

Original Article

Clinical values of serum NGAL combined with NT-proBNP in the early prognosis of type 1 cardiorenal syndrome

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Abstract: Background: Cardiorenal syndrome (CRS) is a condition that defines disorders of the heart and kidneys whereby "acute or chronic dysfunction in one organ may induce acute or chronic dysfunction of the other". Early diagnosis of its biomarkers has a significant impact on the treatment and prognosis of the CRS. Elevated serum NGAL and NT-proBNP levels are independent risk factors for predicting heart and kidney disease. Therefore, we proposed early detection of type 1 CRS using serum NGAL in combination with NT-proBNP. Objective: This study intended to investigate the clinical value of serum NGAL in combination with NT-proBNP in the early diagnosis of type 1 CRS. Methods: In this paper, 80 patients with type 1 CRS and 80 healthy controls admitted to our hospital from January 2019 to August 2020 were retrospectively included, and the predictive value of single index and combined indices for predicting CRS were judged by calculating the correlation between serum NGAL, NT-proBNP and the creatinine levels and plotting receiver operating characteristic (ROC) curves. Results: There was no difference in baseline data between the control and patient groups. Serum NGAL and NT-proBNP in the patient group were significantly higher than those in the control group, and were positively correlated with changes in blood creatinine. The ROC curves showed that serum NGAL and NT-proBNP independently had a high predictive value for CRS, and the combination of the two had a better predictive value. Conclusion: Serum NGAL in combination with NT-proBNP is of high clinical value for the early diagnosis of type 1 CRS.

Keywords: Cardiorenal syndrome, neutrophil gelatinase-associated lipocalin, N-terminal pro b-type natriuretic peptide, prognosis

Introduction

In clinical diseases, kidney and heart, as two important organs of the human body, are often damaged in the pathological process. The two organs have close relationship, including the cross-talk in terms of hemodynamics, neurohormones, and inflammatory factors. Cardiorenal syndrome (CRS), defined as a large group of pathophysiological disorders involving both heart and kidney, in which acute or chronic failure of one organ could cause acute or chronic dysfunction of the other [1]. Five subtypes of CRS are recognized on basis of the speed of disease progression and the order of organs involved [2] and patients may switch between different subtypes as the disease progresses. Cardiorenal syndrome type 1 (CRS1),

also known as acute heart and kidney syndrome (acute CRS), refers to acute renal failure caused by heart failure, such as cardiogenic shock and acute renal failure caused by acute coronary syndrome and acute renal failure caused by acute heart failure. Approximately 14%-40% of hospitalized patients with acute decompensated heart failure (ADHF) progress to occur acute renal injury (AKI) [3].

Currently, the diagnostic criteria for CRS1 are mainly the presence of an increase in serum creatinine of ≥ 0.3 mg/dl in the setting of acute heart failure. However, the sensitivity and specificity of serum creatinine are unsatisfactory and have a delayed effect on the determination of renal function, since the elevated creatinine has already indicated severe renal damage.

Study on cardiorenal syndrome

Therefore, we tried to seek effective markers for the early diagnosis of cardiac and renal damage to achieve the best possible preservation of cardiac and renal function. Neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kD protein secreted by the renal tubular epithelium cells, cardiomyocytes and other specific tissues. The predictive value of NGAL for AKI was evidenced in a variety of pathological settings, such as patients following cardiac surgery and critically ill patients. Meanwhile, blood AKI and urine AKI exhibited the same diagnostic accuracy [4]. Brain natriuretic peptide (BNP) was first isolated from porcine brain tissue, hence named BNP, but was subsequently found to be synthesized and secreted primarily by ventricular myocytes in response to ventricular overload or dilatation, and thus could indicate altered ventricular function [5]. Compared to BNP, N-terminal prohormone of brain natriuretic peptide (NT-proBNP) is relatively stable (half-life of 120 min) and more clinically accessible [6]. It has been found that NT-proBNP levels are usually high in the elderly, and they are also elevated in patients with tachycardia, myocardial ischemia, hypoxemia, renal insufficiency, cirrhosis, sepsis and infections. Also, NT-proBNP is helpful in the diagnosis of heart failure and is considered to be a gold standard biomarker for heart failure similar to BNP [7]. Therefore, we proposed to use serum NGAL combined with NT-proBNP as biomarkers for early diagnosis of patients with CRS1.

