Novel biomarker of Cysteine-rich angiogenic inducer 61 (Cyr61) predicts long-term outcome of ST-elevation myocardial infarction

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Research article

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Abstract

Background: Cysteine-rich angiogenic inducer 61 (Cyr61) is a matricellular protein participating in the angiogenesis, inflammation, and fibrotic tissue repair. Previous study has proven its value in diagnosing and risk stratification of ST-elevation myocardial infarction (STEMI). However, there is no study focusing on Cyr61 and the long-term outcome of STEMI. Methods: A total of 426 patients diagnosed with STEMI were enrolled in this study. Blood sample was acquired 24 hours after the admission. The patients were required long-term follow-up after the discharge, when primary endpoint of all-cause death and secondary endpoint of cardiac complications were observed. Cox hazard ratio model and survival analysis were used to compare the risk of patients with higher level and lower level of Cyr61. Results: We conducted an average of (48.4 ± 17.8) months of follow-up, during which a total of 28 deaths happened (6.6%), while 106 episodes of secondary endpoints occurred (24.9%). Patients with higher quartile (Q4) Cyr61 were at higher risk of death [HR 3.404 95%CI (1.574-7.360), P<0.001] when compared with lower three quartiles (Q1-Q3) Cyr61. In terms of secondary endpoints, patients with Q4 Cyr61 were subject to 4.718 [95%CI (3.189-6.978) , P<0.001] times of risk compared with Q1-Q3 Cyr61. Conclusions: For STEMI Patients, those with increased Cyr61 have higher risk of all-cause death and cardiac complications. Therefore, Cyr61 may be a useful tool in predicting the long-term prognosis of STEMI.

Background

Recent years have witnessed a substantial progress in the diagnosis and treatment of cardiovascular disease (CVD), especially the ST- segment elevation myocardial infarction (STEMI)(1). It is reported that the STEMI accounts for more than 30% of all death (the most common single death cause) and greatly increases the familial and social health burden(2). Despite of the increased successful treatment rates of STEMI patients, they are still confronted with worse prognosis after the incidence of STEMI, including heart failure, recurrence of STEMI, and other complications(3). Multiple tools have been developed to evaluate the risk and predict the prognosis of patients with STEMI, including comprehensive score system and biomarkers, such as SYNTAX score(4), glycated hemoglobin(5), Nardilysin(6) and other biomarkers. However, relative less accuracy and focus on early prognosis have limited the application of those biomarkers and risk stratification, which necessitates more studies to evaluate the value of novel biomarkers in predicting the long-term prognosis of STEMI.

A recent study have demonstrated the great value of Cysteine-rich angiogenic inducer 61 (Cyr61), which is a novel biomarker mostly studied in cancer(7-9), in reflecting myocardial injury severity that improves risk stratification in acute coronary syndrome (ACS) patients(10). As a member of the CCN family of matricellular proteins exerting critical functions in angiogenesis, inflammation, and fibrotic tissue repair(11), Cyr61 is also involved in pathophysiological process of cardiovascular disease(12). It is supposed that Cyr61 participates in the initial stages of ACS reflecting myocardial injury, hence it might be related to early risk of ACS. However, the relationship between serum Cyr61 level and long-term outcome of STEMI has never been investigated.

Methods

Patient

This is a prospective observational cohort study, and patients meeting the following criteria and admitted in the department of cardiology of our hospital were enrolled in this study. Entry criteria were listed as follows: 1. The patient must be adult; 2. The patient must be examined with DSA and diagnosed with STEMI; 3. The patient must receive revascularization treatment; 4. The patient must not die in the hospital due to STEMI; 5. The patient and family must be aware of the study and willing to participate. Exclusion criteria: 1. Patients diagnosed with NSTEMI or unstable angina; 2. Patients in coma or unconsciousness condition; 3. Patients and family cannot cooperate or do not want to cooperate with the follow-up. According to the 2017 ESC Guidelines for the Management of STEMI, STEMI was defined as electrocardiographic ST-segment elevation ≥2 mm in 2 or more contiguous chest leads or ≥1 mm in 2 or more limb leads or new onset of left bundle-branch block, together with chest pain or other typical symptoms and elevated troponin levels >99th percentile(13).

