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Relationship between electrocardiographic changes and EPO level in stable CAD patients with autonomic nerve functional damage

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Abstract. – OBJECTIVE: To investigate the relationship between electrocardiographic changes and erythropoietin (EPO) level in stable coronary artery disease (CAD) patients with autonomic nerve functional damage.

PATIENTS AND METHODS: Clinical data of 96 stable CAD patients who were treated in our hospital from January 2017 to December 2019 were retrospectively analyzed. All patients were grouped according to whether autonomic nerve function damage was combined; the baseline characteristic data and the morphological characteristics of ECG scattergram were compared between 2 groups, and the relationship between ECG scattergram and EPO level & autonomic nerve function was analyzed.

RESULTS: The levels of EPO and red cell volume distributing width (RDW) in stable CAD patients with autonomic nerve dysfunction were significantly higher than that of CAD patients without autonomic nerve dysfunction ($p<0.05$). The length of scattergram in stable CAD patients with autonomic nerve dysfunction was significantly shorter than that of those without autonomic nerve dysfunction ($p<0.05$). The cometary sign proportion of ECG scattergram in stable CAD patients with autonomic nerve dysfunction was significantly lower than that of stable CAD patients without autonomic nerve dysfunction ($p<0.05$). There was negative correlation between EPO levels and scattergram length in stable CAD patients with and without autonomic nerve dysfunction (r=0.44, $p=0.02$). There was no correlation between EPO levels and scatter width in stable CAD patients with and without autonomic nerve dysfunction (r=0.10, $p=0.58$). The results of binary logistic regression analysis showed that EPO level was the independent risk factor for the occurrence of autonomic dysfunction in patients with stable CAD ($p<0.05$). The length of scattergram was the independent protective factor of autonomic nerve function impairment in patients with stable CAD ($p<0.05$). The AUC of EPO level and scattergram was 0.74 and 0.72 respectively, both of which have similar prediction value.

CONCLUSIONS: The level of EPO in stable CAD patients with autonomic nerve dysfunction was related to the change of ECG; and the EPO level and scattergram length can be used to predict the occurrence risk of autonomic nerve dysfunction.

Key Words:
Autonomic nerve function, Coronary heart disease, ECG, Hemopoietin, Relationship.

Introduction

Endogenous EPO is mainly caused by oxygen deficit stimulus, and it has multiple aspects, such as promoting erythrocyte synthesis in bone marrow, anti-inflammation, antioxidant stress, antiapoptosis, promoting neovascularization and adjusting nervous system function. Li et al[1] show that EPO can to some extent alleviate autonomic nerve dysfunction caused by oxygen deficit. Autonomic nerve dysfunction of cardiovascular system can happen in the early stage of multiple chronic diseases, but there are no specific symptoms, or the symptoms are hidden. Autonomic nerve dysfunction of cardiovascular system can

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increase the occurrence risk of long-term cardiovascular events[2]. Early diagnosing autonomic nerve dysfunction of cardiovascular system and giving targeted treatment have important value, which has been widely approved[3]. However, there is still no uniform standard about how to accurately diagnose and evaluate autonomic neuropathy of heart. At present, heart rate variability and Ewing test are mainly applied to clinically assess autonomic nerve function of heart and blood vessels, while Lorenz ECG scattergram is most widespread in heart rate variability[4]. Clinical data of 96 stable CAD patients who were treated in our hospital from January 2017 to December 2019 were retrospectively analyzed. All patients were grouped according to whether autonomic nerve function damage was combined so as to discuss the relationship between ECG change and EPO level in stable CAD patients with autonomic nerve dysfunction.