Materials and methods

Patients

A total of 80 patients with type 1 CRS who were admitted to our hospital from January 2019 to August 2020 were retrospectively included in this study. Inclusion criteria: (1) age ≥ 18 years; (2) admission with acute decompensated heart failure (ADHF) as the primary diagnosis; Eligible patients were to have a left ventricular ejection fraction $\leq 40\%$ and signs and symptoms of HF along with BNP concentration ≥ 400 pg/mL. (3) with increase in serum creatinine ≥ 0.3 mg/dl after admission; and (4) patient signed the informed consent. Exclusion criteria: patients with primary renal disease, endocrine disease (diabetes mellitus, thyroid disease, primary aldosteronism, etc.), malignancy, acute cerebrovascular disease, severe anemia; without

laboratory measurements or medical records; pregnant patients. In addition, 80 healthy volunteers were recruited during the same period. This study was approved by ethics committee of the Second Affiliated Hospital of Nanchang University, and all volunteers signed the informed consent.

Records and assays

Once written informed consent had been obtained, baseline demographic data were recorded and blood samples were obtained for measurements of creatinine (Cr), NGAL, and NT-proBNP within 24 h from hospitalization. These were repeated daily for up to seven days or until discharge. The serum creatine testing was performed by Creatinine (serum) Assay Kit Data (Cayman Chemical, Ann Arbor, Michigan, USA). The NT-proBNP testing was performed by One Step Test for NT-proBNP (Colloidal Gold) (GeteinBiotech, Nanjing, Jiangsu, China). As for the NGAL testing, The Triage NGAL Test (Alere Inc., San Diego, CA, USA) achieved an immunoassay in a single-use plastic cartridge that contains a fluorescently labelled monoclonal antibody against NGAL. The data for the control group were collected from physical examination and questionnaires.

Statistical analysis

Statistical analysis was performed using the SPSS 24.0 (SPSS Inc., Chicago IL, United States). Continuous variables were presented as mean and standard deviation ($\bar{x} \pm s$). Qualitative variables were reported as frequencies or percentages. The differences in baseline data between the two groups were evaluated by the student-t test for quantitative variables and the *Chi*-squared test for qualitative variables. Pearson correlation analysis was performed for correlation analysis. ROC curves were plotted for evaluating the predictive value of NGAL/NT-proBNP for CRS, and the best diagnostic value was evaluated by the Youden index. $P < 0.05$ indicated significant differences.

Results

Clinical characteristics of the study population

The clinical characteristics of the patient and control groups were summarized in **Table 1**, indicating that the baseline was comparable

Study on cardiorenal syndrome

Table 1. Demographic parameters between the control group and the patient group

Demographic parameter	Control group	Patient group	P-value
Number	80	80	
Age (years)	49.29±8.969	49.53±9.461	0.871
Gender (M/F)	38/42	42/38	0.527
BMI (kg/m ²)	21.79±2.70	21.91±2.87	0.77
Baseline Cr (mg/dL)	0.70±0.19	0.68±0.18	0.37
NGAL (ng/mL)	109.40±87.80	243.37±129.72	<0.001
NT-proBNP (ng/L)	296.23±309.65	878.33±473.26	<0.001

BMI: Body mass index; Baseline Cr: baseline serum creatinine; NGAL: Neutrophil gelatinase-associated lipocalin; NT-proBNP: N-terminal prohormone of brain natriuretic peptide.

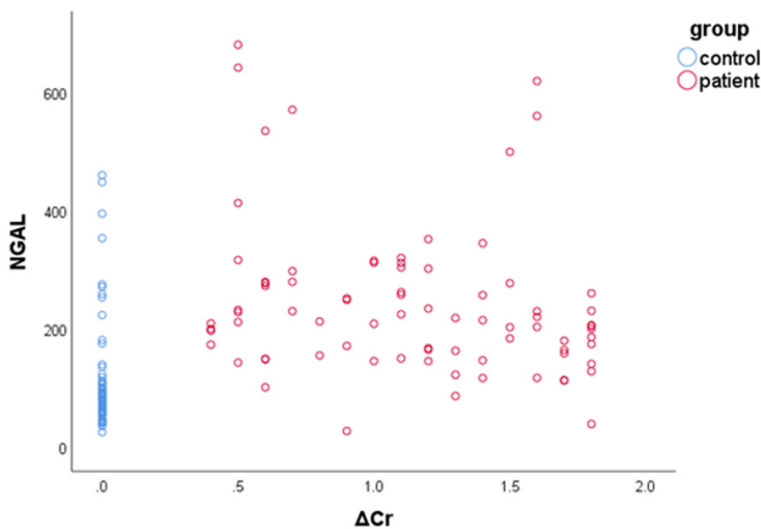


Figure 1. Scatter plot showing the relationship between NGAL and Δ Cr.

