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Wei Gao

Zhongshan Hospital Fudan University

Runda Wu

Zhongshan Hospital Fudan University

Jiasheng Yin

Zhongshan Hospital Fudan University

Yuanji Ma

Zhongshan Hospital Fudan University

Junjie Guo

Affiliated Hospital of Qingdao University

Xin Zhong

Zhongshan Hospital Fudan University

Dong Huang

Zhongshan Hospital Fudan University

Junbo Ge (✉ jbge@zs-hospital.sh.cn)

Zhongshan Hospital Fudan University <https://orcid.org/0000-0002-9360-7332>

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Expression and predictive value of leucine rich α -2 glycoprotein in ischemic heart disease

Wei Gao^{1,*}, Runda Wu^{1,*}, Jiasheng Yin, ^{1,*}, Yuanji Ma^{1,*}, Junjie Guo², Xin Zhong¹, Dong Huang¹, Junbo Ge¹

1. Department of Cardiology, Zhongshan Hospital, Fudan University, Shanghai Institute of Cardiovascular Diseases, Shanghai, China

2. Department of Cardiology, Affiliated Hospital of Qingdao University, Shandong, China.

*These authors contributed equally to this work.

Correspondence to:

Junbo Ge, MD, FESC, FACC, Professor of Medicine/Cardiology

Department of Cardiology, Zhongshan Hospital, Fudan University, Shanghai Institute of Cardiovascular Diseases

180 Fenglin Road, Shanghai 200032, China

Tel: 86-21-64041990

Fax: 86-21-64437078

Email: jbge@zs-hospital.sh.cn

Abstract

Background: The expression and predictive value of leucine rich α -2 glycoprotein (LRG) in ischemic heart disease, including coronary artery disease (CAD), myocardial infarction (MI) and chronic total occlusion (CTO), remain unclear.

Methods: A total of 231 patients were included and divided into 4 groups: control group (N=39), CAD group (N=73), MI group (N=67) and CTO group (N=52). LRG was determined and compared within groups. MI group was then divided into 2 subgroups according to LRG expression. The association between LRG expression and clinical characteristics, peak Troponin T (TNT), left ventricular ejection fraction (LVEF) and in-hospital stay was investigated.

Results: LRG expression in MI group was significantly higher than that in control group (37.9 ± 12.8 vs 25.3 ± 8.9 , $P < 0.001$). LRG expression in CAD and CTO group were similar with that in control group (CAD vs Con, 23.1 ± 6.8 vs 25.3 ± 8.9 , $P = 0.233$; CTO vs Con, 22.0 ± 7.8 vs 25.3 ± 8.9 , $P = 0.082$). In MI group, patients with increased LRG had higher peak TNT (4.80 (2.34 - 10.0) vs 2.46 (0.89 - 4.98), $P = 0.002$), lower LVEF (48.7 ± 10.2 vs 54.8 ± 8.4 , $P = 0.009$) and longer in-hospital stay (10.4 ± 5.4 vs 8.0 ± 2.9 , $P = 0.027$). Linear regression showed that LRG was independently associated with in-hospital stay. ROC curve showed the area of LRG under the curve (AUC) was 0.859.

Conclusions: LRG expression was elevated in MI patients compared with controls, angiography confirmed CAD and CTO patients. In the MI subgroup, patients with elevated LRG had higher peak TNT, lower LVEF and longer in-hospital stay. Whether LRG is associated with major adverse cardiovascular events and long term prognosis needs further investigation.

