Increased circulating levels of tumor necrosis factor-like cytokine 1A and decoy receptor 3 correlate with SYNTAX score in patients undergoing coronary surgery

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Abstract
Objective: Chronic inflammation of the arteries is a critical mechanism responsible for coronary atherosclerosis. We aimed to determine if tumor necrosis factor (TNF)-like cytokine 1A (TL1A) and decoy receptor 3 (DcR3) were involved in promoting atherosclerosis.

Methods: We compared plasma levels of TL1A and DcR3 in patients with coronary artery disease (CAD) undergoing coronary artery bypass grafting (n=40) and patients without CAD group (n=37, normal coronary artery angiogram) by enzyme-linked immunosorbent assay. We also analyzed the correlation between CAD and SYNTAX scores.

Results: Plasma levels of TL1A and DcR3 were significantly higher in the CAD compared with the no-CAD group. Multivariate analysis showed that TL1A and DcR3 were significantly correlated with the presence of CAD, and receiver operating characteristic curve analysis indicated that both TL1A and DcR3 showed high sensitivity and specificity for diagnosing CAD. Furthermore, TL1A was positively and significantly correlated with SYNTAX score in CAD patients.

Conclusions: CAD patients requiring coronary artery bypass grafting have high circulating levels of both TL1A and DcR3, which may thus be useful biomarkers for diagnosing severe CAD. Furthermore, plasma levels of TL1A correlate with SYNTAX score, supporting its potential use as an indicator of the severity of CAD.

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Introduction
Chronic inflammation in the arterial wall, involving several inflammatory mediators, is the critical mechanism leading to coronary atherosclerosis. Tumor necrosis factor (TNF)-like cytokine 1A (TL1A) and its receptors decoy receptor 3 (DcR3) and death receptor 3, which belong to the TNF and TNF receptor superfamilies, are involved in the pathogenesis of chronic inflammation. TL1A was confirmed to be overexpressed both locally and systemically in rheumatoid arthritis, psoriasis, inflammatory bowel disease, and ankylosing spondylitis. Serum levels of DcR3 were also remarkably elevated in a range of conditions including Crohn’s disease, silicosis, active ulcerative colitis, and bacterial infections.

Among patients with rheumatoid arthritis, the TL1A/DcR3 ratio was negatively correlated with the formation of atheromatous plaques in the carotid and femoral arteries. Studies have also shown that plasma TL1A levels were elevated in patients with acute coronary syndrome, compared with those with stable coronary artery disease (CAD), and that the incidence of major adverse cardiovascular events was higher in patients with high DcR3 levels compared with those with intermediate DcR3 levels. However, these two important factors have not been studied in the same patient cohort, particularly among patients undergoing coronary artery bypass grafting (CABG).

Furthermore, although DcR3 levels have been reported to correlate with SYNTAX score, the correlation between TL1A levels and SYNTAX score in CAD patients remains unknown. The SYNTAX score provides a quantitative measure of characteristics of the coronary vasculature, including the complexity, location, number, and functional impact of angiographically obstructive lesions. A pooled analysis revealed that the SYNTAX score was an independent predictor of stent thrombosis, combined ischemic outcomes, and mortality at the first year’s follow up in CABG patients. This score has thus become an important tool for evaluating the severity of CAD, particularly in CABG patients.

The present study was designed to explore the value of circulating levels of TL1A and DcR3 in the same cohort of CAD patients, with an emphasis on their correlations with CAD, and on the levels of these factors and SYNTAX score in CABG patients.

Patients and methods
Study design
Patients who underwent coronary artery angiography at TEDA International Cardiovascular Hospital between January 2016 and March 2016 were enrolled in this study. The research protocol was confirmed by the Institutional Review Board of TEDA International Cardiovascular Hospital. Informed consent was given
prior to the inclusion of patients in the study. Patients were excluded if they had a history of rheumatoid arthritis, ankylosing spondylitis, psoriasis/psoriatic arthritis, systemic lupus erythematosus, systemic sclerosis, Crohn’s disease, malignant tumor, ulcerative colitis, or primary biliary cirrhosis. The patients were divided into a CAD group and a no-CAD group, depending on the presence or absence of CAD confirmed by coronary angiography. All patients in the CAD group had severe CAD (double- or triple-vessel disease and/or left main stenosis) and underwent CABG.

