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Value of Combined Plasma NGAL, Cystatin C and NT-proBNP in the Diagnosis of Cardiorenal Syndrome Type 1

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Abstract

Background: The presence of acute kidney injury in the setting of acute heart failure (AHF) or acute decompensated heart failure (ADHF) is very common occurrence and was termed cardiorenal syndrome 1 (CRS1). Neutrophil gelatinase-associated lipocalin (NGAL) in the blood and urine is one of the earliest biomarkers of acute kidney injury due to ischemia or renal toxicity. Cystatin C is early marker of renal dysfunction. NT-pro BNP is valuable in the diagnosis, prognosis and treatment of acute and chronic heart failure. This study was aimed to evaluate the diagnostic efficacy of the combination of plasma NGAL, Cystatin C and NT-proBNP in diagnosis of CRS1.

Methods: there were 139 patients with AHF or ADHF in the department of Cardiovascular resuscitation and Interventional cardiology at Ho Chi Minh City 115People Hospital from November 2018 to May 2019. This was a prospective cohort study.

Results: there were 48 cases (rate 34.5%) with CRS1, mean age 66.12 ± 15.77, men accounted for 50.4%. There were no significant differences of vital signs on admission, diagnosis, type of heart failure between CRS1 and Non-CRS1 groups. The urea, creatinin on first day (creatininD1) and third day (creatininD3), NT-pro BNP, Cystatin C, NGAL levels were higher in the group with CRS1 than Non-CRS1, the difference was statistically significant p <0.05. The optimal cut-off NGAL for diagnosing CRS1 was> 353.23 ng/ml, Area Under Curve (AUC)was 0.732 (95% CI 0.65-0.80, p <0.001), sensitivity 74.47%, specificity 68.48%, positive predictive value 54.7%, negative predictive value 84%. The optimal cut-off Cystatin C for diagnosing CRS1 was> 1.81 mg/dl, AUC was 0.787 (95% CI 0.71-0.85, p <0.001), sensitivity 75%, specificity 83.52%, positive predictive value 70.6%, negative predictive value 86.4%.The optimal cut-off NT-pro BNP for diagnosing CRS1 was 17681 pg/ml, AUC was0.683 (95% CI 0.59 – 0.76, p < 0.001), sensitivity 53.19%, specificity 77.17%, positive predictive value 54.3%, negative predictive value 76.3%.Combined three biomarker plasma NGAL, Cystatin C and NT proBNP, the specificity of the diagnosis was the highest 95.6%, the positive predictive value was the highest 84.62% in diagnosing CRS1.

Conclusions: The combined plasma NGAL, Cystatin C and NT-pro BNP is high value in the diagnosis of CRS1 in patients with AHF or ADHF.

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Keywords: Neutrophil Gelatinase-Associated Lipocalin (NGAL), Cystatin C, NT-proBNP, Cardio-Renal Syndrome (CRS1) Type 1, biomarkers.

Introduction

Background

Acute kidney injury (AKI) in the setting of acute heart failure (AHF) or acute decompensated heart failure (ADHF) is very common occurrence and was termed cardiorenal syndrome type 1 (CRS1)[14]. CRS is a disorder of the heart and kidneys that can cause acute or chronic dysfunction of one organ to cause another. CRS was divided into 5 types, of which the first type is called acute cardiorenal syndrome, which is an acute cardiac dysfunction leading to damage and/or acute renal dysfunction. The prevalence of cardiorenal syndrome type 1according to studies varies from 32% to 40% in patients hospitalized for episodes of ADHF[11]. It is estimated that in the United States, there will be 320,000 to 400,000 hospitalizations with CRS type 1 every year. Moreover, with the increasing number of heart failure patients, the rate of CRS type 1 will be an important issue in the future.

In the CRS type 1, the diagnosis of acute kidney damage is often delayed because of the creatinine and urine output according to KDIGO (Kidney Disease Improving Global Outcomes). Neutrophil gelatinase-associated lipocalin (NGAL) in the blood and urine is one of the earliest indicators of acute kidney injury due to ischemia or nephrotoxicity. One study showed that using NGAL in urine to diagnose acute kidney injury with 90% sensitivity and 99% specificity[13]. Neutrophil gelatinase-associated lipocalin (NGAL), a protein of the lipocalin super family, is synthesized abundantly in kidney tubules. Its expression is rapidly up regulated by ischemia-reperfusion injury in renal tubular epithelial cells, and NGAL is released into urine in an experimental model. In humans, NGAL has been recognized as a surrogate marker of AKI complicated with various diseases, including sepsis, post–cardiac surgery, and admission to the intensive care unit. In particular, a few studies reported an association between the elevation of serum NGAL levels on admission and consequent AKI in patients with chronic heart failure[13].

Cystatin C is a 13-kDa non-gylcosylated protein that is constitutively produced by all nucleated cells. It is freely filtered by the glomeruli and reabsorbed and catabolised within the proximal tubule. In contrast to plasma creatinine, Cystatin C levels were shown to not be influenced by diet, gender, body muscle mass, inflammation or malignancy. Consequently, cystatin C based formulas estimating glomerular filtration rate were shown to surpass the accuracy of creatinine based equations. Additional studies showed plasma Cystatin C to increase 24 hours earlier than serum creatinine after unilateral nephrectomy in kidney organ donors. Additionally, Cystatin C was shown to be a promising biomarker for the early detection of AKI after cardiac surgery[1].