From Jan 2014 to Jan 2016, a total of 426 STEMI patients were enrolled into the prospective observational cohort study and their blood sample was acquired. All patients and their family had learned about this study and signed informed consent. This study was also approved by the Medical Ethics Committee (the First Affiliated Hospital of Chongqing Medical University). Clinical data were collected from the electric database of the hospital. Routine myocardial injury markers including cTnT as well as CK-MB, and other laboratory indicators including Creatinine, NT-proBNP, hsCRP were also tested.

Sample collection and Cyr61 test

Venous blood sample was acquired at a median of 24 hours after symptom onset and 18 hours after the PCI procedure between 8:00 and 10:00 AM the following morning, which was centrifuged for 10 mins at 2000rpm. Serum sample was stored at -20℃ refrigerator for future study. Human Cyr61/CCN1 Quantikine ELISA Kit (Catalog # DCYR10, R&D Systems, Inc, MN, USA) was used to quantify the serum Cyr61 level according to the manufacturer's protocol. The absorbance value was measured on a microplate reader set at a wavelength of 540nm. Standard curve was plotted to get the absolute value of Cyr61 by comparing to the standard sample(9).

Clinical endpoints

Primary endpoint was defined as all-cause mortality during the follow-up, while secondary endpoint was defined as a composite if adverse events including myocardial infarction, stroke, unscheduled revascularization, or rehospitalization for heart failure.

Follow-up

All enrolled patients were given follow-up by telephone or clinics every year and for a total of 5 years. Follow-up content included survival condition, recent readmission and other complications. Self-dropout or miss contact was considered as censored data.

Statistical analysis

IBM SPSS Statistics, version 19.0 (SPSS, Inc, Armonk, NY) was chosen for statistical analysis in this study. Normality distribution test of the variables was conducted to check the variables distribution condition. Continuous variables meeting the normal distribution were presented as mean ± standard deviations and categorical variables were presented as proportions, while continuous variables unfitting the normal distribution were described as median and interquartile range (IQR). Comparison of continuous variable of different groups was conducted with One-way ANOVA and Tukey's multiple comparisons test of independent samples. Kruskal-Wallis test and Dunn's multiple comparisons test was adopted for the comparison of different groups in non-normal variables of independent samples. Chi-square test were performed in different evaluations of categorical variables.

According to the serum level of Cyr61, patients were divided into four quartiles of Q1, Q2, Q3, Q4 group respectively. Cox hazard ratios (HR) model was adopted as the regression method to compare the relative hazard between patients with Q4 Cyr61and patients with Q1-Q3 Cyr61. Univariate analysis between covariates and endpoints was conducted, when covariates with P value <0.10 was entered into the multivariate analysis. Kaplan-meier survival curves and log rank tests were used to compare the survival status of patients in Q4 and Q1 to Q3. A p-value less than 0.05 was considered statistically significant.

Results

Patient characteristics

Among 426 patients enrolled in this study, a total of 372 patients completed the full term of follow-up, and the average follow-up time was (48.4 ± 17.8) months. During the follow-up, 28 patients died (6.6%), while 106 patients developed secondary endpoints (24.9%). Baseline characteristics and clinical data of patients without endpoints, primary endpoints and secondary endpoints were compared, showed in Table 1. For demographics, patients with primary endpoints or secondary endpoints had significantly higher age (P=0.004), while there was no statistical difference among three groups in gender, BMI and smoking rates (All P>0.05). For other clinical indicators and comorbidities, patients with primary endpoints or secondary endpoints demonstrated higher NYHA grade (P=0.011), lower LVEF (P=0.030), and higher hypertension rates (P=0.023). In terms of laboratory test, only NT-proBNP (P= 0.006) and hsCRP (P<0.001) showed significant difference among three groups.

Serum Cyr61 level

All patients were tested of serum Cyr61 level and comparison of three groups were showed in Figure 1. The serum Cyr61 level of patients without endpoints, primary endpoints and secondary endpoints were 586.5 (327.8, 864), 1014 (519.5, 1628), 1063 (667.3, 1300) ng/L respectively. Cyr61 level of primary endpoints and secondary endpoints were both significantly higher than patients without endpoints ($P<0.001$), while there was no difference between those two groups ($P>0.999$).