Key Words

leucine rich α -2 glycoprotein; coronary artery disease; myocardial infarction; chronic total occlusion

Background

Cardiovascular disease is the leading cause of death and disability worldwide. Coronary artery disease (CAD) with severe stenosis, myocardial infarction (MI) and chronic total occlusion (CTO) are different stages and represent different pathophysiological processes. MI leads to intense inflammation, which plays a role in recovery and remodeling [1]. Although advances in early reperfusion therapies reduced mortality, reperfusion of the myocardial tissue might be hindered by dysfunction of the microcirculation [2]. Chronic total coronary occlusion generally results to good collateral circulation, which improve cellular function and global myocardial performance [3].

leucine rich α -2 glycoprotein (LRG) was initially purified from healthy individuals and subsequent studies have shown that LRG expression increases in response to mediators of acute phase response [4]. It was found to be elevated in patients with autoimmune diseases such as RA, Crohn's disease and ulcerative colitis [5, 6]. Then LRG was demonstrated to promote angiogenesis by modulating endothelial TGF- β signaling [7]. LRG levels are also increased in patients with heart failure [8]. In our previous study, we found LRG was elevated in MI patients compared with healthy controls [9]. However, the prognostic value of LRG in MI patients and its expression in CAD and CTO patients has not been investigated.

In this study, we detected serum LRG expression in controls, CAD patients, MI patients and CTO patients. We found that LRG was elevated in MI patients compared with controls, CAD and CTO patients. The differences of expression of LRG in controls, CAD and CTO patients were not significant. In MI subgroup, patients with elevated LRG had higher peak TNT, lower EF and longer in-hospital stay.

Methods

Study design and patient selection

A total of 231 patients were included in this prospective study. Of these patients, 112 received angiography due to suspected coronary artery disease. After angiography, they were divided into 2 groups: control group (39 patients, 3 main vessels stenosis <50%) and CAD group (73 patients, at least 1 main vessel stenosis $\geq$ 50%). The MI group included 67 patients (48 STEMI and 19 NSTEMI) who were diagnosed with myocardial infarction according to the fourth universal definition of myocardial infarction [10]. The CTO group included 52 patients who had been confirmed as CTO by previous angiography and were hospitalized for interventional therapy.

Laboratory assays and Clinical end points

A venous blood sample was collected for all patients upon admission (within 24 hours) for routine laboratory assays performed by central laboratory at our hospital. Troponin T (TNT) of MI patients was determined by Roche assays upon admission and then every morning in CCU until they began to reduce from peak value. Another 2 ml venous blood sample was collected and centrifuged at 1,500 g for 10 min to precipitate blood cells. The serum was then frozen at -80°C until required for LRG analysis. LRG was determined by Elisa kit purchased from Raybiotech (GA, USA). Echocardiography for MI patients was performed within 24 hours of admission and left ventricular ejection fraction (LVEF) was determined.

Statistical analysis

Normally distributed data are expressed as mean $\pm$ SD, and were compared using the Student's t-test (2 groups) or 1-way ANOVA (n groups). LSD method was used for comparison among

specific 2 groups. Skewed variables are expressed as median and inter quartile range and Mann-Whitney U test was used. Categorical data are expressed as number (percentage) and were compared using the chi-square test. Receiver operating characteristic (ROC) curve and area under the curve (AUC) were used to evaluate predictive value for MI occurrence. Linear regression was used to evaluate association between LRG and other factors. All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). A value of $P < 0.05$ was considered as statistically significant.

Results

The baseline characteristics of the 4 groups are shown in Table 1. Patients in CAD and MI group were older than control and CTO group. The percentages of male, diabetes mellitus (DM), hypertension and percutaneous coronary intervention (PCI) history were significantly lower in control group. LRG expression in MI group was significantly higher than that in control group (37.9 ± 12.8 vs 25.3 ± 8.9 , $P < 0.001$). LRG expression in CAD and CTO group were similar with that in control group (CAD vs Con, 23.1 ± 6.8 vs 25.3 ± 8.9 , $P = 0.233$; CTO vs Con, 22.0 ± 7.8 vs 25.3 ± 8.9 , $P = 0.082$) (shown in Figure 1).