N-terminal fragment of Brain Natriuretic Peptide (NT-pro BNP) derives from the proteolysis of pro-BNP (composed of 108 amino-acids). It consists of 76 amino-acids and recently caused great interest for its possible role in monitoring cardiac in sufficiency and in the stratification of acute coronary syndromes (ACS). Its effects on diuresis and natriuresis (in patients with congestive heart failure) represent a “compensatory” mechanism to the stress of myocytes evolving in

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ventricular dysfunction. In unstable angina NT-pro BNP represents an efficacious marker of the damage caused by cardiac ischemia. The severity of the coronary disease is evidenced by an increase of NT-pro BNP levels. In addition, in acute coronary syndromes (ACS), NT-pro BNP has a immunomodulant role and provides important prognostic information in patients evolving to heart failure[3].

The diagnostic value of plasma NGAL, Cystatin C and NT-pro BNP in diagnosis of CRS1 in AHF or ADHF patients remains poorly understood. We did this study aim to evaluate the diagnostic efficacy of combined plasma NGAL, Cystatin C and NT-pro BNP in diagnosis of CRS1 in AHF or ADHF patients.

Materials and Methods

Selection of participants

Study Population

All patients with AHF or ADHF admitted to Cardiovascular Resuscitation and Interventional Cardiology Department of 115 People Hospital in Ho Chi Minh City from November 2018 to May 2019.

Inclusion criteria for this study were adult inpatients (≥18 years old) with AHF or ADHF with or without CRS type 1

Criteria for diagnosing AHF or ADHF according to Canadian Cardiovascular Society guidelines for the management of heart failure2017[8]

Criteria for diagnosing AKI: according to KDIGO clinical practice guideline for acute kidney injury2012[10]: serum creatinine increased≥ 0,3mg/dL (≥26.5µmol/l) within 48 hoursa 50% increase in serum creatinine from the level on admission during hospitalization. Urine criteria (0.5 mL/kg per hour for 6 hours) were not utilized for AKI diagnosis because of the potential alterations in urine volume induced by therapeutic medication.

Criteria for diagnosing CRS type 1: patients suffered from AHF or ADHF developed AKI within 48 hours[15]

Exclusion criteria were not agree to participation; hospitalization period <2 days; multiple organ failure or septic shock; AKI caused by contrast; renal dialysis; kidney transplant; progressive hepatitis; acute pancreatitis; long-term use of high dose of steroids; cyclosporin; malignancy

Study design

This was a prospective cohort study

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Sample size

This was a diagnostic study, the sample size is calculated by the Buderer formula[2]:

$$n_{se} = \frac{Z^2_{\alpha} x\ P_{se} x\ (1-P_{se})}{w^2 x\ P_{dis}} \quad \text{and} \quad n_{sp} = \frac{Z^2_{\alpha} x\ P_{sp} x\ (1-P_{sp})}{w^2 x\ (1-P_{dis})}$$

where :

n_{se}: estimated sample size to estimate for sensitivity

n_{sp}: estimated sample size to estimate for specificity

P_{se} : the reference sensitivity according to the literature. For NGAL, this sensitivity is 100%[11]

P_{sp}: the reference specificity according to the literature. For NGAL, this specificity is equal to 86.7%[11]

P_{dis}: the rate of CRS type 1 according to F.Fabbian et al is 48.2%[9]

Z : the constant of the normal distribution, with a type I error of 5%, we have $Z^2_{\alpha} = 1.96$

W^2 : the true positive and true negative error of the 95% confidence interval, we choose W = 0.15.

The required sample size n only needs to be larger than n_{se} and n_{sp}

For NGAL, calculate $n_{se} = 31.9$ and $n_{sp} = 38$

So $n \geq 38$ patients. Therefore minimum sample size would be 38 patients

Data Collection

All patients were taken their medical history, meticulous physical examination, assessment of vital signs: pulse, systolic and diastolic blood pressure; jugular venous distention, S3, murmurs, rales, edema. It was then tested: First day serum creatinine (creatininD1) and third day (creatininD3) with Alinity c Creatinine Reagent running on Abbott's Alinity machine; plasma NGAL with Human NGAL ELISA kit 036RUO of BioPorto Diagnostics A/S Copenhagen, Denmark; NT-pro BNP with the Elecsys® pro BNP II reagent kit from Roche Diagnostics, Bromma, Sweden, running on Cobas e411 analyzer, Cystatin C blood sample was taken the next morning in a tube containing EDTA. Cystatin C is measured by means of an immuno-turbidity immunoassay. Cystatin C in the sample (serum, plasma) will combine with latex particles that have been covered on the surface by antibody layer, forming immune conjugate complex. Determine turbidity of this complex by photometric measurement at 546 nm. Based on the standard curve to calculate the concentration of Cystatin C to be analyzed in the sample, the test runs on Siemen machine, these tests were performed at the laboratory department of Medic Medical Center 254 Hoa Hao street, district 10, Ho Chi Minh City, Vietnam. Addition tests: cell blood counts, urea, AST, ALT, electrolytes panel, arterial blood gas were performed at the laborartory department of 115 People Hospital. Electrocardiography, chest X-ray, echocardiography, medications on admission and follow-up during hospital stay: length of hospital stay and in-hospital all-cause mortality. The estimated glomerular filtration rate was calculated using the 2009 CKD-EPI creatinin formula ($eGFR_{CKDEPI}$).

