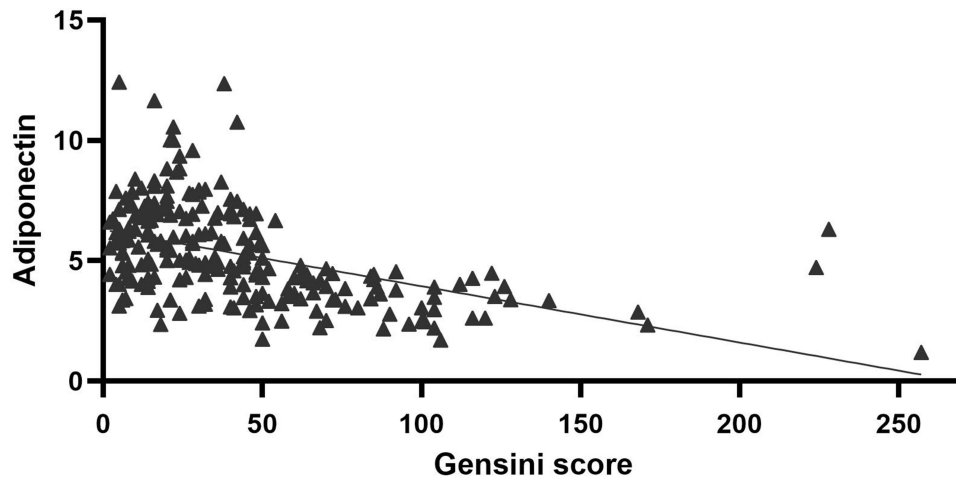


Figure 4 The correlation of serum Adiponectin levels and Gensini score.

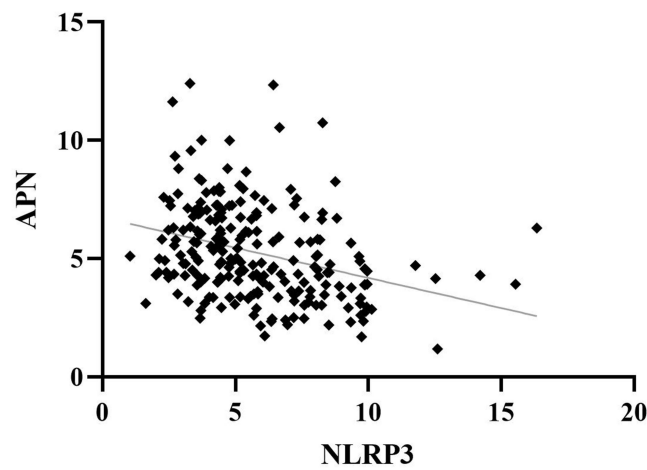
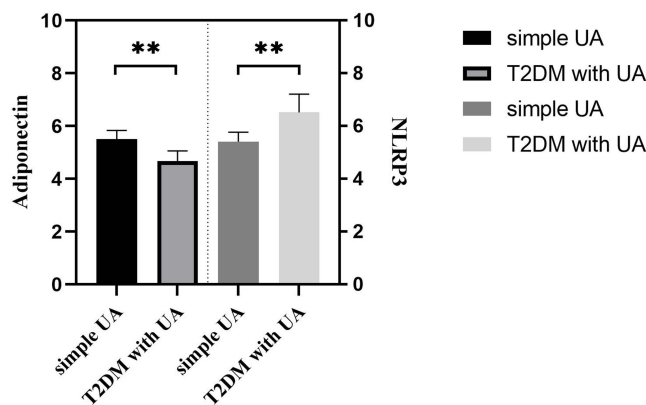
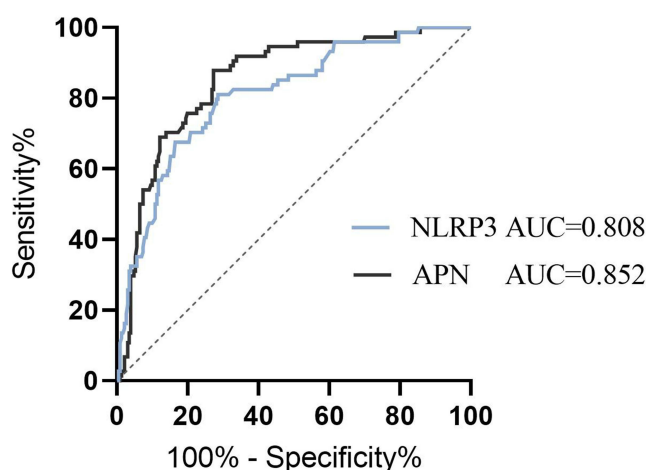


Figure 5 The correlation of serum NLRP3 and Adiponectin levels.



