Clinica Chimica Acta 494 (2019) 8–13

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Clinical determination of serum nardilysin levels in predicting 30-day mortality among adults with malignant cerebral infarction

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ARTICLE INFO

Keywords:
Cerebral infarction
Ischemic stroke
Nardilysin
Prognosis
Severity
Mortality
Inflammation

ABSTRACT

Background: Nardilysin, a kind of metalloendopeptidase, plays an important role in numerous inflammatory diseases. Malignant cerebral infarction (Glasgow coma scale score of < 9) is associated with a high mortality risk. Here, we intended to investigate the relationship between serum nardilysin levels and prognosis of patients with malignant cerebral infarction.

Methods: Serum nardilysin concentrations were quantified at malignant cerebral infarction diagnosis moment in 105 patients and at study entrance in 105 healthy controls. Association of nardilysin concentrations with 30-day mortality and overall survival was estimated using multivariate analyses.

Results: The patients exhibited substantially increased serum nardilysin concentrations, as compared to the controls. Nardilysin concentrations were in pronounced correlation with Glasgow coma scale scores and serum C-reactive protein concentrations. Serum nardilysin was independently predictive of 30-day mortality and overall survival. Under receiver operating characteristic curve, its high discriminatory ability was found.

Conclusions: Rising serum nardilysin concentrations following malignant cerebral infarction are strongly related to stroke severity, inflammatory extent and a higher risk of mortality, substantializing serum nardilysin as a potential prognostic biomarker for malignant cerebral infarction.

1. Introduction

Ischemic stroke is the most common cerebrovascular disease, while cerebral infarction is the most lethal type of ischemic stroke, resulting in severe health burden, death and disability [1–3]. Although the mechanisms underlying brain injury following ischemic stroke were very complex and even remain unclear, it is admitted that inflammation should be involved in this process [4–6]. Inflammation can damage blood-brain barrier, induce brain edema, cause neuronal cell death, and eventually impair neurological function [7–9]. Malignant cerebral infarction (MCI) is complete blockage of the middle cerebral artery, carotid artery or cerebral cortical branches, characterized by severe brain edema, intracranial hypertension and a high risk of mortality [10–15]. Clinically, Glasgow coma scale (GCS) score can be estimated to reflect impaired consciousness accurately [16]. Generally, MCI is diagnosed when cerebral infarction patients have GCS score of < 9 [17–21]. Of note, in recent decades, circulating biomarkers have drawn researchers' attention for the assessment of MCI severity and prognosis [17–21].

N-arginine dibasic convertase, abbreviated as nardilysin, is a metalloendopeptidase of the M16 family, which functions to ectodomain shedding of multiple membrane proteins such as tumor necrosis factor-α and heparin-binding epidermal growth factor-like growth factor [22–24]. Nardilysin plays an important role in inflammation, metaplasia and tumors [25–28]. There is the accumulating evidence showing the role of nardilysin in diabetes, liver fibrosis, and autoimmune arthritis [28–31]. Recently, this biomarker has been reported to be valuable in early diagnosis of acute coronary syndrome [32]. Also, its increased serum concentration at admission is associated with a higher risk of all-cause mortality for ST-elevation myocardial infarction patients [33]. Interestingly, nardilysin may have roles in human brain diseases, such as Alzheimer's disease, Down syndrome, schizophrenia, mood disorders and alcohol abuse [34–39]. In addition, although it is unclear regarding the exact role of nardilysin in cardiovascular

Abbreviations: GCS, Glasgow coma scale; MCI, malignant cerebral infarction

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https://doi.org/10.1016/j.cca.2019.03.1608
Received 17 February 2019; Received in revised form 7 March 2019; Accepted 8 March 2019
Available online 11 March 2019
0009-8981/

Table 1
Characteristics of patients with malignant cerebral infarction.