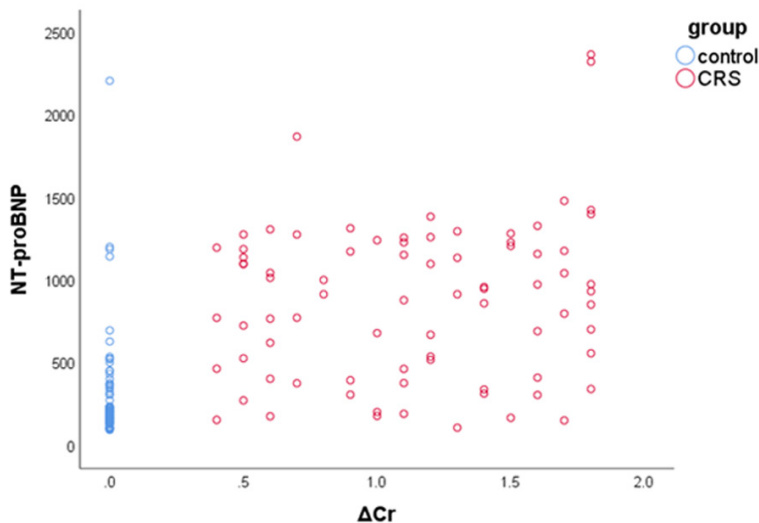


Figure 2. Scatter plot showing the relationship between NT-proBNP and Δ Cr.

between two groups. The NGAL and NT-proBNP in the patient group were significantly higher than those in the control group ($P<0.05$).

Correlation analysis

The outcome measures of serum Cr were 0.70 ± 0.19 (mg/dL) and 1.81 ± 0.52 (mg/dL) respectively for the control group and patient group. The Δ Cr were significantly higher in the patient group than the control group ($P<0.05$). We correlated NGAL/NT-proBNP and Δ Cr in the patient group. The results showed a significant positive correlation between NGAL/NT-proBNP and Δ Cr (Figures 1, 2; Table 2).

ROC curves of NGAL/NT-proBNP for predicting CRS1

AUC of NGAL predicting type 1 CRS was 0.875 and the optimal diagnostic value was 114.37 ng/mL, with a sensitivity of 0.95 and a specificity of 0.812. AUC of NT-proBNP predicting type 1 CRS was 0.872 and the optimal diagnostic value was 376.72 ng/mL, with a sensitivity of 0.825 and a specificity of 0.825. The AUC of two joint indicators was 0.917, with a sensitivity of 0.925 and a specificity of 0.8, which was significantly higher than the predictive value of index alone (Figure 3; Tables 3, 4).

Discussion

The pathogenesis of CRS is complex, involving the heart and kidney, two major organs of the body, which interacts with each other to promote disease progression through a variety of pathways and mechanisms. Among them,

Table 2. Analysis of the correlation between serum NGAL, NT-proBNP with Δ Cr

		NGAL	NT-proBNP
Δ Cr	r	0.371	0.565
	P-value	<0.001	<0.001

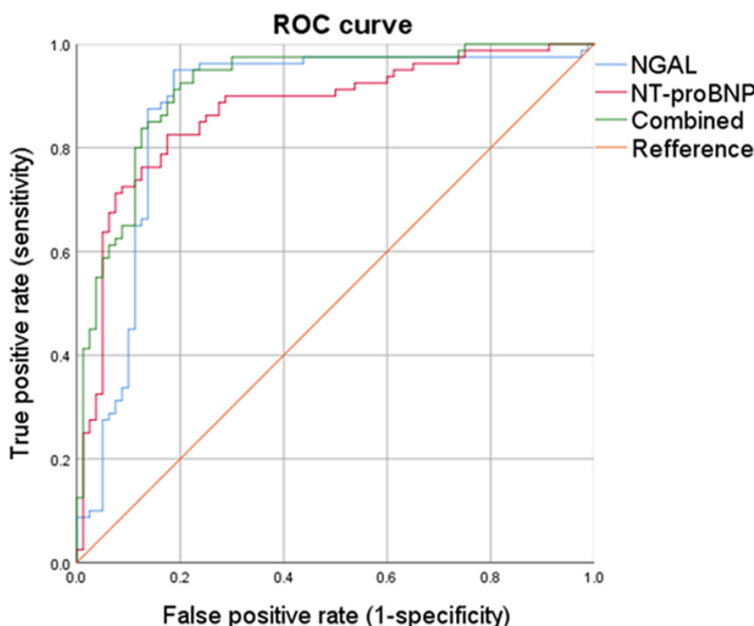


Figure 3. Receive operating characteristic curve analysis of the prediction of type 1 CRS by serum NGAL, NT-proBNP and combined NGAL with NT-proBNP.

Table 3. Parameters of receive operating characteristic curves

Parameter	AUC	SD	P-value	95% Confidence interval	
				Lower limit	Upper limit
NGAL	0.875	0.032	<0.001	0.813	0.937
NT-proBNP	0.872	0.029	<0.001	0.815	0.929
Combined	0.917	0.023	<0.001	0.872	0.961

overactivation of the neurohormonal system and elevated venous pressure are the main contributing factors [8]. For example, the RAAS, the sympathetic nervous system, and the arginine-vasopressin system, which are initiated at the beginning of the course as a protective response to maintain steady state, are instead involved in degradation and decompensation, causing further damage to the heart and kidneys [9]. The treatment option of CRS is mainly targeted at primary organ failure, and the prognosis of patients is poor [10]. Therefore, early diagnostic options, early intervention, and timely protective treatment of the heart and kidneys are crucial for patient prognosis.