Patients and Methods

Clinical Data

The study was approved by our hospital Ethics Committee. Clinical data of 96 stable CAD patients who were treated in our hospital from January 2017 to December 2019 were retrospectively analyzed. All patients were grouped according to whether autonomic nerve function damage was combined. Total score of Ewing≥2 was used as the criterion of combined autonomic nerve dysfunction. There were 52 cases with combined autonomic nerve dysfunction, and 44 without combined autonomic nerve dysfunction. Inclusion criteria: conform to CAD diagnostic criteria[5]; stenosis degree of main blood vessel branches of coronary artery trunk, anterior descending branch, circumflex branch, right coronary artery or above≥50% in coronary angiography (CAG) examination, and drug therapy could not gain obvious remission; age≥18 years old; complete clinical data. Exclusion criteria: take drugs influencing autonomic nerve function recently; NYHA grading≥III; hyperthyroidism; combined with pulmonary disease; severe hepatic and renal dysfunction; severe nervous system disease; malignant tumor; anemia or blood transfusion in recent 4 weeks; severe general infection. The research program conforms to the requirements of Declaration of Helsinki. Besides, patients and their relatives were informed and agreed.

Coronary Angiogram Examination

All patients completed CAG via radial artery approach, and Seldinger method was used. The same group of doctors completed the operation and judged the results. Gensini scoring was applied to assess coronary artery stenosis and blood vessel severity. Coronary stenosis ratio < 25%: 1 mark; 26%-49%: 2 marks; 50%-74%: 4 marks; 75%-89%: 8 marks; 90%-99%: 16 marks; complete occlusion: 32 marks. Meanwhile, coronary artery segment coefficient was combined to score lesions of left main coronary artery, left anterior descending branch, left circumflex artery and right coronary artery, and calculate the sum[6].

EPO Detection

3 ml venous blood was collected and centrifuged for 10 min at the speed of 3000 r/min. Then, the supernatant was taken. 0.3 ml sample was taken and kept at 80°C for the test. Chemiluminescent immunoassay was used to determine EPO level, and the kit was provided by Guangzhou Aodesen Biotechnology Co., Ltd.

ECG Scattergram Examination

All patients received 24 h dynamic ECG examination. Lorenz scattergram was gained after automatic analysis and calibration by the software. The length, width, and morphological characteristic type were recorded.

Statistical Analysis

SPSS 21.0 (IBM, Armonk, NY, USA) software was used for data processing. Measurement data were compared with *t*-test, expressed with ($\bar{x} \pm s$). Enumeration data were compared with χ^2-test or exact probability method, expressed with %. Correlation analysis was tested with Pearson. Binary Logistic regression model was applied for multiple-factor analysis. ROC curve was drawn to assess the prediction value. $p<0.05$ means the difference has statistical significance.

Results

Baseline Data Comparison of Stable CAD Patients with and Without Nerve Function Damage

The levels of EPO and RDW in stable CAD patients with autonomic nerve dysfunction were significantly higher than that of CAD patients

Table I. Baseline data comparison of stable CAD patients with and without nerve function damage.

Indicato	Combined with autonomic nerve dysfunction (n = 52)	Not combined with autonomic nerve dysfunction (n = 44)	p
Age (year)	58.78 ± 11.53	56.35 ± 9.47	0.63
Male (No.)	32	24	0.41
Primary hypertension (No.)	22	16	0.41
T2DM (No.)	18	12	0.44
Smoking (No.)	14	8	0.33
Hb (g/L)	135.11 ± 12.67	136.57 ± 13.50	0.95
MCV (fl)	92.33 ± 4.90	94.48 ± 5.69	0.19
TC (mmol/L)	4.41 ± 0.80	4.49 ± 0.87	0.91
TG (mmol/L)	2.17 ± 0.45	1.75 ± 0.64	0.34
HDL-C (mmol/L)	1.29 ± 0.43	1.34 ± 0.48	0.20
LDL-C (mmol/L)	2.49 ± 0.68	2.56 ± 0.76	0.73
FBG (mmol/L)	6.09 ± 1.16	5.32 ± 1.25	0.12
Folic acid (ng/ml)	6.23 ± 2.52	6.94 ± 3.25	0.41
VB12 (pg/ml)	405.73 ± 113.27	392.60 ± 106.28	0.77
Serum Fe (μmol/L)	15.09 ± 4.28	20.59 ± 6.23	0.20
SF (ng/ml)	187.50 ± 90.73	182.59 ± 104.82	0.32
EPO (mIU/ml)	13.59 ± 3.82	10.53 ± 4.76	0.00
RDW (%)	13.04 ± 2.57	12.27 ± 2.31	0.00
Gensini score (mark)	46.82 ± 10.44	47.90 ± 11.51	0.15
Scattergram width (ms)	0.78 ± 0.10	0.75 ± 0.13	0.84
Scattergramlength (ms)	148.81 ± 20.45	162.97 ± 24.64	0.01