	All patients (n = 105)	Non-survivors (n = 50)	Survivors (n = 55)	P value
Gender male	61 (58.1%)	28 (56.0%)	33 (60.0%)	NS
Age (y)	63 (55–74)	70 (60–74)	63 (55–73)	0.036
Body mass index (kg/m^2)	25.1 (23.8–26.8)	25.3 (24.0–26.6)	25.1 (23.2–26.8)	NS
Smoking	49 (46.7%)	25 (50.0%)	24 (43.6%)	NS
Atrial fibrillation	75 (71.4%)	37 (74.0%)	38 (69.1%)	NS
Hypertension	79 (75.2%)	34 (68.0%)	45 (81.8%)	NS
Diabetes mellitus	25 (23.8%)	14 (28.0%)	11 (20.0%)	NS
Hyperlipidemia	28 (26.7%)	16 (32.0%)	12 (21.8%)	NS
Cardiac insufficiency	22 (21.0%)	12 (24.0%)	10 (18.2%)	NS
Coronary artery disease	15 (14.3%)	7 (14.0%)	8 (14.6%)	NS
Chronic kidney disease	9 (8.8%)	4 (8.0%)	5 (9.1%)	NS
Thrombolysis	34 (32.4%)	19 (38.0%)	15 (27.3%)	NS
Midline shift (mm)	9 (4–12)	10 (5–13)	7 (3−11)	0.027
Hemorrhagic transformation	20 (19.1%)	13 (26.0%)	7 (12.7%)	NS
Decompressive craniectomy	22 (21.0%)	14 (28.0%)	8 (14.6%)	NS
Glasgow coma scale score	6 (5–7)	5 (4–6)	7 (6–8)	< 0.001
Systolic arterial pressure (mmHg)	170 (157–187)	172 (157–190)	159 (156–186)	NS
Diastolic arterial pressure (mmHg)	101 (95–106)	103 (96–106)	99 (89–105)	NS
Blood glucose (mmol/L)	11.9 (10.0–15.9)	13.2 (10.8–18.5)	11.8 (8.5–14.2)	0.003
Serum C-reactive protein (mg/L)	13.3 (11.7–17.5)	16.3 (11.9–22.0)	12.2 (9.7–13.4)	< 0.001
Lactic acid (mmol/L)	1.24 (1.14–1.39)	1.25 (1.12–1.38)	1.24 (1.14–1.40)	NS
Blood white blood cell count ($\times 10^9$/L)	8.1 (6.6–10.8)	8.9 (6.6–10.9)	7.5 (6.1–9.9)	NS
Blood platelet count ($\times 10^9$/L)	141 (119–175)	129 (116–158)	157 (126–179)	0.026
Serum nardilysins > 3.34 ng/mL	61 (58.1%)	44 (88.0%)	17 (30.9%)	< 0.001

Continuous variables and categorical variables were reported in form of median (25th to 75th percentiles) and frequency (percentage) respectively. Intergroup comparisons were done using the chi-square test or Mann–Whitney U test as appropriate.

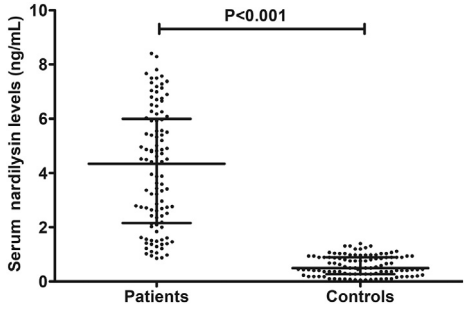

Fig. 1. Graph displaying the significant difference of serum nardilysin concentrations between malignant cerebral infarction patients and controls. This shows that serum nardilysin concentrations were significantly higher in malignant cerebral infarction patients than in controls.

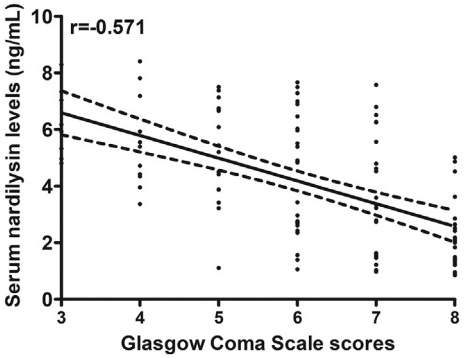

Fig. 2. Graph illustrating the substantial correlation of serum nardilysin concentrations with Glasgow coma scale score at the moment for diagnosis of malignant cerebral infarction. This shows that serum nardilysin concentrations were strongly correlated with Glasgow coma scale score at the diagnosis moment of malignant cerebral infarction.

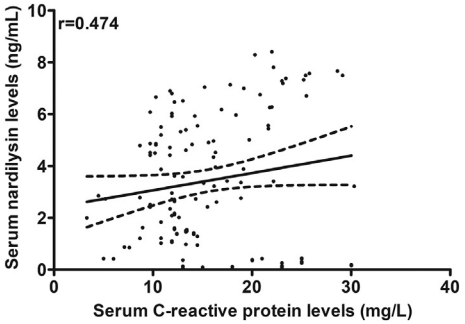

Fig. 3. Graph outlining the strong association of serum nardilysin concentrations with serum C-reactive protein concentrations at the moment for diagnosis of malignant cerebral infarction. This shows that serum nardilysin concentrations were intimately related to serum C-reactive protein concentrations at the diagnosis moment of malignant cerebral infarction.

diseases, for instance, acute myocardial infarction and acute coronary syndrome, it has been strongly supposed that nardilysin should play a role in their inflammatory processes [32,33].