We divided the MI patients into 2 groups according to LRG expression and their characteristics are shown in Table 2. There were no differences of age, male, DM, hypertension and PCI history between the 2 groups. Patients with increased LRG had higher peak TNT (4.80 (2.34 - 10.0) vs 2.46 (0.89 - 4.98), $P = 0.002$), lower LVEF (48.7 ± 10.2 vs 54.8 ± 8.4 , $P = 0.009$) and longer in-hospital stay (10.4 ± 5.4 vs 8.0 ± 2.9 , $P = 0.027$).

Linear regression showed that LRG was independently associated with in-hospital stay (Table 3). LRG was not independently associated with other factors, including gender, age, DM, Hypertension, PCI history LVEF and peak TNT. We plotted ROC curve to evaluate the ability of LRG to predict MI. As shown in Figure 2, the area of LRG under the curve (AUC) was 0.859. The best cut off value was 28.3ug/mL with the biggest sum of sensitivity and specificity (0.826 and 0.795, respectively).

Discussion

In this study, LRG expression was demonstrated to be elevated in MI patients compared with controls, angiography confirmed CAD and CTO patients. There was no significant difference of LRG expression in controls, angiography confirmed CAD and CTO patients. In the MI subgroup, patients with elevated LRG had higher peak TNT, lower LVEF and longer in-hospital stay. Linear regression analysis revealed an independent association between LRG and in-hospital stay.

LRG belongs to the leucine-rich repeat (LRR) family and is expressed during granulocyte differentiation [10]. LRG plays important roles in protein-protein interaction, signaling, and cell adhesion [11]. Then it was found to increase in response to mediators of acute phase response [4] and promote angiogenesis [7]. Thus LRG level was elevated in inflammatory diseases [12] and some cancers [13-15]. Recent study also found elevation of LRG is associated with arterial stiffness, endothelial dysfunction, peripheral vascular disease and CAD [16-18].

Myocardial infarction leads to comprehensive pathophysiological process, including intense inflammation and angiogenesis [19]. In our previous study [9], we performed a proteomics research in early-onset myocardial infarction patients using iTRAQ coupled with LC-MS/MS. Among the 538 proteins identified and quantified, LRG was up-regulated in the early-onset MI

group compared with healthy controls. During the validation stage, LRG was confirmed to be elevated in MI group. We also found that LRG was positively correlated with C-reactive protein. In this current study, we further compared LRG expression in angiography confirmed CAD and MI patients with non-CAD controls. The result of LRG expression in MI patients compared with control and CAD patients was similar with the previous study.

The elevated LRG expression might promote angiogenesis at border zone. A previous study showed that angiogenesis and cardiac function were impaired in LRG-deficient mice. Prognosis was improved by transplantation of bone marrow cells, which can produce LRG protein [20]. However, the predictive value of LRG in MI patients has not been investigated. We divided the MI patients into 2 groups according to LRG expression and found higher LRG group had increased peak TNT, decreased EF and longer in-hospital stay. Further analysis revealed an independent association between LRG level and in-hospital stay. Future studies investigating predictive value for clinical events are warranted. It sounds inconsistent that LRG promote angiogenesis while, at the same time, correlates with worse cardiac function and longer in-hospital stay. We think it might be just like BNP, which is associated with heart failure and able to act as a therapeutic agent. Whether administration of LRG can improve prognosis needs to be studied.

CTO is defined as an atherosclerotic complete vessel occlusion with Thrombolysis in Myocardial Infarction (TIMI) grade 0 flow within the occluded segment for more than 3 months. Its prevalence ranges from 30-50% [21] and around 50% CTO patients had MI history [22]. There is no evidence of association between CTO and inflammation. The most distinct character of CTO is coronary collateralization (including arteriogenesis and angiogenesis), which can improve prognosis [23]. The coronary collateralization of CTO patients are influenced by some factors, such as cytokines, miRNAs and diabetes [24]. In this study, we for the first time investigated LRG expression in CTO patients and found no difference between CTO patients and control/CAD patients.