Primary endpoints

Showed in Table 2, univariate Cox regression analysis between covariates and primary endpoint was conducted to screen the risk factors, which found that age, smoking, NYHA level, LVEF, heart failure, hypertension, NT-proBNP and hsCRP were associated with primary endpoint, which were used to adjust the HR in multivariate Cox hazard regression analysis. Multivariate regression analysis was further conducted to analyze the hazard ratio of primary endpoint for patient with Q4 compared with Q1-3 Cyr61 level, which was demonstrated in Table 3. The results showed that patient with Q4 Cyr61 level were subjected to 3.404 95%CI(1.574-7.360) times risk of death. Survival curve (Figure 2) also presented the significantly different survival condition between patients with Q4 and Q1-3 Cyr61 level ($P<0.001$).

Secondary endpoints

We analyzed the hazard ratios of developing secondary endpoints for patient with Q4 compared with Q1-3 Cyr61 level, which was demonstrated in Table 3. The results showed that patients with Q4 Cyr61 level were subjected to higher risk of myocardial infarction [4.743 95%CI (2.653-8.477)], stroke [10.911 95%CI (2.108-56.472)], Emergent revascularization [3.245 95%CI (1.678-6.276)] and readmission due to heart failure [7.359 95%CI (2.714-19.949)]. Figure 3 illustrated the survival curve of secondary endpoints between patients with Q4 and Q1-3 Cyr61 level, which also validated that patients with Q4 Cyr61 level had higher rates of suffering secondary endpoints ($P<0.001$).

Discussion

In this study, by measuring the serum Cyr61 level after the incidence of STEMI and conducting a long-term follow-up, we found that the serum Cyr61 level was strongly associated with all-cause death and other adverse events. It is found that STEMI patients who died during follow-up had a significantly higher serum Cyr61 level, and patients with Q4 Cyr61 level were subjected to 3.404 times risk of death and 4.718 times risk of developing adverse events. Other variables were also found to be covariates affecting the

incidence of primary endpoint, such as age, smoking, NYHA level, LVEF, heart failure, hypertension, NT-proBNP and hsCRP.

Cyr61, also named CCN1, belongs to CCN superfamily of matricellular proteins, which exerts critical functions in angiogenesis, inflammation, and fibrotic tissue repair(14). Previous studies have proven the potential of Cyr61 as a biomarker in certain diseases. Terada et al found that Cyr61 expression levels are associated with a lower risk of prostate cancer, and can serve as serum-based biomarker for differentiating lethal and non-lethal prostate cancer(7). Another study demonstrates that CYR61 promotes breast cancer lung metastasis by facilitating tumor cell extravasation and protecting from anoikis during initial seeding to the lung(15). Cyr61 is not only associated with cancer, but also vital in some autoimmune diseases(16, 17). However, the role of Cyr61 in cardiovascular system, especially in CAD has seldomly been explored. Klingenberg and colleagues firstly identified the differentially expressed biomarker of Cyr61 in gene expression profiling, and validated its expression level in ischemia/reperfusion murine models, then they demonstrated that serum Cyr61 improved risk stratification for all-cause mortality when added to the reference GRACE risk score for ACS patients(10). It is found that Cyr61 concentration was the highest in STEMI patients compared with NSTEMI patients and stable ACS patients, meaning that Cyr61 concentration correlated well with the severity of the myocardial infarction, hence might also be well with long-term outcomes.

Several tools have been developed to stratify the risk and predict the long-term outcomes of STEMI. Tarasov and his colleagues evaluate the prognostic value of SYNTAX score in patients with STEMI undergoing PCI, which showed that patients with a SYNTAX score over 23 points has a higher rate of the combined endpoint of death, myocardial infarction, and target vessel revascularization (OR 2.8) as compared with patients with a lower SYNTAX score(4). The SYNTAX scoring system is a unique tool for assessing the complexity of coronary artery disease, including coronary lesion information and demographic characteristics. Our study also found differences in age, BMI, LVEF, hypertension, and some laboratory findings between patients with different endpoints, which correlates well with the SYNTAX scoring system. Other biomarkers have been previously studied to assess their value in assessing long-term risks. He J reports that the neutrophil to lymphocyte ratio (NLR) is a useful and powerful predictor of mortality and adverse outcomes in Chinese STEMI patients(18). Ritschel's study confirmed a close relationship between circulating levels of IL-6 and gp130 and long-term clinical outcomes of STEMI. Compared with the above studies, our study divided the results into primary and secondary endpoints, rather than combining all adverse events, which helped to more accurately illustrate specific relationships(19). Higher serum Cyr61 level indicates a more severe myocardial infarction condition, implying worse long-term outcomes including heart failure, revascularization and recurrent myocardial infarction. Notably, we found that patients with higher Cyr61 level were also at higher risk of stroke, which could be explained by similar atherosclerosis in coronary artery and cerebral artery.