2. Materials and methods

2.1. Subjects

Between January 2014 and July 2017, we performed a prospective, observational study at the Taizhou First People's Hospital, Taizhou, China. Patients admitted to our hospital and meeting the following criteria would be enrolled in this study. According to entry criteria, the patients should (1) be > 18 y; (2) be diagnosed with massive cerebral infarction by computed tomography or magnetic resonance imaging; (3) have GCS score < 9; (4) have available determination of serum nardilysin concentrations at MCI diagnosis moment; (5) be aware of the study and willing to participate; (6) have no loss to follow-up. We excluded patients with pregnancy, coexisting inflammatory or malignant diseases, or previous stroke, brain tumor or brain trauma. Simultaneous, subjects free of other diseases were selected as controls. The study was approved by the ethics committee of the Taizhou First People's Hospital and conducted in according with the Declaration of

Helsinki. The family members signed the informed consent.

2.2. Assessment

We collected some demographic, clinical and biochemical variables for each patient. Demographic data included age, sex and body mass index. Vascular risk factors consisted of smoking, atrial fibrillation, hypertension, diabetes mellitus, hyperlipidemia, cardiac insufficiency, coronary artery disease and chronic kidney disease. Severity was evaluated using GCS scores [17–21]. Other variables contained thrombolysis, midline shift, hemorrhagic transformation, decompressive craniectomy, glycemia, lactic acid, blood platelet count, blood white blood cell count and serum C-reactive protein concentrations. The study end-point was death within 30 days after MCI.

2.3. Nardilysin test

Upon the MCI diagnosis, patient blood sample was acquired immediately. And, at study entry, control blood sample was collected at once. Serum samples were separated from blood by centrifuging at $3000 \times g$ for 10 min, and afterwards, were preserved at $-80\,^{\circ}C$ until measurement. Every 3 months, nardilysin concentrations were gauged in duplicate samples using an enzyme-linked immunosorbent assay kit (MyBioSource, Inc.). In accordance with the manufacturer's instructions, the absorbance value was measured on a microplate reader set at a wavelength of 540 nm. Standard curve was plotted to obtain the absolute value of nardilysin via comparing to the standard sample. For statistical analyses, we calculated the mean values of two measurements. All determinations were completed by the same laboratory technician inaccessible to all clinical data.

2.4. Statistical analysis

Data were analyzed statistically using The Statistical Package for the Social Sciences ver 20.0. Continuous and categorical variables were presented as medians (interquartile ranges [IQRs]) and frequencies (percentages) respectively. Intergroup comparisons for continuous and categorical variables were done utilizing Wilcoxon–Mann–Whitney test and chi-square test respectively. Bivariate correlation analysis was completed using Spearman correlation coefficients. A receiver operating characteristics curve was established to identify an optimal cut-off points of nardilysin concentrations, which yielded the corresponding sensitivity and specificity values to discriminate the patients at risk of death within 30 days following MCI. To investigate its predictive value, area under curve (AUC) and the corresponding 95% confidence interval (CI) values were reported. 30-day overall survival was estimated using the Kaplan–Meier method. Serum nardilysin concentrations were dichotomized in terms of the cut-off value generated from ROC curve. Using the log-rank test, survival time was compared between patients with serum nardilysin concentrations more than the cut-off value and those with serum nardilysin concentrations less than the cut-off value. At first, univariate analysis was performed to verify the factors associated with 30-day mortality or 30-day overall survival. Then, those factors, which were significant ($P < .1$) in univariate analysis, were incorporated into multivariate binary logistic regression model or multivariate Cox's proportional hazard model to identify variables independently associated with 30-day mortality or overall survival. Odds ratio (OR) and hazard ratio (HR) values and their corresponding 95% CI values were presented. All $p < .05$ were considered statistically significant.

3. Results

3.1. Study population characteristics

During the study period, a total of 134 patients fulfilled entry criteria. According to the exclusion criteria, twenty-nine patients were removed on account of the following reasons: pregnancy (3 cases), coexisting inflammatory or malignant diseases (12 cases) and previous stroke, brain tumor or brain trauma (14 cases). Ultimately, 105 patients were eligible for the study requirements. Alternatively, 105 healthy individuals were enrolled as the controls. In terms of age, gender and body mass index, there were not significant differences between the controls and the eligible patients.