Samples

Plasma samples were collected from all patients after overnight fasting. Venous blood samples were acquired from a peripheral vein and 2 mL blood was immediately centrifuged at 2500 × g for 15 minutes. The supernatant was then divided into aliquots and transferred to microtubes, and stored at −80°C until the tests were carried out.15

Enzyme-linked immunosorbent assay (ELISA)

TL1A and DcR3 levels in plasma were measured using an ELISA kit (CUSABIO, Wuhan, China) according to the manufacturer’s instructions, as described previously.16

Clinical features

All patients’ medical records were reviewed to detect any histories of smoking, hypertension, myocardial infarction, diabetes mellitus (DM), or hyperkinemia. DM was defined as fasting plasma glucose >126 mg/dL or use of hypoglycemic agents. Hypertension was graded into three levels according to the 1999 WHO-ISH Guidelines for the Management of Hypertension.17 Total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, uric acid, blood glucose, and hemoglobin were measured using an automatic biochemical analyzer (Hitachi, Tokyo, Japan) and automatic blood cell analyzer (Sysmex, Kobe, Japan).

SYNTAX score calculation

SYNTAX scores for patients in the CAD group were calculated by two expert interventional cardiologists who were blinded to the patient’s clinical data. Each coronary lesion with a stenosis diameter >50% in vessels with a diameter >1.5 mm was scored using the online SYNTAX score calculator V2.28 (http://www.syntaxscore.com).18 The final SYNTAX score for each patient was the average of the two scores calculated by the two respective cardiologists.

Statistical analysis

Data were analyzed using IBM SPSS Statistics, version 23.0 (IBM Corp., Armonk, NY, USA). Continuous variables with a normal distribution were presented as mean (standard deviation) and differences were tested using Students’ t-tests, and data with a nonparametric distribution were presented as median (interquartile range) and compared using Mann–Whitney U tests. Categorical variables were compared using χ2 tests. Correlations between plasma levels of TL1A and DcR3 and the presence of CAD were analyzed using multivariate logistic regression models. Receiver operating characteristic (ROC) curve analysis was undertaken to evaluate the predictive powers of TL1A and DcR3 for CAD. Area under the curve (AUC) comparisons were made using the Z-test. The established risk for the combination of the two tests was calculated as: log hazard ratio = (β1 × TL1A) + (β2 × DcR3). Univariate linear regression was performed to assess the correlations.
between TL1A and DcR3 and SYNTAX scores in the CAD group. Values of \( P < 0.05 \) were considered significant.

**Results**

**Clinical and demographic characteristics**

A total of 77 patients were enrolled in this study, including 40 in the CAD group and 37 in the no-CAD group. No patients were excluded after enrolment. The demographic and baseline clinical data are shown in Table 1.

**Differences in TL1A and DcR3 levels between the CAD and no-CAD groups**

Plasma levels of TL1A and DcR3 were significantly higher in the CAD compared with the no-CAD group (4.4±1.8 ng/mL versus 2.7±1.9 ng/mL; \( P < 0.001 \); Figure 1; 15.3 ±6.0 ng/mL versus 7.3±3.0 ng/mL, \( P < 0.001 \); Figure 2, respectively).

**Table 1.** Characteristics of patients in the CAD and no-CAD groups.

| Variable                  | CAD (n=40) | no-CAD (n=37) | \( P \) value |
|---------------------------|------------|---------------|--------------|
| Age, years, mean (SD)     | 61.8 (7.2) | 57.4 (8.7)    | 0.02         |
| Male sex (%)              | 22 (55.0)  | 25 (67.6)     | 0.26         |
| BMI (kg/m²), mean (SD)    | 26.2 (3.3) | 25.7 (3.3)    | 0.51         |
| MI, n (%)                 |            |               |              |
| AMI                       | 10 (25)    | –             | –            |
| OMI                       | 5 (12.5)   | –             | –            |
| Smoking, n (%)            | 21 (52.5)  | 13 (35.1)     | 0.13         |
| Hypertension*             | 11/3/10/16 | 19/2/10/9     | 0.26         |
| Diabetes mellitus, n (%)  | 17 (42.5)  | 5 (13.5)      | 0.005        |
| Hyperkemina, n (%)        | 30 (75.0)  | 13 (35.1)     | <0.001       |
| Uric acid (µmol/L), mean (SD) | 324.7 (77.8) | 338.2 (78.9) | 0.45         |
| Hemoglobin (g/L), mean (SD)| 138.4 (15.7) | 141.9 (11.2) | 0.27         |
| SYNTAX score              | 51.4 (17.9) | –             | –            |

*No hypertension/hypertension grade 1/2/3
CAD, coronary artery disease; SD, standard deviation; BMI, body mass index; MI, myocardial infarction; AMI, acute myocardial infarction; OMI, old myocardial infarction
We further evaluated the associations between TL1A and DcR3 levels and the presence of CAD by multivariate logistic regression analysis. After adjusting for traditional risk factors including gender, body mass index, age, smoking, hypertension, DM, and hyperkinemia, levels of both TL1A ($P=0.005$) and DcR3 ($P=0.001$) were independently associated with CAD (Table 2).