Limitations

Limitations of this study must be noted. Firstly, the 95% confidential interval of endpoints were too wide than expected, which could be attributed to small sample scale. Despite of the significant results, this study still necessitates a large-scale cohort study to consolidate the results before application. Secondly, the sample time of this study was within a period rather than fixed time, which might affect the serum level and the results. Last, although some clinical factors had been included in the Cox regression model to adjust the HR value, other confounding factors might be neglected, such as lesion type, time from STEMI to revascularization.

Conclusions

In conclusion, by observing the long-term clinical outcomes of patients with STEMI stratified by serum Cyr61 level, we can safely come to the conclusions that STEMI Patients with increased Cyr61 have higher risk of all-cause death and cardiac complications. Therefore, Cyr61 may be a useful tool in predicting the long-term prognosis of STEMI.

Declarations

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Tables

Table 1 Demographical characteristics and clinical data of the patients enrolled stratified by the endpoints

Variables	Patients without endpoints (n=292)	Patients with primary endpoint (n=28)	Patients with secondary endpoint (n=106)	P value
Demographics				
Age(y, Mean ± SD)	62.6 ± 15.6	72.0 ± 10.9	64.5 ± 12.0	0.004
Gender(%male)	225 (77.1%)	24 (85.7%)	83 (78.3%)	0.570
BMI(kg/m-2)	24.6 ± 3.2	25.2 ± 2.8	24.2 ± 3.4	0.221
Smoking(%)	108 (37.0%)	5 (17.9%)	41 (38.7%)	0.109
NYHA				0.011
	70 (24.0%)	5 (17.9%)	27 (25.5%)	
	94 (32.2%)	7 (25.0%)	36 (34.0%)	
	98 (33.6%)	6 (21.4%)	32 (30.2%)	
	30 (10.3%)	10 (35.7%)	11 (10.4%)	
LVEF	49.3 ± 9.5	44.2 ± 13.1	49.3 ± 10.0	0.030
Comorbidities				
Heart failure	78 (26.7%)	13 (46.4%)	27 (25.5%)	0.070
Hypertension	70 (24.0%)	13 (46.4%)	33 (31.1%)	0.023
Diabetes mellitus	38 (13.0%)	4 (14.3%)	17 (16.0%)	0.741
Chronic kidney disease	15 (5.1%)	3 (10.7%)	4 (3.8%)	0.336
Chronic lung disease	38 (13.0%)	3 (10.7%)	10 (9.4%)	0.609
Cerebrovascular disease	16 (5.5%)	1 (3.6%)	8 (7.5%)	0.641
Tumor	9 (3.1%)	1 (3.6%)	3 (2.8%)	0.978
Laboratory test at admission				
Peak cTnT (ng/dL)	3.32 (4.82)	5.87 (4.38)	4.43(3.50)	0.223
Peak CK-MB (ng/dL)	555.1 (252.5)	471.9 (268.5)	585.3 (294.3)	0.429
Creatinine (umol/L)	104.0 (57.3)	98.0 (65.8)	93.5 (57.3)	0.244
NT-proBNP (ng/L)	63.3(86.4)	107.2(102.5)	79.2(96.0)	
hsCRP (mg/L)	21.6(17.7)	30.9 (15.9)	25.3(16.6)	<0.001

BMI, Body mass index; NYHA, New York Heart Association; LVEF, left ventricle ejection fraction;

Table 2 Univariate Cox regression analysis between covariates and primary endpoint (Q4 vs Q1 to Q3)

Covariates	HR	95%CI	P value
Age	1.046	1.017-1.076	0.002
Gender(male to female)	1.798	0.624-5.183	0.277
BMI	1.061	0.943-1.194	0.324
Smoking	0.379	0.144-0.997	0.049
NYHA	1.607	1.087-2.377	0.017
LVEF	0.950	0.915-0.987	0.008
Heart failure	2.281	1.085-4.793	0.030
Hypertension	2.534	1.205-5.325	0.014
Diabetes mellitus	1.016	0.353-2.930	0.140
Chronic kidney disease	1.780	0.537-5.897	0.345
Chronic lung disease	0.790	0.238-2.617	0.700
Cerebrovascular disease	0.559	0.076-4.116	0.568
Tumor	1.459	0.198-10.738	0.711
Peak cTnT	1.166	0.962-1.414	0.118
Peak CK-MB	0.998	0.996-1.001	0.205
Creatinine	1.001	0.999-1.004	0.234
NT-proBNP	1.001	1.000-1.003	0.070
hsCRP	1.081	1.038-1.127	<0.001