without autonomic nerve dysfunction (p<0.05). The length of scattergram in stable CAD patients with autonomic nerve dysfunction was significantly shorter than that of those without autonomic nerve dysfunction (p <0.05), as shown in Table I.

Comparison of Morphological haracteristics of ECG Scattergram in Stable CAD Patients with and Without Nerve Function Damage

The cometary sign proportion of ECG scattergram in stable CAD patients with autonomic nerve dysfunction was significantly lower than that of stable CAD patients without autonomic nerve dysfunction (p<0.05), as shown in Table II.

Correlation Analysis of EPO Level, ECG Scattergram Length and Width

There was negative correlation between EPO levels and scattergram length in stable CAD patients with and without autonomic nerve dysfunction (r=0.44, p=0.02). There was no correlation between EPO levels and scattergram width in stable CAD patients with and without autonomic nerve dysfunction (r=0.10, p=0.58).

Independent Influencing Factors of Autonomic Nerve Dysfunction in CAD Patients

The results of binary logistic regression analysis showed that EPO level was the independent risk factor for the occurrence of autonomic dy-

Table II. Comparison of morphological characteristics of ECG scattergram in stable CAD patients with and without nerve. function damage.

Morphological characteristic type of ECG scattergram	Combined with autonomic nerve dysfunction (n = 52)	Not combined with autonomic nerve dysfunction (n = 44)	p
Comet	20	40	0.00
Short rod	12	2	0.09
Long rod	8	0	0.08
Torpedo	6	0	0.14
Triangle	4	0	0.23
Complex	2	2	0.81

sfunction in patients with stable CAD (p<0.05). The length of scattergram was the independent protective factor of autonomic nerve function impairment in patients with stable CAD (p<0.05), as shown in Table III.

Prediction Effect of Autonomic Nerve Dysfunction in CAD Patients

ROC curves were drawn. Autonomic nerve function was used as the state variable, and EPO level and scattergram length were regarded as test variables, respectively. The AUC of EPO level and scattergram was 0.74 and 0.72 respectively, both of which have similar prediction value.

Discussion

Biological phenomena of human body have diurnal rhythm. Adverse events of heart and blood vessels and ECG parameter change cannot be avoided. Multiple studies verify that maintenance and regulation of diurnal rhythm phenomenon of cardiovascular system most automatic nervous system are mostly related to automatic nervous system[7,8]. However, there is no "golden standard" for the assessment of autonomic neuropathy of heart, and it is mostly evaluated through heart rate variability (HRV) and Ewing. Lorenz ECG scattergram examination is the most common HRV test means. Heart rate variation is not nonlinear change rule, so chaos characteristics exist. HRV is deemed to reveal heart system characteristics more accurately. Ewing test is widely applied in the clinical evaluation of cardiac autonomic nerve function[9].