**ROC curve analysis**

In ROC curve analysis, TL1A demonstrated an AUC of 0.776 (95% confidence interval [CI]: 0.668–0.884, $P<0.001$), with an optimal cut-off level for diagnosing CAD of 2.83 ng/mL, which showed high specificity (85.00%) and sensitivity (64.86%). The AUC for DcR3 was 0.899 (95% CI: 0.833–0.965, $P<0.001$), with an optimal cut-off level of 9.93 ng/mL, with a high sensitivity (82.50%) and specificity (86.49%). The combination of TL1A and DcR3 demonstrated an AUC of 0.944 (95% CI: 0.898–0.991, $P<0.001$), which was better than that for either TL1A ($P=0.001$) or DcR3 ($P=0.052$) alone (Figure 3).

**Correlations of TL1A and DcR3 with SYNTAX score in CAD patients**

Bivariate correlations indicated that plasma TL1A levels were positively correlated with SYNTAX score in the CAD group ($r=0.36$, $P=0.02$; Figure 4a), whereas DcR3 level showed no such correlation (Figure 4b).

**Discussion**

The results of the current study provide the first evidence demonstrating that circulating TL1A and DcR3 levels were significantly elevated in CAD patients compared with individuals without CAD, suggesting a direct correlation between these proteins and the presence of CAD. Furthermore, TL1A levels were positively correlated with SYNTAX score, suggesting a possible predictive value of TL1A for the severity of CAD.

**Importance of TL1A and DcR3**

TL1A is expressed predominantly by endothelial cells and is a member of the TNF

| Table 2. Variables associated with CAD in univariate and multivariate logistic regression analyses. |
|-----------------------------------------------|
| Variable | Univariate | Multivariate |
|---------|-------------|--------------|
| Age | 1.1 | 1.0–1.2 | 0.03 | 0.9 | 0.9–1.1 | 0.74 |
| Male sex | 0.6 | 0.2–1.5 | 0.26 | 1.4 | 0.2–12.3 | 0.76 |
| BMI, kg/m² | 1.1 | 0.9–1.2 | 0.51 | 0.9 | 0.7–1.2 | 0.40 |
| Smoking | 2.0 | 0.8–5.1 | 0.13 | 3.5 | 0.5–26.0 | 0.22 |
| Hypertension | 1.4 | 0.9–2.0 | 0.07 | 1.1 | 0.6–2.2 | 0.77 |
| Diabetes mellitus | 4.7 | 1.5–14.7 | 0.007 | 3.1 | 0.4–22.6 | 0.27 |
| Hyperlipemia | 5.5 | 2.1–14.8 | 0.001 | 2.1 | 0.3–13.5 | 0.43 |
| TL1A, ng/mL | 1.8 | 1.3–2.4 | <0.001 | 2.1 | 1.3–3.6 | 0.005 |
| DcR3, ng/mL | 1.6 | 1.3–2.0 | <0.001 | 1.8 | 1.3–2.6 | 0.001 |

*Adjusted for age, gender, BMI, smoking, hypertension, diabetes mellitus, hyperkinemia, TL1A and DcR3 levels CAD, coronary artery disease; CI, confidence interval; OR, odds ratio, BMI, body mass index; TL1A, tumor necrosis factor-like cytokine 1A; DcR3, decoy receptor 3.
ligand superfamily. TL1A exists as a membrane-bound protein that can also be processed into a soluble form when expressed ectopically. Similarly, DcR3 is a secreted member of the TNF receptor superfamily, which neutralizes the TNF ligands LIGHT, FasL, and TL1A. The interaction between TL1A and DcR3 is important in inflammation, apoptosis, homeostasis, host defense, and autoimmunity.

**Elevated TL1A and DcR3 levels in CAD patients**

TL1A and DcR3 have been reported to be present in atherosclerotic plaques. An in vitro study showed that TL1A promoted the expression of scavenger receptors and suppressed the expression of members of the ABC transporter family.