Study limitations

The present study has several limitations. The sample size was relatively small and we did not investigate the major adverse cardiovascular events (MACE), such as cardiac death, non-fatal acute myocardial infarction, revascularization and stroke. The MACE rate was quite low during in-hospital follow up. The study observation time was limited to in-hospital stay; therefore, analysis of long-term prognosis is warranted.

Conclusions

In this study, LRG expression was demonstrated to be elevated in MI patients compared with controls, angiography confirmed CAD and CTO patients. There was no significant difference of LRG expression in controls, angiography confirmed CAD and CTO patients. In the MI subgroup, patients with elevated LRG had higher peak TNT, lower LVEF and longer in-hospital stay. Linear regression analysis revealed an independent association between LRG and in-hospital stay. Whether LRG is associated with major adverse cardiovascular events and long term prognosis needs further investigation.

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Availability of data and materials

All data are freely available for scientific purpose. The data can be found from authors.

Authors' contributions

WG, RW, JY and YM designed the study and performed the research. WG and RW wrote the manuscript. JG, XZ and DH performed the research. JG designed and supervised the study.

Ethics approval and consent to participate

Informed consent was obtained from all patients and this study was approved by the Ethics Committee of Zhongshan Hospital Fudan University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Prabhu SD, Frangogiannis NG. The Biological Basis for Cardiac Repair After Myocardial Infarction: From Inflammation to Fibrosis. *Circ Res*. 2016;119:91-112.
2. Badimon L, Borrell M. Microvasculature Recovery by Angiogenesis After Myocardial Infarction. *Curr Pharm Des*. 2018;24:2967-2973.
3. McEntegart MB, Badar AA, Ahmad FA, Shaukat A, MacPherson M, et al. The collateral circulation of coronary chronic total occlusions. *EuroIntervention*. 2016;11:e1596-1603.
4. Shirai R, Hirano F, Ohkura N, Ikeda K, Inoue S. Up-regulation of the expression of leucine-rich alpha(2)-glycoprotein in hepatocytes by the mediators of acute-phase response. *Biochem Biophys Res Commun*. 2009;382:776-779.
5. Serada S, Fujimoto M, Terabe F, Iijima H, Shinzaki S, et al. Serum leucine-rich alpha-2 glycoprotein is a disease activity biomarker in ulcerative colitis. *Inflamm Bowel Dis*. 2012;18:2169-2179.
6. Serada S, Fujimoto M, Ogata A, Terabe F, Hirano T, et al. iTRAQ-based proteomic identification of leucine-rich alpha-2 glycoprotein as a novel inflammatory biomarker in autoimmune diseases. *Ann Rheum Dis*. 2010;69:770-774.
7. Wang X, Abraham S, McKenzie JAG, Jeffs N, Swire M, et al. LRG1 promotes angiogenesis by modulating endothelial TGF-beta signalling. *Nature*. 2013;499:306-311.
8. Watson CJ, Ledwidge MT, Phelan D, Collier P, Byrne JC, et al. Proteomic analysis of coronary sinus serum reveals leucine-rich alpha2-glycoprotein as a novel biomarker of ventricular dysfunction and heart failure. *Circ Heart Fail*. 2011;4:188-197.