BMI, Body mass index; NYHA, New York Heart Association; LVEF, left ventricle ejection fraction;

Table 3 Hazard Ratio (HR) of endpoint for patients with Q4 level of Cyr61 compared with Q1 to Q3 level of Cyr61

	Unadjusted HR(95%CI)	P value	Adjusted* HR(95%CI)	P value
Primary endpoint	3.957 (1.881-8.322)	<0.001	3.404 (1.574-7.360)	0.002
Secondary endpoint				
Myocardial infarction	4.743 (2.653-8.477)	<0.001	4.926 (2.720-8.919)	<0.001
Stroke	10.911 (2.108-56.472)	0.004	8.972 (1.711-47.032)	0.009
Emergent revascularization	3.245 (1.678-6.276)	<0.001	3.011 (1.523-5.953)	0.002
Readmission due to heart failure	7.359 (2.714-19.949)	<0.001	8.174 (2.951-22.638)	<0.001
Overall	4.697 (3.203-6.888)	<0.001	4.718 (3.189-6.978)	<0.001

*HR adjusted by covariates of Age, smoking, NYHA, Heart failure, Hypertension, NT-proBNP and hs-CRP

Figures

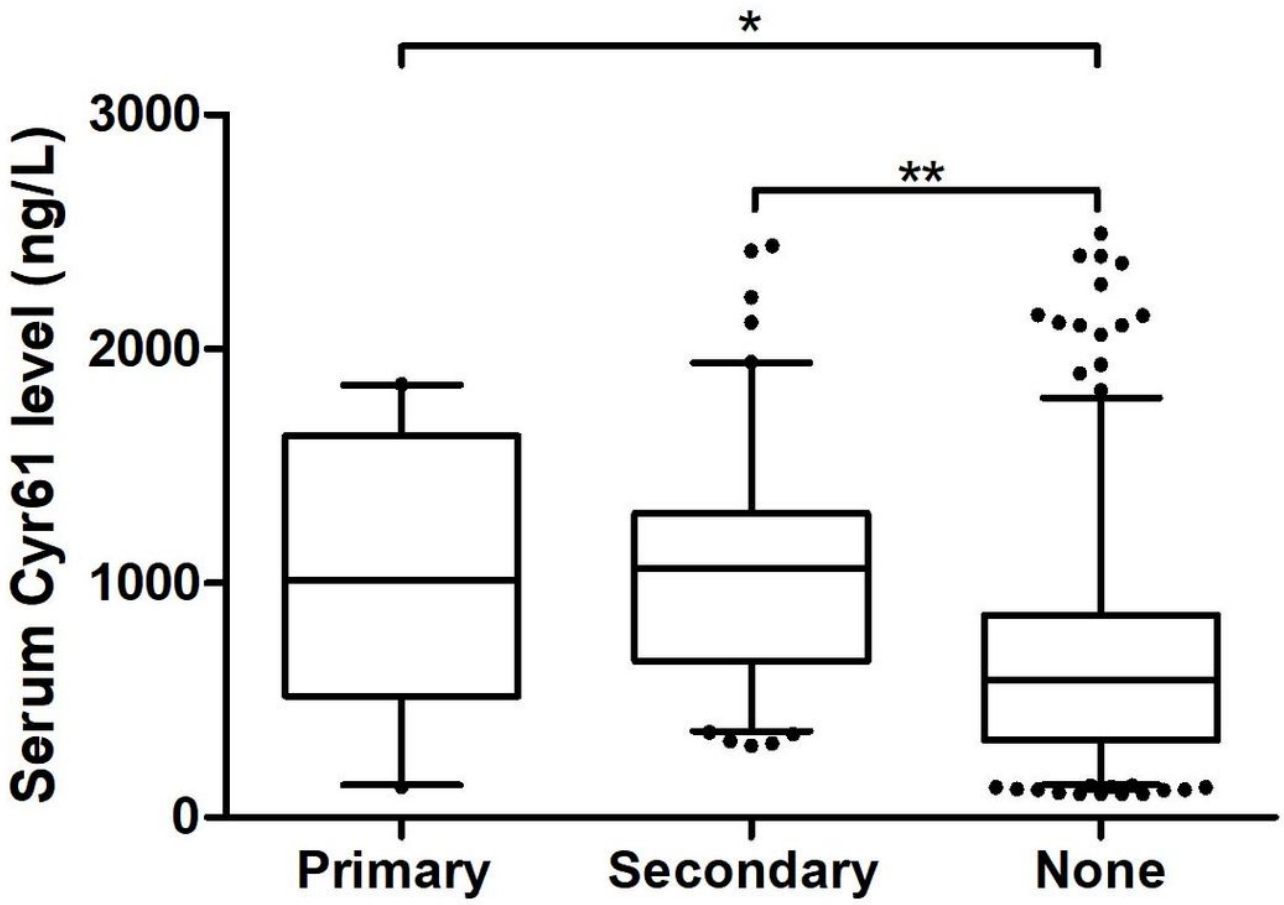

Figure 1

Serum Cyr61 level of patients with different endpoints. *P<0.05.