**Figure 6** The serum NLRP3 and Adiponectin levels in simple UA and T2D with UA.



**Figure 7** The value of NLRP3 and Adiponectin in predicting UA+T2D.

And patients with diabetes are in a state of hyperglycemia for a long time, which can increase the production of ROS by multiple mechanisms such as increasing the production of AGEs, and further increase vascular permeability, thus accelerating atherosclerosis.<sup>12</sup> The inflammasome is one of the important components involved in the inflammatory response, its essence is a multiprotein complex, and the NLRP3 inflammasome has been the most widely studied. The NLRP3 inflammasome is a complex composed of NLRP3, apoptosis-related microparticle protein (ASC), and Caspase-1 precursor, which can be initiated and activated by PAMPs and DAMPs. After activation, it converts pro-IL-1 $\beta$  and pro-IL-18 into IL-1 $\beta$  and IL-18, and then participates in the inflammatory response.<sup>13</sup> Abnormal activation of NLRP3 inflammasome has been demonstrated to cause or aggravate the development of varieties of diseases, such as atherosclerosis, diabetes, cerebral hemorrhage, NAFLD, CAPS, Rheumatoid Arthritis.<sup>5,14,15</sup> Inhibition of NLRP3 or loss of gene expression improves the process of cardiac remodeling and myocardial fibrosis.<sup>16</sup> Nowadays, NLRP3 has been recognized as a novel biomarker of cardiovascular risk.

As an important component in maintaining vascular homeostasis, adiponectin can reduce the expression of adhesion molecule, inhibit lipid deposition and the formation of foam cells, and as an insulin sensitizer, adiponectin can also improve insulin resistance.<sup>17</sup> Its level decreased significantly in inflammatory-related diseases such as coronary heart disease and diabetes.<sup>18,19</sup> The activation of NLRP3 requires the participation of both initiation signal and activating signal, in which the initiation signal is mediated by the NF- $\kappa$ B pathway, the activation signal associated with ROS production, K<sup>+</sup> excretion and lysosomal membrane permeability, and the lack of adiponectin can increase the production of ROS and promote the expression of NF- $\kappa$ B, decrease the phosphorylation levels of AMPK, JNK, and Erk1/2, and

further activate the NLRP3 inflammasome.<sup>5,20</sup> Several studies have shown that APN and APN receptor agonists are able to inhibit the inflammation by inhibiting NLRP3 expression through a variety of signaling pathways, thus playing a protective role in inflammation-related diseases such as coronary heart disease, diabetes, and obesity.<sup>21–23</sup> AdipoRon is a kind of adiponectin receptor agonist, which can protect against inflammation and antioxidant stress by binding and activating adiponectin receptors AdipoR1 and AdipoR2.<sup>24</sup>

However, the correlation among the levels of NLRP3 and APN and the severity of coronary artery lesions has not been studied. In this study, the subjects were first divided into UA and control group, and the degree of coronary lesions was evaluated according to the Gensini score. The results showed that the levels of serum HbA1c and NLRP3 were significantly increased and the level of adiponectin was significantly decreased in patients with UA, which were closely correlated with Gensini score. There were significant difference in fasting blood glucose and HbA1c between the two groups. The subjects in observation group were further divided into UA complicated with T2D group and simple UA group. In them, NLRP3 was further increased in patients with UA complicated with T2D, while APN was on the contrary.

Studies concluded that the levels of serum NLRP3 and APN are independent risk factors for patients with UA complicated with T2D, and have high predictive value for the occurrence of atherosclerosis, which provides a new perspective for further research and understanding of the pathogenesis of UA complicated with T2D, and also provides a theoretical basis for the development of targeted treatment strategies.

## Limitations

This research has some limitations. Firstly, this is a single-center retrospective research. Secondly, the sample size is small, there is no long-term follow-up, and the effect on the long-term survival rate of patients has not been confirmed. A multicenter prospective study with a large sample size is needed to further confirm its reliability.

## Conclusion

This study demonstrated that serum NLRP3 and Adiponectin were related to the occurrence and development of UA and T2D. There are high expression level of NLRP3 and low expression of Adiponectin in patients complicated with T2D, and there is a strong correlation between them.

## Ethics Approval

The study was approved by the Medical Ethics Committee of Xuzhou Medical University's Affiliated Hospital in compliance with the Helsinki Declaration. Ethics number is XYFY2023-KL324. All patients provided written informed consent for participation in the study.

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## Disclosure

The authors declare that they have no conflicts of interest in this work.

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