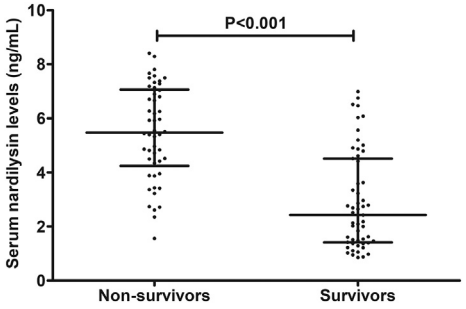

Fig. 4. Graph showing the difference of serum nardilysin concentrations between the dead and the alive within 30 days following malignant cerebral infarction. This shows that serum nardilysin concentrations were substantially increased in the dead, as compared to the alive at 30 days after malignant cerebral infarction.

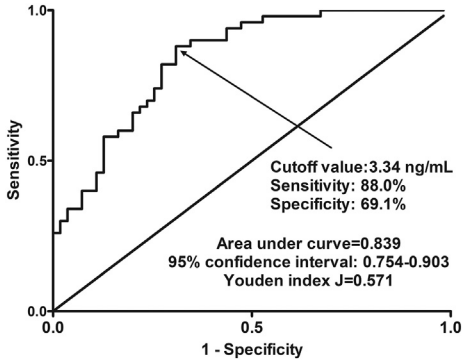

Fig. 5. Graph portraying the predictive ability of serum nardilysin concentrations for 30-day mortality under receiver operating characteristic curve in patients with malignant cerebral infarction. This shows that serum nardilysin concentrations exhibited a considerably high predictive value for 30-day mortality after malignant cerebral infarction in accordance with area under receiver operating characteristic curve.

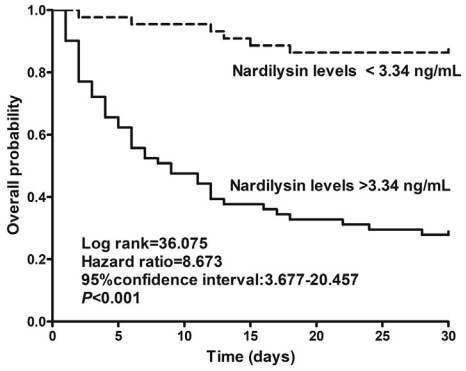

Fig. 6. Graph depicting the difference of 30-day survival probability between patients with serum nardilysin concentrations > 3.34 ng/mLand the remainders based on survival curve among patients with malignant cerebral infarction. This shows that malignant cerebral infarction patients with serum nardilysin concentrations > 3.34 ng/mL displayed a remarkably shorter 30-day survival overall time than other remaining ones based on survival curve.

Table 2
Factors associated with 30-day mortality and overall survival using univariable analysis.

Factors	30-day mortality		30-day overall survival	
	OR (95% CI)	*P* value	HR (95% CI)	*P* value
Gender male	0.848 (0.390–1.844)	NS	0.870 (0.498–1.520)	NS
Age (y)	1.041 (0.999–1.085)	NS	1.031 (0.999–1.064)	NS
Body mass index (kg/m^2)	1.080 (0.924–1.261)	NS	1.060 (0.948–1.186)	NS
Smoking	1.292 (0.599–2.787)	NS	1.177 (0.676–2.050)	NS
Atrial fibrillation	1.273 (0.543–2.986)	NS	1.262 (0.671–2.374)	NS
Hypertension	0.472 (0.191–1.170)	NS	0.590 (0.325–1.069)	NS
Diabetes mellitus	1.556 (0.630–3.842)	NS	1.383 (0.746–2.564)	NS
Hyperlipidemia	1.686 (0.704–4.038)	NS	1.354 (0.747–2.453)	NS
Cardiac insufficiency	1.421 (0.553–3.652)	NS	1.395 (0.729–2.671)	NS
Coronary artery disease	0.956 (0.320–2.860)	NS	1.034 (0.465–2.299)	NS
Chronic kidney disease	0.870 (0.220–3.437)	NS	0.932 (0.336–2.590)	NS
Thrombolysis	1.634 (0.717–3.724)	NS	1.385 (0.782–2.452)	NS
Midline shift (mm)	1.090 (1.005–1.181)	0.036	1.054 (1.000–1.110)	0.049
Hemorrhagic transformation	1.796 (0.692–4.661)	NS	1.388 (0.738–2.612)	NS
Decompressive craniectomy	2.285 (0.865–6.034)	NS	1.746 (0.940–3.243)	NS
Glasgow coma scale score	0.213 (0.120–0.378)	< 0.001	0.445 (0.362–0.547)	< 0.001
Systolic arterial pressure (mmHg)	1.017 (1.000–1.035)	NS	1.012 (1.000–1.025)	NS
Diastolic arterial pressure (mmHg)	1.040 (1.000–1.082)	0.050	1.027 (0.998–1.058)	NS
Blood glucose (mmol/L)	1.167 (1.061–1.285)	0.002	1.093 (1.034–1.155)	0.002
Serum C-reactive protein (mg/L)	1.282 (1.144–1.437)	< 0.001	1.157 (1.100–1.217)	< 0.001
Lactic acid (mmol/L)	0.339 (0.034–3.387)	NS	0.548 (0.101–2.966)	NS
Blood white blood cell count ($\times 10^9$/L)	1.119 (0.972–1.288)	NS	1.098 (0.996–1.210)	NS
Blood platelet count ($\times 10^9$/L)	0.990 (0.980–1.000)	NS	0.992 (0.984–1.000)	0.048
Serum nardilysins > 3.34 ng/mL	16.392 (5.870–45.775)	< 0.001	8.673 (3.677–20.457)	< 0.001