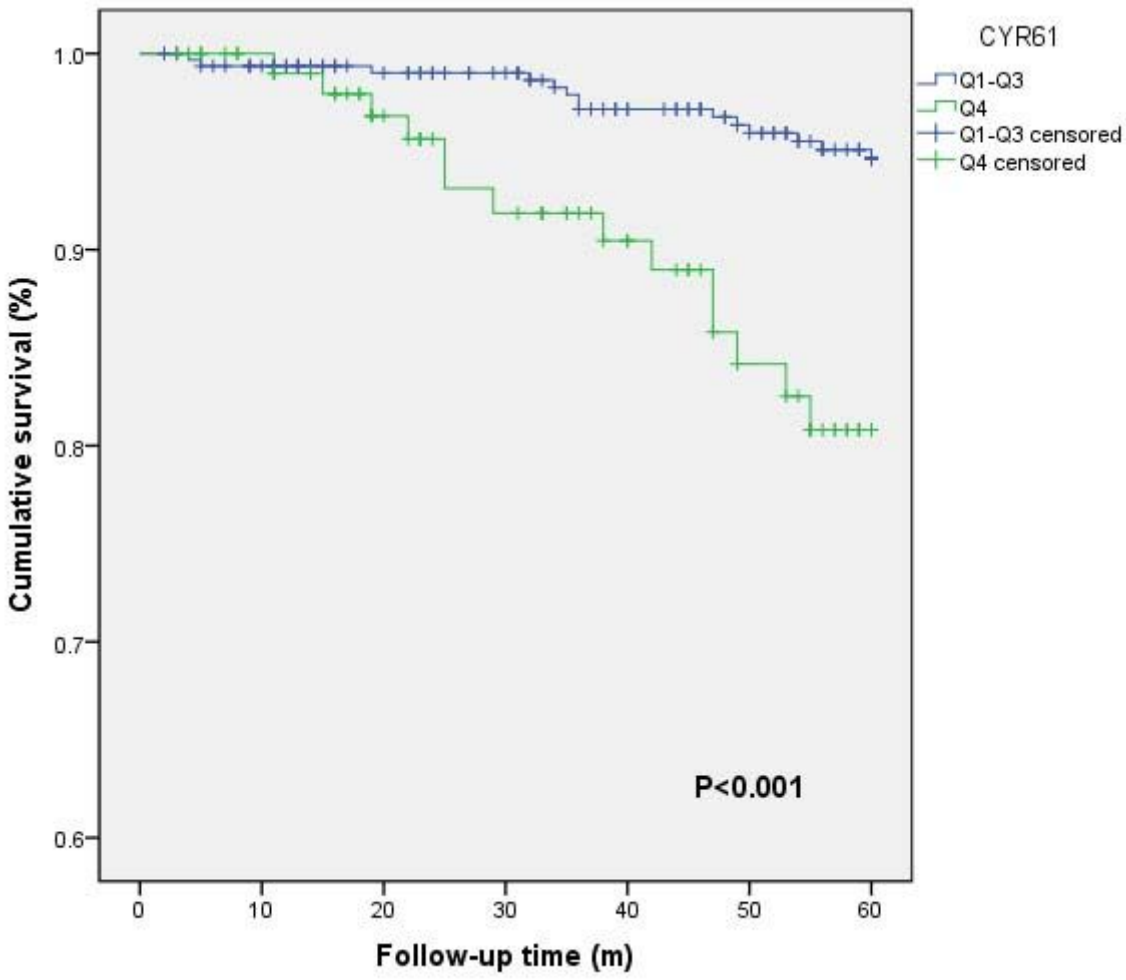

Figure 2

Survival curves of the primary endpoint for the patients with highest Q4 level Cyr61 and patients with Q1-Q3 level Cyr61. Log rank test was used for the comparison.