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Statistical Analysis

Data were processed using IBM SPSS Statistics Version 25 software, Med Calc @ version 19.0.5 software. A statistical significance level of 0.05 was used. All hypothesis testing was two-tailed. Discrete variables are expressed as counts (percentage) and continuous variables as means ± standard deviation (SD) or median and in terquartile range [IQR]. Comparision the medium of the two groups by the t-test; comparision two rates by the chi-square test; The diagnostic accuracy of the different biomarker was evaluated using receiver operating characteristic (ROC) curve analysis; using a ROC curve and calculate the AUC. The cut-off value is chosen at the highest score of You den (J) with J = Sensitivity + Specificity - 1. Calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV). The comparison of areas under (AUC) the ROC curve was performed as recommended by de Long et al.

Evaluating the correlation between two normal distribution continuous variables by Pearson and Spearman correlations if those not in normal distribution. Binary univariate logistic regression analysis between CRS1 and qualitative variables not in the normal distribute on. The variables with p value < 0.1 were selected in the multivariate logistic regression model by Wald test with backward-stepwise method.

The study was carried out according to the principles of the Declaration of Helsinki. It was approved by the local ethical committee. Written informed consent was obtained from all participants.

Results

During November 2018 and May 2019, 172 patients were initially diagnosed with AHF or ADHF. After follow-up, 33 cases were excluded from the study because they did not meet the inclusion criteria, we eventually collected 139 cases of AHF or ADHF metinclusion criteria and no exclusion criteria. Among 139 cases, there were 48 cases of diagnosis of CRS type 1 accounting for 34.5%. We were divided into two groups with CRS type 1 (CRS1, n = 48) and no CRS type 1 (Non-CRS1, n = 91). Among the group with CRS type 1, there were 04 cases without EF evaluation, 01 case without cell blood count; group without CRS type 1 had 07 cases without evaluation of EF, 01 case without cell blood count.

Demographic and clinical characteristics

Detailed baseline characteristics of the study population were summarized in **Table 1**. Mean age was 66.12 ± 15.77; minimum 20 years old and maximum 96 years old. Male/Female ratio: 1.01; BMI, median and interquartiles of the two groups were 23.44 [21.56 - 25.05], statistically significant diffence p < 0.05.

The majority of patients with a history of arterial hypertension accounted for 63.3%, followed by medical history of diabetes 36.7%, heart failure 32.6% and chronic kidney disease 15.8%. There were no differences in medical history between two groups with CRS1 and Non-CRS1, p > 0.05. However, there was a difference in the patients with Hx chronic kidney disease between the two groups, statistically significant p < 0.05.

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There were 60 cases (43.2%) were diagnosed with acute pulmonary edema; 38.8% were ADHF; 16.5% were cardiogenic shock; 56 patients (40.9%) acute myocardial infarction. There were 65 cases (50.8%) of heart failure with preserved EF ≥ 50%; 26.6% heart failure reduced EF < 40%; 22.7% heart failure mid-range EF 40-49%. There was no difference in vital signs at admission, diagnosis, type of EF-based heart failure between two groups, $p > 0.05$.

There were similarities in laboratory values at admission: neutrophil, hemoglobin, liver enzymes (AST, ALT), troponin I, arterial blood gases (pH, HCO_3^-, pCO_2, pO_2), Na^+, K^+ concentration between two the groups. However, the concentration of Urea, CreatininD1 and D3, plasm NGAL and NT-proBNP in the CRS1group were higher than the Non-CRS1 group, the differences were statistically significant $p < 0.05$. eGFR by creatinine on first day ($eGFR_{CKDEPID1}$)and third day ($eGFR_{CKDEPID3}$) in CRS1group lower than Non-CRS1group, $p < 0.05$.

The majority of patients using furosemide diuretics accounted for 77.7%, the mean dose 40 mg. Nitrates were used in 85 patients (61.2%). Only one patient (0.7%) used beta-blockers, up to 18.7% received noradrenaline. There were 2 patients (1.4%) indicated continuous renal replacement therapy in the CRS1 group, but the differences between two groups were not statistically significant $p > 0.05$. There were similarities in treatment at admission between two groups.

The length of hospital stay of the two groups with the median was 9 days, the in terquartile was 7-12 days. Length of hospital stay in the CRS1 group was longer than in the Non-CRS1 group, but this difference was not statistically significant $p > 0.05$. In-hospital mortality or serious illness was 21 cases, accounting for 15.1%. In-hospital mortality/serious illness were higher in the CRS1 group compared with the Non-CRS1 group, $p < 0.05$.