9. Xuan C, Li H, Li LL, Tian QW, Wang Q, et al. Screening and Identification of Pregnancy Zone Protein and Leucine-Rich Alpha-2-Glycoprotein as Potential Serum Biomarkers for Early-Onset Myocardial Infarction using Protein Profile Analysis. *Proteomics Clin Appl*. 2019;13:e1800079.
10. O'Donnell LC, Druhan LJ, Avalos BR. Molecular characterization and expression analysis of leucine-rich alpha2-glycoprotein, a novel marker of granulocytic differentiation. *J Leukoc Biol*. 2002;72:478-485.
11. Takahashi N, Takahashi Y, Putnam FW. Periodicity of leucine and tandem repetition of a 24-amino acid segment in the primary structure of leucine-rich alpha 2-glycoprotein of human serum. *Proc Natl Acad Sci U S A*. 1985;82:1906-1910.
12. Naka T, Fujimoto M. LRG is a novel inflammatory marker clinically useful for the evaluation of disease activity in rheumatoid arthritis and inflammatory bowel disease. *Immunol Med*. 2018;41:62-67.
13. Zhang Q, Huang R, Tang Q, Yu Y, Huang Q, et al. Leucine-rich alpha-2-glycoprotein-1 is up-regulated in colorectal cancer and is a tumor promoter. *Onco Targets Ther*. 2018;11:2745-2752.
14. Yamamoto M, Takahashi T, Serada S, Sugase T, Tanaka K, et al. Overexpression of leucine-rich alpha2-glycoprotein-1 is a prognostic marker and enhances tumor migration in gastric cancer. *Cancer Sci*. 2017;108:2052-2060.
15. Linden M, Lind SB, Mayrhofer C, Segersten U, Wester K, et al. Proteomic analysis of urinary biomarker candidates for nonmuscle invasive bladder cancer. *Proteomics*. 2012;12:135-144.
16. Pek SL, Tavintharan S, Wang X, Lim SC, Woon K, et al. Elevation of a novel angiogenic factor, leucine-rich-alpha2-glycoprotein (LRG1), is associated with arterial stiffness, endothelial dysfunction, and peripheral arterial disease in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2015;100:1586-1593.
17. Bos S, Phillips M, Watts GF, Verhoeven AJM, Sijbrands EJG, et al. Novel protein biomarkers associated with coronary artery disease in statin-treated patients with familial hypercholesterolemia. *J Clin Lipidol*. 2017;11(3):682-693.
18. Yin X, Subramanian S, Hwang SJ, O'Donnell CJ, Fox CS, Courchesne P, et al. Protein biomarkers of new-onset cardiovascular disease: prospective study from the systems approach to biomarker research in cardiovascular disease initiative. *Arterioscler Thromb Vasc Biol*. 2014;34:939-945.
19. Frangogiannis NG. Pathophysiology of Myocardial Infarction. *Compr Physiol*. 2015;5:1841-1875.
20. Kumagai S, Nakayama H, Fujimoto M, Honda H, Serada S, et al. Myeloid cell-derived LRG attenuates adverse cardiac remodelling after myocardial infarction. *Cardiovasc Res*. 2016;109:272-282.
21. Koelbl CO, Nedeljkovic ZS, Jacobs AK. Coronary Chronic Total Occlusion (CTO): A Review. *Rev Cardiovasc Med*. 2018;19:33-39.
22. Han YL, Zhang J, Jing QM, Wang SL, Ma YY, et al. [Coronary intervention in 1148 patients with chronic total coronary occlusion]. *Zhonghua Xin Xue Guan Bing Za Zhi*. 2005;33:299-302.
23. Vo MN, Brilakis ES, Kass M, Ravandi A. Physiologic significance of coronary collaterals in chronic total occlusions. *Can J Physiol Pharmacol*. 2015;93:867-871.
24. Shen Y, Ding FH, Dai Y, Wang XQ, Zhang RY, et al. Reduced coronary collateralization in type 2 diabetic patients with chronic total occlusion. *Cardiovasc Diabetol*. 2018;17:26.

Table 1 Clinical characteristics and LRG expression of the 4 groups

	Con (N=39)	CAD (N=73)	MI (N=67)	CTO (N=52)	P value*
Age	59.5±9.3	66.3±10.8	64.9±11.3	57.5±12.7	<0.001
Male	12 (30.8%)	57 (78.1%)	56 (92.9%)	48 (71.6%)	<0.001
DM	3 (7.7%)	23 (31.5%)	18 (26.9%)	18 (26.9%)	0.019
Hypertension	8 (20.5%)	42 (57.5%)	32 (47.8%)	31 (46.3%)	<0.001
PCI history	2 (5.1%)	22 (30.1%)	8 (11.5%)	33 (49.3%)	<0.001
LRG (ug/ml)	25.3±8.9	23.1±6.8	37.9±12.8	22.0±7.8	<0.001 [#]

Con: control, CAD: coronary artery disease, MI: myocardial infarction, CTO: chronic total occlusion, DM: diabetes mellitus, PCI: percutaneous coronary intervention, LRG: leucine rich α -2 glycoprotein

* Normally distributed data are expressed as mean $\pm$ SD and compared using 1-way ANOVA.