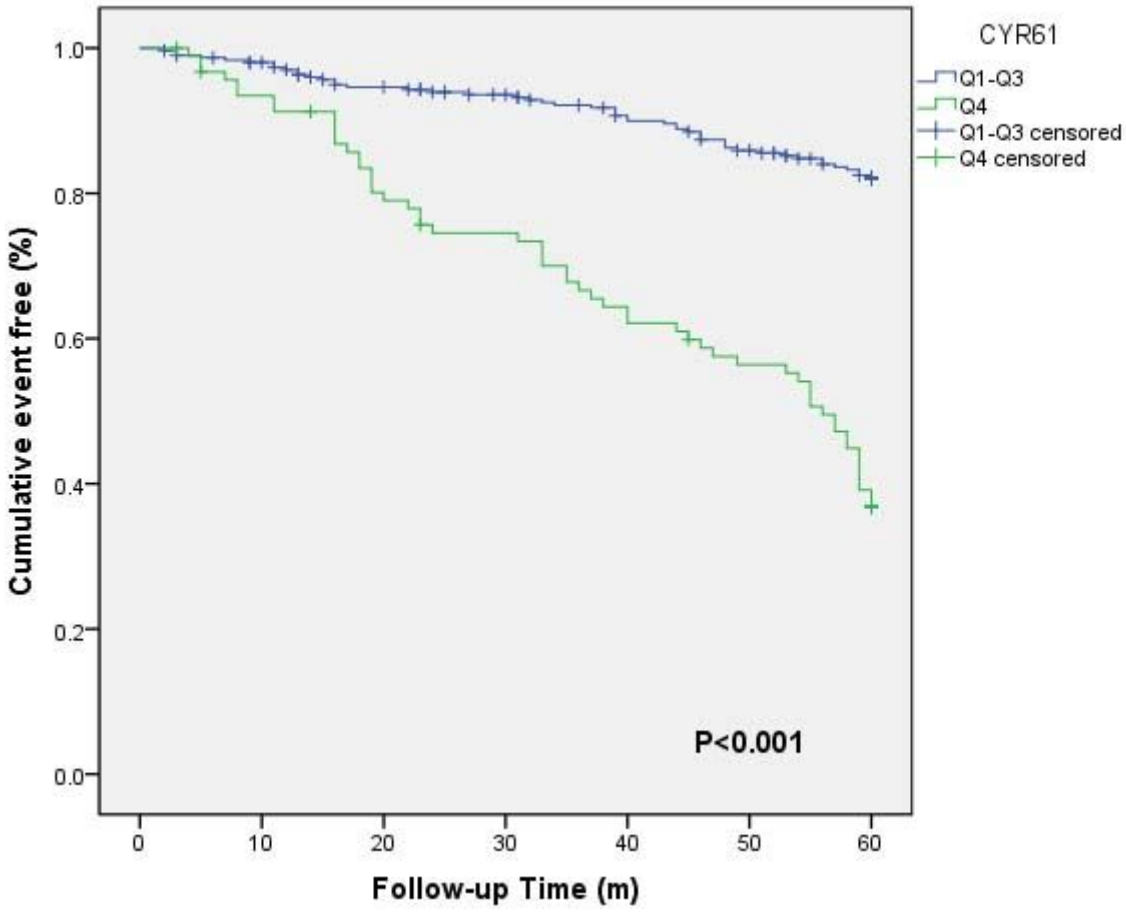

Figure 3

Survival curves of the secondary endpoint for the patients with highest Q4 level Cyr61 and patients with Q1-Q3 level Cyr61. Log rank test was used for the comparison.