Table 1. Demographic and clinical characteristics

Varables	**Total (n=139)**	**CRS1 (n=48)**	**Non-CRS1 (n=91)**	**P value**
Age (years)	66.12 ± 15.77	64.06 ± 15.29	67.19 ± 15.98	0.27
Male	70(50.4)	24(51.4)	46(50)	0.95
Body Mass Index* (kg/m^2)	**23.44 [21.56 – 25.05]**	**24.29 [22.5 - 25.82]**	**23.44[21.33 - 24.38]**	**0.037**
Medical History				
Aterial Hypertension	88 (63.3)	34 (70.8)	54 (59.3)	0.18
Diabetes mellitus	51 (36.7)	20 (42.6)	31 (33.7)	0.38
Dyslipidemia	9 (6.5)	4 (8.5)	5 (5.4)	0.49
Smoking	14 (10.1)	5 (10.4)	9 (9.9)	0.92
Alcohol drink	1 (0.7)	1 (2.1)	0 (0)	0.17
IHD/old MI	42 (30.2)	15 (31.3)	27 (29.7)	0.85
DCM	5 (3.6)	2 (4.2)	3 (3.3)	0.56
Valve heart diseases	25 (18)	5 (10.4)	20 (21.9)	0.092
Heart Failure	45 (32.6)	17 (35.4)	28 (30.8)	0.61
CKD	**22 (15.8)**	**12 (25)**	**10 (10.9)**	**0.031**
Stroke	10 (7.2)	4 (8.3)	6 (6.6)	0.74
Vital signs at admission				
Heart rate (beats/min)	102 [88 – 114]	98 [84 -115]	104 [90 – 114]	0.89
BP (mmHg)				
Systolic	120 [90 – 140]	120 [90 – 140]	110 [100 – 140]	0.79
Diastolic	70 [60 – 80]	70 [60 – 80]	70 [60 – 80]	0.29
Mean	86.67 [70-100]	86.67 [70-100]	86.67 [73.33-100]	0.58
Oxygen saturation (%)**	90 [86-95]	90 [87-96]	90 [86-94]	0.53
Diagnosis				
APE	60 (43.2)	15 (31.3)	45 (49.5)	
Cardiogenic shock	23 (16.5)	9 (18.8)	14 (15.4)	0.11
ADHF	54 (38.8)	24 (50)	30 (32.9)	
Others	2 (1.4)	0 (0)	2 (2.2)	0.66
Acute MI	56 (40.9)	18 (37.5)	38 (41.8)	
Laboratory values				
EF*** based-HF				
EF reduced	34 (26.6)	9 (20.9)	25 (29.4)	
EF mid-range	29 (22.7)	13 (30.2)	16 (18.8)	0.29
EF preserved	65 (50.8)	21 (48.8)	44 (51.8)	
Neutrophil# (K/µL)	7.84 [5.50 -10.71]	8.5 [5.37 -11.96]	7.73 [5.50–10.32]	0.39
Hb (g/dl)#	11.60 [9.98 – 13.53]	10.8 [9.13 – 13.38]	12.15 [10.4–13.60]	0.087
AST (UI/l)##	47.49 [28.98-104.83]	48.2 [30.2-106.33]	46.9 [28.58-104.83]	0.41
ALT (UI/l)##	29.7 [17.86-79.04]	33.11[17.78-85.64]	28.02 [18.08-69.20]	0.94
Ure (mmol/l)###	**9.82 [6.20 – 14.53]**	**12.67 [8.51–19.27]**	**8.09 [5.45–11.67]**	**< 0.01**
CreatininD1(mg/dl)	**1.31 [0.99 – 2.24]**	**2.44 [1.47–4.09]**	**1.08 [0.83–1.47]**	**< 0.01**
$eGFR_{CKD-EPID1}$	**47 [23 – 75.75]**	**22 [13– 44]**	**64 [38.25–84.05]**	**< 0.01**
Creatinin D3	**1.29 [0.87- 2.32]**	**2.84 [1.38-4.8]**	**1.07 [0.8 -1.44]**	**< 0.01**
$eGFR_{CKD-EPID3}$	**50 [23.25 – 79]**	**19.5 [11 – 47.5]**	**67 [38–86.50]**	**< 0.01**
Na^+ (mmol/l)	137.4 [133.48-140.48]	136.8 [130.55-138.8]	138.4[135.03-141.05]	0.49