Table 3
Factors associated with 30-day mortality and overall survival using multivariable analysis.

Factors	30-day mortality		30-day overall survival	
	OR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (y)	1.098 (1.008–1.197)	0.032	1.045 (1.007–1.084)	0.021
Midline shift (mm)	1.107 (0.955–1.284)	NS	1.036 (0.981–1.094)	NS
Decompressive craniectomy	2.647 (0.425–16.492)	NS	1.422 (0.712–2.838)	NS
Glasgow coma scale score	0.275 (0.126–0.602)	0.001	0.550 (0.425–0.711)	< 0.001
Systolic arterial pressure (mmHg)	1.004 (0.968–1.041)	NS	0.998 (0.983–1.014)	NS
Diastolic arterial pressure (mmHg)	1.029 (0.949–1.115)	NS	1.002 (0.968–1.038)	NS
Blood glucose (mmol/L)	1.076 (0.898–1.289)	NS	0.974 (0.908–1.044)	NS
Serum C-reactive protein (mg/L)	1.030 (0.904–1.173)	NS	1.038 (0.836–1.288)	NS
Blood white blood cell count ($\times 10^9$/L)	1.192 (0.933–1.522)	NS	1.044 (0.921–1.183)	NS
Blood platelet count ($\times 10^9$/L)	0.988 (0.973–1.004)	NS	0.995 (0.987–1.004)	NS
Serum nardilysin > 3.34 ng/mL	10.563 (1.985–56.215)	0.006	4.842 (1.782–13.153)	0.002

Among all patients containing 44 females and 61 males, the median age was 63 years (IQR, 55–74 y) and there was 25.1 kg/m^2 (IQR, 23.8–26.8 kg/m^2) at the median body mass index. Other clinical, laboratory and radiological parameters for the patients are displayed in Table 1. Totally, 50 patients (47.6%) were deceased within 30 days following MCI.

3.2. Serum nardilysin concentrations and its association with other variables

Just as depicted in Fig. 1, the patients had remarkably high median serum nardilysin concentrations, as compared to the controls. Also, among all patients, with decreasing GCS score, serum nardilysin concentrations were markedly increased (Fig. 2); in addition, there was a positive correlation between serum C-reactive protein concentrations and serum nardilysin concentrations (Fig. 3).

3.3. Assessment for 30-day mortality

Just as illustrated in Fig. 4, serum nardilysin concentrations were profoundly higher in the dying than the alive within 30 days after MCI. Then, we config.d a ROC curve (Fig. 5) to assess the discriminatory capability of serum nardilysin concentrations for the development of death within 30 days following MCI. It was demonstrated that serum nardilysin concentrations substantially distinguished the patients at risk of 30-day mortality after MCI (AUC, 0.839; 95% CI, 0.754–0.903). Alternatively, a suitable cutoff value of serum nardilysin concentrations (3.34 ng/mL) was generated from the ROC curve, which differentiated between non-survivors and survivors within 30 days with 88.0% sensitivity and 69.1% specificity (Youden index J, 0.571). Based on the preceding cutoff value of serum nardilysin concentrations (3.34 ng/mL), all patients were dichotomized. In Fig. 6, the patients with serum nardilysin concentrations > 3.34 ng/mL showed significantly shorter mean 30-day overall survival time than the remainders.