NGAL, first discovered in mature neutrophil granules, is also widely expressed, including in kidney cells, liver cells, cardiomyocytes, neurons, many immune cells and smooth muscle cells [11, 12]. In models of acute kidney injury, NGAL is primarily derived from renal cells; smaller amounts are derived from extrarenal sources, such as circulating immune cells. NGAL has been shown to be a small secreted glycoprotein. It was evidenced that NGAL is involved in iron transport and plays a bacteriostatic and chemotactic role in immune function [13]. NGAL is also a growth factor that promotes tissue proliferation and differentiation. Pathologically, NGAL has been widely recognized as a biomarker for acute kidney injury [14, 15]. Compared with traditional markers of renal injury, such as creatinine and blood urea nitrogen levels, NGAL is more sensitive to changes in NGAL [16]. In an animal model of ischemia-reperfusion, a significant increase in NGAL levels was observed within 3 hours of ischemia when changes in blood creatinine levels remain insignificant [17]. Moreover, changes in NGAL levels caused by renal injury persist

over a long period of time, leaving a long window for clinical diagnosis. In one animal study on renal injury, both urinary cystatin C and NGAL were elevated following renal damage, but cystatin C levels recovered more rapidly, while NGAL levels remained consistently high [18]. In addition to being a biomarker for kidney injury, NGAL has also been shown to be associated with the development of cardiovascular disease. Serum NGAL levels were found to be higher in patients with acute myocardial infarction and chronic heart failure than in healthy subjects [19]. In conclusion, NGAL is involved in the pathology of renal and cardiac diseases through multiple mechanisms and is a sensi-

Study on cardiorenal syndrome

Table 4. Cut-off values for NGAL and NT-proBNP

	Cut off	Sensitivity	Specificity	Youden index
NGAL	114.3700	0.950	0.813	0.138
	112.8000	0.950	0.800	0.150
	115.0000	0.938	0.813	0.125
	117.1300	0.925	0.813	0.113
	142.1400	0.875	0.863	0.013
NT-proBNP	376.72	0.825	0.825	0.000
	538.30	0.713	0.925	-0.213
	374.96	0.825	0.813	0.013
	378.26	0.813	0.825	-0.013
	459.12	0.763	0.875	-0.113
Combined		0.925	0.800	0.125
		0.950	0.775	0.175
		0.913	0.813	0.100
		0.938	0.775	0.163
		0.925	0.788	0.138

tive biomarker of renal injury and myocardial damage.

BNP belongs to the natriuretic peptide family, which also includes atrial natriuretic peptide (ANP) and c-type natriuretic peptide (CNP), of which CNP is mainly secreted by the vascular endothelium [20]. ANP and BNP are mainly produced by cardiomyocytes, and ventricular wall stress is the main stimulus for the synthesis and secretion of BNP-related peptide, which is involved in the regulation of cardiovascular homeostasis [21]. The secretion of natriuretic peptide is accompanied by an increase in the severity of left ventricular dysfunction [22], and in patients with acute coronary syndromes, BNP levels are also increased without significant ventricular dilation [23]. In addition, BNP levels are also elevated in patients with renal failure or pulmonary hypertension [24]. In summary, the synthesis and secretion of BNP in cardiomyocytes are regulated by a multifaceted interaction of mechanical, neurohormonal, and immune factors. Heart failure and myocardial infarction can lead to upregulation of NT-proBNP levels in local ventricular cells, which subsequently flow into the systemic circulation. Compared with BNP, NT-proBNP has a longer half-life, is more stable in the blood, and has a higher plasma concentration [25]. Also, NT-proBNP is mainly metabolized by renal clearance, while BNP is only metabolized in small amounts by the kidney, making NT-proBNP more advantageous in the assessment of renal

function. NT-proBNP is effective in terms of diagnosis and prognosis of heart failure [26].

In conclusion, serum NGAL and NT-proBNP are key factors in the pathology of heart failure and kidney injury, and both can independently indicate the severity of heart or renal failure. In type 1 CRS, both indicators could predict the occurrence of CRS, while the combined testing of both indicators could significantly improve the predictive accuracy and timely prevent permanent damage to the heart and kidneys. However, the conclusions of this study have some limitations such as limited sample size and short follow-up time. A large multicenter cohort clinical study is needed to confirm the conclusions.

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Disclosure of conflict of interest

None.

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Study on cardiorenal syndrome

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