[#] The P value was calculated by 1-way ANOVA. P value of CAD vs Con, MI vs Con and CTO vs Con was 0.233, <0.001 and 0.082, respectively. LSD method was used for comparison among specific 2 groups.

Table 2 Clinical characteristics in MI group divided into 2 groups according to LRG expression

	LRG<34.81 (N=33)	LRG $\geq$ 34.81 (N=34)	P value
Age	62.4±10.7	67.4±11.4	0.071
Male	28 (84.8%)	28 (82.4%)	0.783
DM	11 (33.3%)	7 (20.6%)	0.239
Hypertension	15 (45.5%)	17 (50%)	0.710
PCI history	5 (15.2%)	3 (8.8%)	0.425
Peak TNT (ng/ml)*	2.46 (0.89-4.98)	4.80 (2.34-10.0)	0.002
LVEF (%)	54.8±8.4	48.7±10.2	0.009
In-hospital stay (days)	8.0±2.9	10.4±5.4	0.027
LRG (ug/ml)	29.0±4.6	46.4±12.4	<0.001

LRG: leucine rich α -2 glycoprotein, DM: diabetes mellitus, PCI: percutaneous coronary intervention, TNT: Troponin T, LVEF: left ventricular ejection fraction

*Peak TNT was skewed variable and expressed as median and inter quartile range. Mann-Whitney U test was used for statistical analysis.

Table 3 Linear regression analysis LRG and other factors

	B	95% CI	P value
Gender	0.132	-8.619-8.883	0.976
Age	0.198	-0.098-0.494	0.186
DM	-1.235	-8.772-6.253	0.742
Hypertension	4.994	-1.219-11.208	0.113
PCI history	0.723	-9.659-11.105	0.890
Peak TNT	0.681	-0.409-1.771	0.216
LVEF	0.063	-0.320-0.446	0.743
In-hospital stay	0.813	0.029-1.597	0.042

DM: diabetes mellitus, PCI: percutaneous coronary intervention, TNT: Troponin T,
LVEF: left ventricular ejection fraction

Fig. 1

Serum LRG expression in control, CAD, MI and CTO groups. LRG expression in MI group was significantly higher than that in control group (37.9 ± 12.8 vs 25.3 ± 8.9 , $P < 0.001$). LRG expression in CAD and CTO group were similar with that in control group (CAD vs Con, 23.1 ± 6.8 vs 25.3 ± 8.9 , $P = 0.233$; CTO vs Con, 22.0 ± 7.8 vs 25.3 ± 8.9 , $P = 0.082$).