We determined associations of clinical, biochemical and radiographic parameters with 30-day mortality and overall survival using univariate analyses. It was thereof found that serum nardilysin concentrations > 3.34 ng/mL and other variables listed in Table 1 and Table 2 had intimate relation to 30-day mortality and overall survival. Afterwards, the aforementioned significant variables ($P < .1$) were incorporated to the two multivariate models, and it was thereby documented that serum nardilysin concentrations > 3.34 ng/mL and age in addition to GCS score still retained independently associated with 30-day mortality and overall survival (Table 3).

4. Discussion

MCI patients are at high risk of mortality and almost half of patients will die within 30 days after ischemic stroke [17–21]. Among this group of MCI patients, 30-day mortality was 54.5%, which is similar to the previously reported data [17–21]. Up to now, decompressive craniectomy might be a good choice for such patients [10–13]. However, because of the poor prognosis, some relatives decline decompressive craniectomy for it. Thus, early prognostic prediction might aid in its clinical decisions.

To the best of our knowledge, the current study is the first series to determine serum nardilysin concentrations in MCI patients. Subsequently, it was revealed that non-survivors exhibited apparently higher serum nardilysin concentrations than survivors. We also demonstrated that serum nardilysin concentrations were profoundly associated with degree of systemic inflammation indicated by serum C-reactive protein concentrations, clinical severity as defined by GCS scores and short-term mortality in patients with MCI.

Nardilysin belongs to the family of zinc-metalloendopeptidases that cleave doublets of basic amino acids at the N-terminal side of arginine residue of neuropeptides in vitro [22–24]. In vertebrates, nardilysin has been shown to localize to different cellular compartments, including the cell surface, nucleus, and cytosol in different cells or tissues [22–24]. Nardilysin has multiple functions, including ectodomain shedding of cell surface proteins and transcriptional coregulation in the nucleus [22–24]. In human diseases, its inflammatory properties have been studied greatly. Reportedly, nardilysin regulated activation of tumor necrosis factor-alpha and subsequent production of inflammatory cytokines in gastric cancer cells [40]. Also, nardilysin crucially regulated gastric inflammation caused by Helicobacter felis infection or forced expression of prostaglandin E2 in K19-C2mE mice [26]. Likewise, gene deletion or silencing of nardilysin in macrophages or THP-1 cells resulted in the reduction of tumor necrosis factor-alpha shedding [28]. Such evidence shows that nardilysin might be involved in inflammatory response of human diseases. In fact, nardilysin can be expressed widely in human brain neurons [41] and also participates in human brain diseases, e.g., Alzheimer's disease, Down syndrome, schizophrenia, mood disorders and alcohol abuse [34–39]. However, there are no previous data regarding serum nardilysin concentrations in patients with brain injury. Our study found that serum nardilysin concentrations were apparently higher in the deceased than in the alive. Intriguingly, the current study showed that serum C-reactive protein concentrations were intimately related to serum nardilysin concentrations. Herein, we speculated that nardilysin might be linked to inflammation after ischemic stroke.

Another interesting novel finding of our study was that serum nardilysin concentrations were closely correlated with GCS scores. Because GCS scores can accurately reflect the clinical severity of brain injury [17–21], serum nardilysin concentrations, to some extent, were able to assess the severity of cerebral infarction. However, it is unclear whether nardilysin in the peripheral blood of patients is derived from injured brain injury. So, this sort of hypothesis warrants to be affirmed in future. Additionally, we found that serum nardilysin concentrations were in strong association with early mortality in patients with MCI after controlling for CGS and other variables in two multiple analysis models. It was also revealed that serum nardilysin was a good predictor of 30-day mortality under receiver operating characteristic curve. Taken together, serum nardilysin might have the potential to assess the severity and early mortality following MMACAI.

5. Conclusions

The novel findings of our study are that non-surviving MCI patients have higher serum nardilysin concentrations than surviving MCI patients, and that there is an association between high serum nardilysin concentrations and early mortality of MCI patients. Hence, serum nardilysin might present a promising prognostic biomarker for MCI.

Acknowledgements

The authors thank all staffs in Department of Neurology and Department of Neurologic Intensive Care Unit at The Huangyan Hospital of Wenzhou Medical University (Taizhou, China) for their technical support.

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