K^+ (mmol/l)	4.05 [3.54-4.49]	4.15 [3.58-4.59]	3.96 [3.52-4.44]	0.54
NGAL (ng/ml)	**327.13[205.38-516.76]**	**511.63 [338.27-587.94]**	**262.59[193.07-401.11]**	**< 0.001**
Cystatin C (mg/l)	**1.47 [1.13-2.26]**	**2.38 [1.81-3.03]**	**1.31 [1.06-1.63]**	**<0.000**
NT-proBNP (pg/ml)	**8814 [3860-26419]**	**20131[6350-35000]**	**6378[2935.25-17177.50]**	**1**
Troponin I$^{\$}$ (pg/ml)	6156.18±13176.59	6575.08 ±13505.34	5941.86 ± 13080.16	**0.005**
pH$^{\$\$}$	7.40 ± 0.087	7.39 ± 0.099	7.42 ± 0.079	0.79
$HCO_3^{-\$\$}$ (mmol/l)	21.8 [17.85-24.98]	20.03 [16.4-23.7]	22.6 [19.1-25.98]	0.08
$pCO_2^{\$\$}$ (mmHg)	35 [29.08-40.03]	35 [27.85-40.95]	35 [29.98-39.48]	0.25
$pO_2^{\$\$}$ (mmHg)	76 [61.75-111]	75 [60-110.5]	77 [62.75-111]	0.67
				0.77
Therapy at admission				
Furosemide	108 (77.7)	36 (75)	72 (79.1)	0.58
Furosemide dose (mg)	40 (20-40)	40 (20-40)	40 (20-40)	0.50
ACEIs/ARBs use	14 (10.1)	4 (8.3)	10 (10.98)	0.62
Beta blockers	1 (0.7)	0 (0)	1 (1.1)	0.66
Dobutamin	19 (13.8)	7 (14.6)	12 (13.2)	0.84
Dopamin	7 (5)	3 (6.3)	4 (4.4)	0.64
Noradrenaline	26 (18.7)	9 (18.8)	17 (18.7)	0.99
Nitrates	85 (61.2)	28 (58.3)	57 (62.6)	0.62
Conventional oxygen	110 (79.1)	41(85.4)	69 (75.8)	0.19
Ventilation Invasive	12 (8.6)	5 (10.4)	7 (7.7)	0.59
Mechanicalventilation	13 (9.4)	5 (10.4)	8 (8.8)	0.75
CRRT	2 (1.4)	2 (4.2)	0 (0)	0.051
Hospital length of stay (days)	9 [7 – 12]	10 [7 – 12]	8 [7 – 12.75]	0.33
In-hospital all-cause mortality/serious illness	**21 (15.1)**	**12 (25)**	**9 (9.9)**	**0.018**

Data are presented as n (%); medium± SD; median [interquartile range]
*n=113; **n= 131; ***n=128; $^{\#}$n=137; $^{\#\#}$n=115; $^{\#\#\#}$n=134; $^{\$}$n=130; $^{\$\$}$n=117; EF reduced < 40%; EF mid-range 40-49%; EF preserved ≥ 50%. APE: Acute Pulmonary Edema; BP: blood Pressure; MI: Myocardial Infraction; IHD: Ischemic Heart Disease; DCM: Dilated cardiomyopathy CCRT: Continous Renal Replacement Therapy. ACEIs: Angiotensionogen Coverting Enzyme Inhibitors; ARBs: Angiotensin II Receptor Blockers.Bold indicated statistically significant. Serious illness: high risk of mortality patients were resusciated but their families asked to be discharged before death in hospital.

The value of plasma NGAL, Cystatin C and NT-pro BNP in diagnosing CRS1

The diagnostic accuracy of the three biomarkers was evaluated using receiver operating characteristic (ROC) curve analysis. The optimal cut-off point of NGAL to diagnose CRS1 was > 353.23 ng/ml, the area under the AUC curve is 0.732 (95% CI 0.65-0.80, p<0.001), sensitivity 74.47%, specificity 68.48%, positive predictive value 54.7%, negative predictive value 84%. The optimal cut-off Cystatin C for diagnosing CRS1 was > 1.81 mg/dl, AUC was 0.787 (95% CI 0.71-0.85, p <0.001), sensitivity 75%, specificity 83.52%, positive predictive value 70.6%, negative predictive value 86.4%. The optimal cut-off NT-proBNP for diagnosing CRS1 was 17681 pg/ml, AUC was 0.683 (95% CI 0.59 – 0.76, p < 0.001), sensitivity 53.19%, specificity 77.17%, positive predictive value 54.3%, negative predictive value 76.3%**(Table 2).**

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Table 2. Cut-off point, sensivity, specificity, AUC of plasma NGAL, Cystatin C and NT-pro BNP in diagnosing CRS1

Variable	Cut-off point	Sensivity Se (%)	Specificity Sp (%)	Area Under Curve(AUC)	Confident Interval (CI) 95%	P value
NGAL (ng/ml)	>353.23	74.47	68.48	0.73	0.65 -0.80	<0.001
Cystatin C (mg/l)	> 1.81	75	83.52	0.79	0.71-0.85	< 0.001
NT-pro BNP (pg/ml)	> 17681	53.19	77.17	0.68	0.59 – 0.76	< 0,001

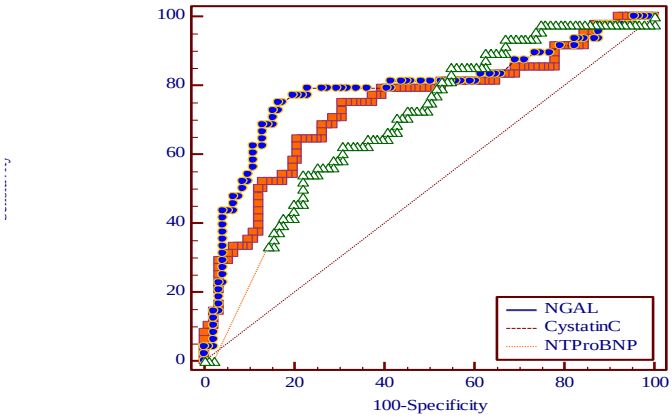

Figure 1. Cut-off point, sensivity, specificity of plasma NGAL, Cystatin C and NT-pro BNP for diagnosing CRS1

The correlation between CRS1 and some factors

To investigate the correlation between CRS1 and several factors, we conducted Pearson correlation analysis if variables was normal distribution and otherwise Spearman rank was used. As a result, there were 7 variables correlating to CRS1 (**Table 3**).