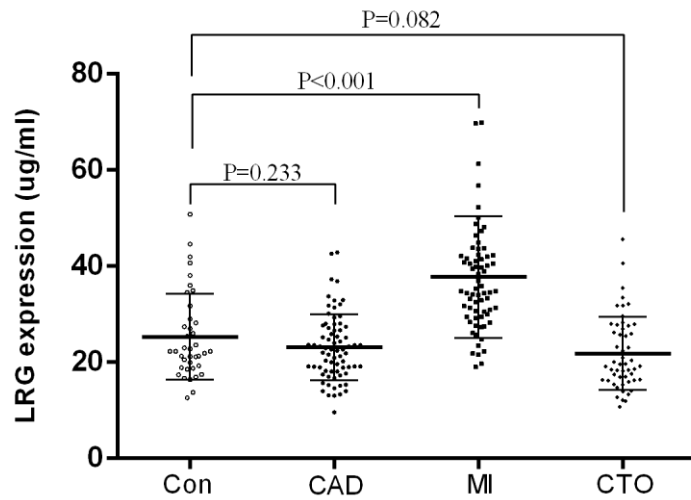
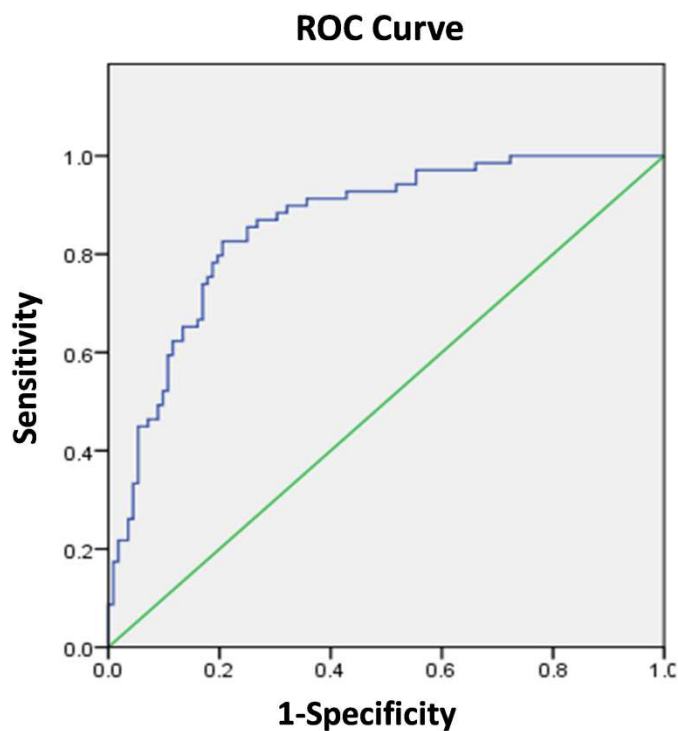


Fig. 2

ROC curve of LRG to predict MI. The area of LRG under the curve (AUC) was 0.859. The best cut off value was $28.3 \mu\text{g/mL}$ with the biggest sum of sensitivity and specificity (0.826 and 0.795, respectively).