As a soluble TL1A receptor, DcR3 can facilitate monocyte binding to endothelial

![Figure 3](image_url)

**Figure 3.** Predictive performances of TL1A (red dashed line), DcR3 (purple dotted line), and the combination of TL1A and DcR3 (blue solid line) for CAD. The AUCs of TL1A, DcR3, and the combination of TL1A and DcR3 were 0.776 (95% CI: 0.668–0.884, P < 0.001), 0.899 (95% CI: 0.833–0.965, P < 0.001), and 0.944 (95% CI: 0.898–0.991, P < 0.001), respectively.

![Figure 4](image_url)

**Figure 4.** Circulating TL1A levels (a) were positively and significantly correlated with SYNTAX score (r = 0.36, P = 0.02), but DcR3 levels (b) showed no such correlation.
cells via enhancing actin reorganization and the expression of interleukin-8, intercellular adhesion molecule 1, and vascular cell adhesion molecule-1 in monocytes,24 thereby mediating the recruitment and migration of inflammatory cells. This may be the key mechanism of DcR3 in the pathogenesis of CAD.

**Associations of TL1A and DcR3 levels with CAD**

A previous study demonstrated an association between DcR3 and outcome in patients with acute respiratory distress syndrome.24 In the current study, we showed that plasma levels of DcR3 and TL1A were both significantly associated with the development of CAD according to multivariate analysis \( (P=0.005 \text{ and } 0.001, \text{ respectively}) \). Furthermore, ROC analysis revealed that TL1A and DcR3 levels were both effective predictors for diagnosing CAD. The combination of TL1A and DcR3 levels showed greater discriminatory power than either TL1A or DcR3 alone. Given that circulating TL1A and DcR3 levels are easy to test in the clinical setting, these two inflammatory factors may be potentially useful biomarkers for CAD.

**TL1A and DcR3 levels and SYNTAX score**

In the present study, we found a positive correlation between plasma TL1A levels and SYNTAX score \( (P=0.022) \). TL1A levels have previously only been correlated with Gensini score,25 and to the best of our knowledge, this is the first study to demonstrate a correlation with SYNTAX score.

In contrast, although circulating DcR3 levels have previously been shown to correlate with SYNTAX score,12 the present study found no such correlation, and further studies with larger sample sizes are therefore needed to clarify this relationship.

Acute symptoms of CAD usually occur as a result of thrombotic reactions caused by the rupture of unstable plaques.21,26 The stability of atherosclerotic plaques is maintained by a fibrous cap composed of vascular smooth muscle cells and extracellular matrix molecules. However, TL1A can promote the synthesis of matrix metalloproteinases 1, 9, and 13 in monocytes via combining with DR3.27 These matrix metalloproteinases may then degrade the fibrous cap, leading to plaque rupture and consequently acute symptoms. This mechanism may explain the positive correlation between TL1A levels and SYNTAX score.

**Clinical significance**

The current results clarify the mechanism underlying the pathogenesis of CAD. Plasma, which can be easily obtained from patients in a clinical setting, reflects processes occurring in anatomic compartments,28 as recently indicated by plasma protein changes in CABG patients.29 Our results suggest that plasma levels of TL1A and DcR3 may represent promising new and convenient diagnostic indicators for the existence and severity of CAD, as well as offering potential pharmacological targets in patients with CAD.

**Limitations of the study**

Although plasma levels of TL1A and DcR3 were shown to be elevated in CAD patients in the present study, the study also had some limitations. To reduce possible technical errors, we used the same ELISA kit for the samples from both CAD patients and control, non-CAD subjects. However, the kit hosted a maximum of 80 samples, thus limiting the sample size. Further studies with larger sample sizes may therefore be required to validate these results. Nevertheless, the present study clearly demonstrated correlations between these two
proteins and severe CAD requiring CABG, as well as showing a correlation between plasma TL1A levels and SYNTAX score.

**Conclusion**

In summary, the present study demonstrated that CAD patients requiring CABG have high circulating levels of both TL1A and DcR3, and that plasma levels of TL1A are correlated with SYNTAX score. Circulating levels of TL1A and DcR3 may thus be useful biomarkers for diagnosing severe CAD. Furthermore, TL1A levels may be developed as an indicator of the severity of CAD.

**Declaration of conflicting interest**

The authors declare that there is no conflict of interest.

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