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Table 3. The correlation between CRS1 and some factors

Variables	Coefficients Pearson r or Spearman rho	P value
Age (years)	-0.11	0.20
Sex (Male/Female)	-0.01	0.91
Heart rate (beats/min)	-0.09	0.29
Systolic blood pressure (mmHg)	-0.032	0.71
Diastolic lood pressure (mmHg)	0.14	0.11
Mean blood pressure (mmHg)	0.004	0.96
Hb (g/dl)	-0.13	0.14
Ure (mmol/l)	**0.32**	**< 0.001**
Creatinin D1(mg/dl)	**0.38**	**< 0.001**
$eGFR_{CKD-EPID1}$	**-0.48**	**< 0.001**
NGAL (ng/ml)	**0.40**	**< 0.001**
Cystatin C (mg/l)	**0.48**	**< 0.001**
NT-proBNP (pg/ml)	**0.22**	**0.01**
Hx Chronic Kidney Disease	**0.19**	**0.025**
Hx Hypertension	0.10	0.23
Hx Diabetes mellitus	0.087	0.31
Hx Heart failure	0.022	0.79
Atrial fibrilation	-0.03	0.71

Bold indicated statistically significant. Hx: history

Univariable and multivariable logistic regression between CRS1 and some variables

Seven variables correlated with CRS1 were analysed by univariable logistic regression. The variables with p value < 0.1 were selected in the multivariate logistic regression model by Wald test with backward-stepwise method. During multivariable regression analysis $eGFR_{CKD-EPID1}$ remained the strongest independent predictor of CRS1 (OR 0.96; 95%CI 0.94-0.98; p <0.001). Plasma NGAL, Cystatin C and NT-pro BNP failed to predict the occurrence of early CRS1 **(Table 4).**

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Table 4. Univariable and multivariable logistic regression between CRS1 and some variables

Univariable logistic regression				
Predictors	**β**	**SE**	**Odds Ratio(CI 95%)**	**P value**
Ure (mmol/l)	0.10	0.031	1.11 (1.04-1.18)	0.001
Creatinin D1(mg/dl)	0.63	0.16	1.87 (1.38-2.54)	< 0.001
$eGFR_{CKD\text{-}EPI\ D1}$	-0.045	0.009	0.96 (0.94-0.97)	< 0.001
NGAL (ng/ml)	0.005	0.001	1.005 (1.0029-1.0074)	< 0.001
Cystatin C (mg/l)	1.14	0.24	3.13 (1.93-5.06)	< 0,001
NT-proBNP (pg/ml)	0.000	0.000	1.00	0.016
Hx CKD	1.034	0.47	2.81 (1.11-7.11)	0.029
Multivariable logistic regression				
Predictors	**β**	**SE**	**Odds Ratio(CI 95%)**	**P value**
$eGFR_{CKDEPI}$	-0.047	0.009	0.95 (0.94-0.97)	0.0000

Multivariable analysis included all significant candidate variables ($p< 0.1$) identified in univariate analysis.

Combination NGAL, Cystatin C and NT-proBNP in diagnosing CRS1

Plasma NGAL, Cystatin C and NT-proBNP levels dichotomized at the cut-off points in diagnosing CRS1. NGAL-, Cystatin C-, NT-proBNP-denotes NGAL, Cystatin C, NT-proBNP levels below the cut-off point; NGAL+, Cystatin C+, NT-proBNP+ denoted NGAL, Cystatin C, NT-proBNP levels above the cut-off point. We built 2x2 tables for each combination (NGAL and Cystatin C; NGAL and NT-proBNP; Cystatin C and NT-proBNP; NGAL, Cystatin C and NT-proBNP) to calculate sensivity, specificity, PPV, NPV, LR(+), LR(-).

When a combination of 2 or 3 biomarkers was used, the sensitivity of the diagnosis would decrease, while the specificity of the diagnosis would increase compared to one biomarker. Combination NGAL and Cystatin C had highest sensivity 70.83% and Likelihood ratio (+) 6.45. When combining all three biomarkers NGAL, Cystatin C and NT-proBNP, the specificity of diagnosis was highest 95.6%, positive predictive value was highest 84.62%. The results were presented in **Table 5**.