Figures

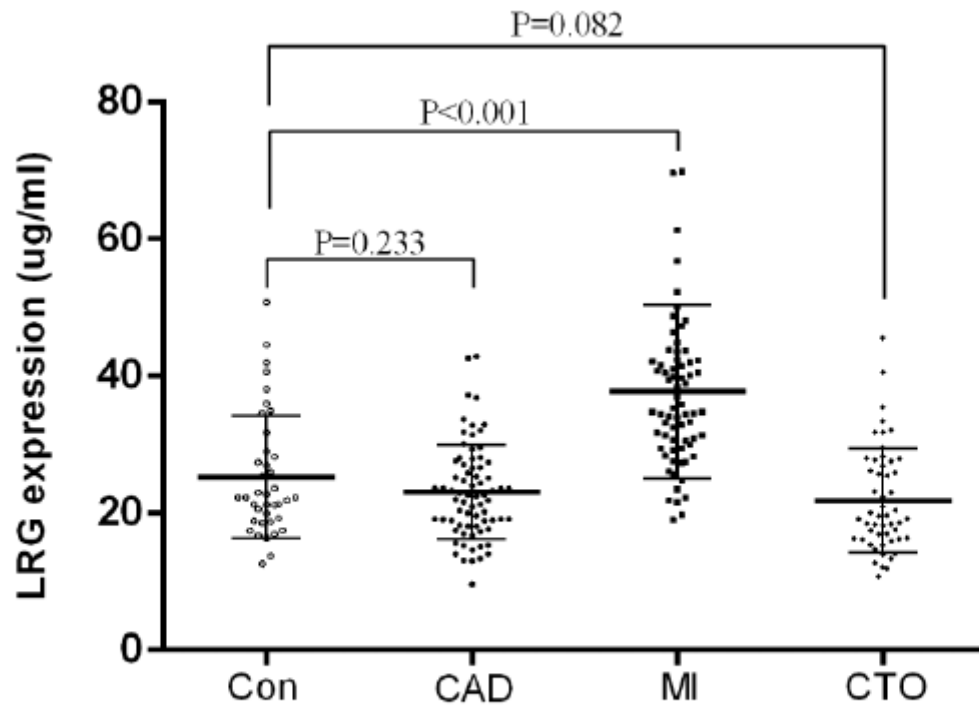


Figure 1

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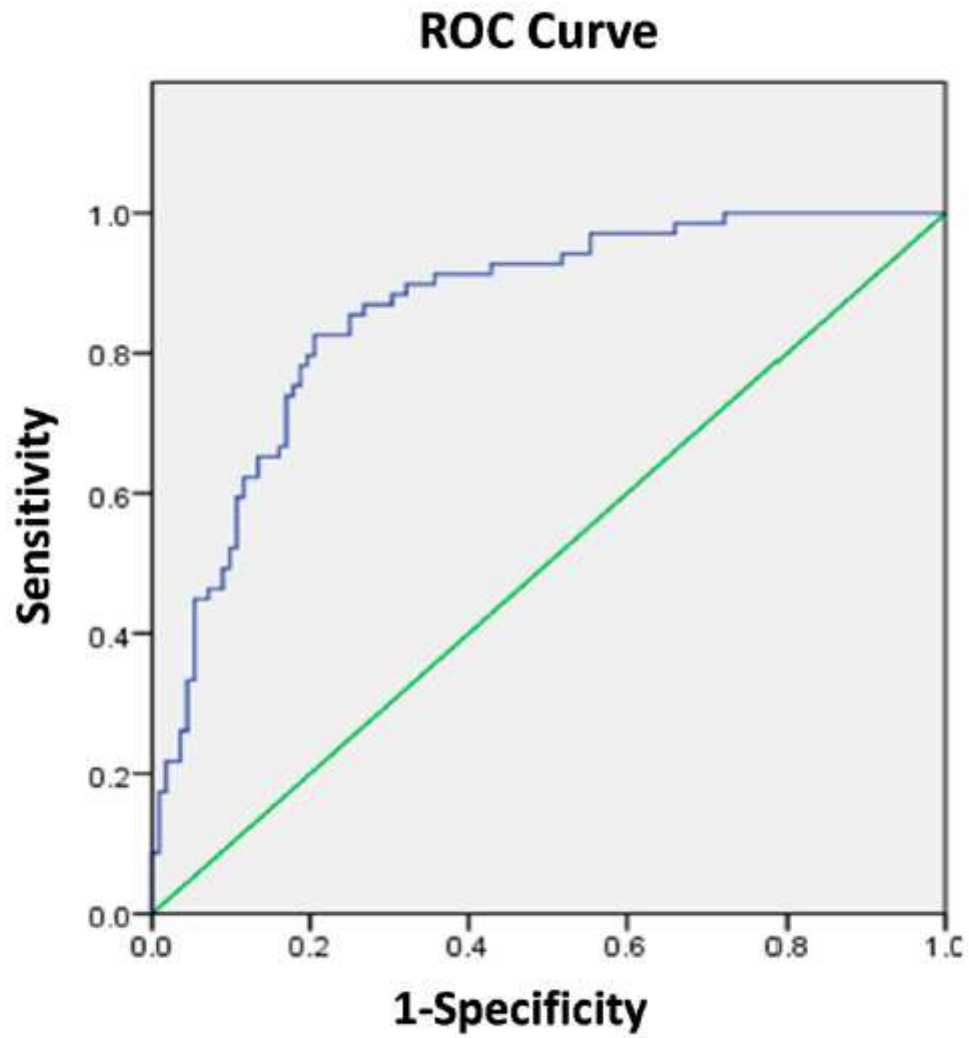


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