In this study, clinical data of 96 stable CAD patients who were treated in our hospital from January 2017 to December 2019 were retrospectively analyzed. All patients were grouped according to whether autonomic nerve function damage was combined. Total score of Ewing≥2 was used as the criterion of combined autonomic nerve dysfunction. There were 52 patients with combined autonomic nerve dysfunction, and 44 without combined autonomic nerve dysfunction. Morphological characteristic types of ECG scattergram include comet, short rod, long rod, torpedo, triangle and complex shape. Scattergram length reflects total dispersion degree of heart rate, while width can reflect interphase difference of adjacent RR, i.e., instantaneous heart rate change trend. The cometary sign shows the head end of the scattergram is small, and the corresponding interphase is short with small distribution area, indicating that arrhythmia degree is slow when the heart rate increases, and reflecting sympathetic nerve activity. The corresponding interphase of tail end is long with large distribution area, indicating arrhythmia degree is large when the heart rate is slow and reflecting vagus activity. The torpedo sign hints sympathetic nerve and vagus dysfunction[10]. In the results of this study, the cometary sign proportion of ECG scattergram in stable CAD patients with autonomic nerve dysfunction was significantly lower than that of stable CAD patients without autonomic nerve dysfunction (p<0.05), which is consistent with previous reports[11]. This verifies that the heart loses normal regulation function of autonomic nerve so that RR interphase no longer changes with heart rate variation. Although Lorenz ECG scattergram examination is visual and simple, there is no uniform graph diagnosis criterion, and the subjectivity is stronger. Quantitative analysis method is also required to improve diagnosis accuracy[12]. ECG scattergram is quantitatively analyzed according to mathematical features of scattergram shape. Traditional indicators include length and width. The results of this study showed that the length of scattergram in stable CAD patients with autonomic nerve dysfunction was significantly shorter than that of those without autonomic nerve dysfunction (p<0.05). Meanwhile, the width comparison difference of two groups of scattergrams had no statistical significance (p>0.05), indicating that the length of Lorenz scattergram can visually reflect the functions of sympathetic nerve and vagus and then distinguish the state of autonomic nerve function. Moreover, EPO level of stable CAD patients with neurological dysfunction was significantly higher than that of those

Table III. Independent influencing factors of autonomic nerve dysfunction in CAD patients.

Indicator	β	SE	Wald	df	p	OR	95% CI
EPO	0.25	0.72	0.10	1	0.82	0.42	0.44-3.60
Scattergram length	1.27	0.55	6.55	1	0.01	1.75	1.48-9.25
Constant	0.03	0.01	9.60	1	0.00	1.04	1.02-1.10

without neurological dysfunction ($p<0.05$). The results of binary logistic regression analysis further showed that EPO level was the independent risk factor for the occurrence of autonomic dysfunction in patients with stable CAD ($p<0.05$). At the same time, ROC curve was drawn. Autonomic nerve dysfunction was used as the state variable, while EPO level and scattergram length were deemed as the test variables, respectively. The AUC was 0.74 and 0.72, respectively. Both own similar prediction value. The above data reveal that autonomic nerve function of CAD patients is closely related to EPO level and scattergram length. EPO level can to certain degree regulates autonomic nerve function.

Multifunctional cytokine EPO has been verified to stabilize internal environment of the body and protect the functions of nervous system. Relevant experimental studies verify that EPO can well protect the functions of nervus centralis and peripheral nervous system and relieve autonomic neuropathy degree of diabetes model rats[13,14]. Besides, it can give play to synergistic stimulus function of P13K/Akt signal path together with IGF-1. At present, it is believed that EPO mainly protects nervous system functions through forming a compound by EPOR andβreceptor subunit. EPOR on excitatory neuron can activate multiple signal paths represented by P13K/Akt and resist abnormal oxidative stress and apoptosis, thus effectively protecting nerves. A study also verifies that, peripheral Schwann cells can release EPO after axonal injury, and combine with EPOR to inhibit axon damage[15]. Additionally, *in vitro* phosphorylated Akt/total Akt of sympathetic neurons has obvious EPO level dependence, that is, this specific value increases with the rise of EPO level, indirectly demonstrating that EPO level is correlated with sympathetic functions[16].

This study also has limitations: it is a small-sample and monocentric retrospective study, and selection bias cannot be avoided; some possible influencing indicators are not included, and chaos problem may exist; there is lack of research on the relationship between EPO and ECG change. Thus, more rigorous and in-depth studies are required.

Conclusions

Altogether, the above reported data indicated that the level of EPO in stable CAD patients with autonomic nerve dysfunction was related to the change of ECG; and the EPO level and scattergram length can be used to predict the occurrence risk of autonomic nerve dysfunction.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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