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Table 5. Combination NGAL, Cystatin C and NT-pro BNP in diagnosing CRS1

Combination	Sensivity (%)	Specificity (%)	PPV (%)	NPV (%)	Likelihood Ratio(+)	Likelihood Ratio(-)
NGAL+and NTproBNP+	45.83	86.81	64.71	75.24	3.21	0.64
NGAL+and Cystatin C+	70.83	89.01	77.27	85.26	6.45	0.33
Cystatin C+and NTproBNP+	50	91.2	75	77.57	5.56	0.55
NGAL+, Cystatin C+, and NTproBNP+	45.83	95.6	84.62	76.99	2.81	0.65

NGAL (+): >353,23 ng/ml; Cyastatin C (+): >1,81 pg/ml; và NTproBNP (+): 17681pg/ml

Discussion

The mean age of patents was 66.12 ± 15.77. The percentage of female patients with AHF or ADHF in our study was 49.6% lower than the study of the author Breidthardt T et al[16] which mean age was 79 [71-85]. When compared with other studies, our results are similar to those of author Belziti César A et al[7] which percentage of women was 43%. The male rate was similar to that of Margarida et al[6] was 47.9% by Nakada et al[13], 59.6% by Alan S. Maisel et al[12], 61% ($p > 0.05$); however, the male rate was lower than that of Aghel et al[5] 68% ($p < 0.05$).

Tachycardia at admission with median was 102beats/minute and in terquartile was [88-114]. This result is higher than the research results of Margarida et al[6]. Systolic blood pressure 120 [90-140]mmHg, diastolic blood pressure 70 [60-80]mmHg is lower than the research results of Nakada et al. systolic blood pressure 144.1 ± 34.5 and diastolic 81.8 ± 19.4[13]. This is explained by the fact that our study included all patients with AHF and ADHF while the study by Nakada et al. only in patients with ADHF. Diagnosed acute pulmonary edema accounts for 43.2%; 38.8% were diagnosed with ADHF; cardiogenic shock accounted for 16.5%. 56 patients (40.9%) were diagnosed with acute myocardial infarction. There are similarities in vital signs at admission, diagnosis between two groups with CRS1 and Non-CRS1. This is also explained by the fact that both groups were patients with AHF or ADHF.

There was a similarity in subclinical features at admission: left ventricular ejection fraction EF. neutrophil, hemoglobin between the two groups CRS1 and Non-CRS1. However, ure concentration, creatininD1 and D3, NT-proBNP, NGAL in the CRS1 group were higher than the Non-CRS1 group. Sodium concentration, $eGFR_{CKDEPID1}$, $eGFR_{CKDEPID3}$ in the CRS1 group were lower than the Non-CRS 1 group, $p < 0.05$. This result was similar to the research result of Nakada et al. with Hb 11.6 ± 2.4 g/dl; Na 138.6 ± 4.3mEq/l; eGFR 45.9 ± 24.3ml/min/1.73m^2[13]. Cystatin C level in our study was similar to result of study of the author Breidthardt T et al[1].

Plasma NGAL concentrations in the CRS1 group 506.49 [322.51-591.80] ng/ml was higher than in the Non-CRS1 group 1263.89 [193.07-409.46] ng/ml, $p < 0.001$. Cut-off point was > 353.23 ng/ml. the area under the AUC curve was 0.732 (95% CI 0.65-0.80, $p < 0.001$), sensitivity

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74.47%, specificity 68.48%, positive predictive value 54.7%, negative predicted value 84%. The plasma NGAL concentration and cut-off point for the diagnosis of CRS1 in our study was higher than that of the author Margarida et al[6] in the CRS1 group which was 212 [189- 307]ng/ml and in the Non-CRS1 group was 83 [60-136] ng/ml with a cut-off point > 170 ng/ml. This may be explained by the different NGAL test kit, so the results will be different. The optimal cut-off Cystatin C for diagnosing CRS1 was > 1.81 mg/dl, AUC was 0.787 (95% CI 0.71-0.85, p <0.001), sensitivity 75%, specificity 83.52%, positive predictive value 70.6%, negative predictive value 86.4%. AUC of Cystatin C for predict CRS1 was higher than result of study of the author Breidthardt T et al[1] AUC=0,67 (0.58-0.76). The AUC=0.68 of NT-pro BNP lowest among 3 biomarkers was poor for for preditingCRS1. Natriuretic peptides have a clearly established role in the diagnosis and risk stratification of patients presenting with acute and chronic heart failure, but their diagnostic ability CSR1 was poor.

When entering the biomarkers into a univariate binary regression analysis plasma NGAL and Cystatin C levels as well as NT-pro BNP predicted the occurrence of early CRS1**(Table 4)**. During multivariable regression analysis admission $eGFR_{CKDEPID1}$ remained the strongest independent predictor of CRS1. All of 3 biomarkers failed to predict the occurrence of early CRS1. But their diagnostic ability can be further improved in multimarker approaches integrating different pathophysiological pathways: NGAL predict acute kidney injury, Cystatin C predict renal dysfunction and NT-pro BNP can diagnose, prognosis, treatment follow-up in acute anf heart failure. Therefore, in this study, the combined assessment of NGAL, Cystatin C and BNP identified combined (NGAL+Cystatin C) had highest sensivity 70.83%, Likelihood Ratio highest 6.45; combined (NGAL+Cystatin C and NT-pro BNP) had highest specificity 95.6%, Sackett et al[4] introduced Mnemonic SpPIn i.e when specificity is high, a positive test rules in the diagnosis. Therefore, combination of 3biomarker NGAl, Cystatin C and NT-pro BNP had high value in diagnosing CRS1 when all of them were positive.

Study Limitations

There are several limitations to the study. First, this study was conducted in a single center in Vietnam, limiting the external validity to other centers with different settings. Second, most patients are seriously ill so they have not been fully assessed for hospitalization because ADHF may not be admitted to cardiac resuscitation department. Third, some kidney diseases (such as urinary tract infections or immune diseases) can also lead to an increase in NGAL levels. Although we had tried to eliminate these patients with a history and physical examination, they were still not completely controlled. Fourth, we did not measure hemodynamics or more accurate measurements of glomerular filtration rate to directly link the increased NGAL level to the compensatory kidney condition. Fifth,

Our sample size is still relatively small and there were some missing data. Sixth, we only evaluate for CRS1 within 48 hours, so we can skip cases with CRS1 after 48 hours to 7 days. Lastly, we only tested plasma NGAL once in the first day but did not test after 48 hours and before discharge to assess the variability of plasma NGAL concentration compared with creatinine concentration.

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Conclusion

The combined plasma NGAL, Cystatin C and NT-pro BNP had high value in the diagnosis of CRS1 in patients with AHF or ADHF

Disclosures

Nothing to declare

Ethical Approval

The study was approved by the Medical Ethics Committee of Hue University of Medicine and Pharmacy, Hue University (ethical approval code: H2018/13).

References

Breidthardt T., Z. Sabti, R. Zilleret al. (2017), "Diagnostic and prognostic value of cystatin C in acute heart failure", Clin Biochem. 50(18), pp. 1007-1013.

Buderer Nancy M Fenn (1996), "Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity", Academic Emergency Medicine. 3(9), pp. 895-900.

Federico Cacciapuoti (2010), "Natriuretic Peptide system and cardiovascular disease", Heart views : the official journal of the Gulf Heart Association. 11(1), pp. 10-15.

Sackett D Haynes R, Guyatt G, Tugwell P. (1992), Clinical Epidemiology: A Basic Science for Clinical Medicine, Boston, MA: Little Brown.

Arash Aghel, Kevin Shrestha và Wilfried Mullens (2010), "Serum neutrophil gelatinase-associated lipocalin (NGAL) in predicting worsening renal function in acute decompensated heart failure", J Card Fail. 16, pp. 49-54.

Margarida Alvelos (2011), "Neutrophil Gelatinase-Associated Lipocalin in the Diagnosis of Type 1 Cardio-Renal Syndrome in the General Ward", Clin J Am Soc Nephrol. 6(3), pp. 476-481.

César A Belziti, Rodrigo Bagnati, Paola Ledesmaet al. (2010), "Worsening renal function in patients admitted with acute decompensated heart failure: incidence, risk factors and prognostic implications", Revista Española de Cardiología (English Edition). 63(3), pp. 294-302.

Justin A. Ezekowitz, Eileen O'Meara, Michael A. McDonaldet al. (2017), "2017 Comprehensive update of the Canadian Cardiovascular Society guidelines for the management of heart failure", Canadian Journal of Cardiology. 33(11), pp. 1342-1433.

F. Fabbian, M. Pala, A. De Giorgiet al. (2011), "Clinical Features of Cardio-Renal Syndrome in a Cohort of Consecutive Patients Admitted to an Internal Medicine Ward", The Open Cardiovascular Medicine Journal. 5, pp. 220-225.

John A Kellum, Norbert Lameire, Peter Aspelinet al. (2012), "KDIGO clinical practice guideline for acute kidney injury", Kidney Int. 2(1), pp. 138.

Johan P.E. Lassus, Markku S. Nieminen, Keijo Peuhkurinenet al. (2010), "Markers of renal function and acute kidney injury in acute heart failure: definitions and impact on outcomes of the cardiorenal syndrome", Eur Heart J. 31, pp. 2791-2798.

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Alan S. Maisel, Christian Mueller, Robert Fitzgeraldet al. (2011), "Prognostic utility of plasma neutrophil gelatinase associated lipocalin in patients with acute heart failure: The NGAL EvaLuation Along with B-type NaTriuretic Peptide in acutely decompensated heart failure (GALLANT) trial", Eur J Heart Fail. 13, pp. 846-851.

Yasuki Nakada, Rika Kawakami, Masaru Matsuiet al. (2017), "Prognostic Value of Urinary Neutrophil Gelatinase-Associated Lipocalin on the First Day of Admission for Adverse Events in Patients With Acute Decompensated Heart Failure", J Am Heart Assoc. 6, pp. e004582.

.Kurt W. Prins, Thenappan Thenappan, Jeremy S. Markowitzet al. (2015), "Cardiorenal syndrome type 1: Renal Dysfunction in Acute Decompensated Heart Failure", J Clin Outcomes Manag. 22(10), pp. 443-454.

Claudio Ronco và Luca Di Lullo (2016), "Cardiorenal Syndrome in Western Countries: Epidemiology, Diagnosis and Management Approaches", Kidney Dis,. 2, pp. 151-163.

Breidthardt Tobias, Socrates Thenral, Noveanu Markuset al. (2011), "Effect and clinical prediction of worsening renal function in acute decompensated heart failure", Am J Cardiol. 107(5), pp. 730-5.