Differential Performance of Neutrophil Gelatinase-Associated Lipocalin and Cystatin C to Predict Ischemic Stroke

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Research

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Abstract

Objective Both cystatin C (CysC) and neutrophil gelatinase-associated lipocalin (NGAL) are markers of kidney injury and may also be marker candidates for neuroinflammation. The aim of this article is to explore the relationship between kidney injury and ischemic stroke (IS).

Methods 498 IS patients were enrolled, and 173 IS-related disease control (DC) patients and 293 healthy control (HC) subjects were randomly selected. We analyzed the relationship between the levels of serum kidney function markers (including NGAL, Cre, Ure, CysC and eGFR) and the occurrence of IS.

Results When they were admitted to the hospital, the NGAL level of patients with first-onset IS was higher than that of both HC group ($z=5.964$, $P<0.001$) and DC ($z=12.191$, $P<0.001$); The level of CysC of them was higher than that of HC group ($z=5.762$, $P<0.001$), and was the similar with that of DC group ($z=1.663$, $P=0.289$). The partial correlation coefficient between NGAL and the occurrence of IS was the highest ($r_p=0.341$, $P<0.001$) in IS patients with normal kidney function. However, the partial correlation coefficient between CysC and IS was the highest ($r_p=0.460$), $P<0.001$) in IS patients with chronic kidney disease (CKD). For patients with normal kidney function, only NGAL was a risk factor for IS [OR(95%CI)=6.54(3.75,11.41)], and had the certain predictive performance AUC=0.734($z=12.928$, $P<0.001$). However, for CKD patients, CysC has better predictive performance for IS occurrence AUC=0.835 ($z=11.343$, $P<0.001$) and risk assessment ability [OR(95%CI)=5.97(2.45, 14.56)] than NGAL.

Conclusion IS is related to kidney injury and neuroinflammation. NGAL and CysC are suitable for IS prediction in patients with normal kidney function and CKD, respectively. Researchers should pay attention to the changes of NGAL and CysC for the prevention and treatment of stroke in these two types of patients, respectively.

Background

Stroke is a sudden disorder of cerebral blood circulation. According to the guidelines of the American Heart Association/American Stroke Association (AHA/ASA), it is clinically classified into hemorrhagic stroke (HS) and ischemic stroke (IS). IS is the most common type. According to a report by GBD Stroke Collaborators in 2019, stroke is the second leading cause of death and disability in the world [1], and seriously threatens human life and health. Even if a patient with a stroke survives after treatment, it may seriously affect the patient's life quality. The microvascular system of physiological structure of the brain is very similar to that of the kidney. Thus, both of them are particularly vulnerable to ischemia-reperfusion injury. Studies have confirmed the diseases that cause chronic kidney disease (CKD) and acute kidney injury (AKI), such as hypertension [2], diabetes mellitus (DM) [2], coronary artery disease (CAD) [3], hyperuricemia [4] and other chronic diseases, are also an important risk factor for stroke. Therefore, stroke patients may often be accompanied by kidney injury. In addition, patients with kidney injury may also have a high risk of stroke. In recent years, many studies have confirmed that renal function impairment is closely related to the occurrence, recurrence and outcome of stroke [5-6]. Therefore, some clinician predicted the possibility of stroke occurrence through changes in some traditional renal function markers [7]. Traditional renal function markers, such as creatinine (Cre), have many influencing factors (especially those whose serum levels are greatly affected by the compensatory ability of the kidneys). The Cre level does not increase until the renal function decreases by more than 50%, resulting in poor sensitivity to reflect renal function impairment. The Cre level of patients may still be at a normal level even if a stroke occurs. Therefore, the Cre level is poor to predict the occurrence of stroke. The estimated glomerular filtration rate (eGFR) is a more specific indicator for evaluating kidney injury. However, it is controversial to use eGFR as a predictive marker for stroke [7, 8].

With the continuous recognition of renal function impairment, many newly discovered biomarkers have been used in clinical diagnosis of nephropathy in recent years. Among them, cystatin C (CysC) and neutrophil gelatinase-associated lipocalin (NGAL) are favored by scholars. CysC is an ideal endogenous marker for evaluating glomerular filtration rate (GFR) recommended by Food and Drug Administration to the world in 2002. Its formula for calculating eGFR in combination with Cre has been recommended by Kidney Disease Improving Global Outcomes (KDIGO) for preliminary screening of CKD patients. [9]. There are many reports about the predictive ability of CysC on IS[10,11]. Studies have shown that CysC can promote the occurrence of IS through inflammation induction, cell or cytokine chemotaxis, autophagy induction, lipid oxidation and deposition [31]. However, there is no guideline or consensus recommending to use CysC to predict the occurrence of IS.

NGAL is a biomarker discovered in recent years that can predict the occurrence of AKI. It can reflect the severity of AKI to a certain extent and predict the prognosis of the disease. It has better sensitivity and specificity than Cre in the early diagnosis of AKI. Thus, it is recommended as a potential AKI marker by the tenth Acute Dialysis Quality Initiative Consensus Conference [12]. NGAL has been used for laboratory diagnosis of AKI in many clinical laboratories in China. In recent years, clinical applications have found that NGAL can not only be used for the early prediction of the occurrence of AKI [13], but also reflect the progress of CKD. In addition, its serum level gradually increases with the continuous progress of CKD [14]. Many studies have found that NGAL can also be used as a marker of neuroinflammation and related behavioral dysfunction [15,16]. The increase of NGAL in brain tissue can aggravate amyloid- β -induced toxicity, leading to neurodegenerative changes in the brainstem nucleus [17]. Iron chelator has the potential to improve neurodegenerative changes and treat central nervous system diseases, but its neuroprotective effect may be related to patient's NGAL level [18]. In clinical practices, some patients have experienced symptoms such as mild neuroinflammation and related behavioral dysfunction before the occurrence of stroke. As the progress of stroke patients continue, these symptoms will become significant. At present, few studies have reported whether NGAL as a marker of AKI and neuroinflammation is related to the occurrence of stroke.

Materials And Methods

Study Population (1) **Patients.** From March 2017 to October 2020, 498 IS patients were first diagnosed in the Stroke Center of Mianyang Central Hospital, including 280 males and 218 females, aged from 23 to 64, with a median age of 49-year-old. Inclusion criteria: (a) IS symptoms occur for the first time, such as limb weakness or numbness, facial numbness or angle of mouth, slurred speech or difficulty in understanding language, eyes staring to one side, loss or blurred vision in one or both eyes, dizziness and vomiting, severe headaches that are rare in the past, vomiting, disturbance of consciousness or convulsions. (b) The diagnosis complied with the AHA/ASA "2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke" [19]. The cranial computed tomography examination on admission ruled out the cerebral hemorrhage, but found low-density cortical infarcts or wedge-shaped lacuna edges, band-shaped low-density infarcts, unclear boundary between the basal cortex and surrounding white matter, or the boundary of the lenticular nucleus or even the entire insular lobe structure, without intracranial space-occupying lesions; or the cortex layered punctate or flaky high signal (fresh lesions) was found by diffusion-weighted magnetic resonance imaging (DWMRI). (c) Albumin to creatinine ratio (ACR) and eGFR historical examination records can be retrieved 3 months before the admission. Exclusion criteria: (a) HS patients complied with the AHA/ASA "Guidelines for the Management of Spontaneous Intracerebral Hemorrhage" [20]; (b) Relapsed IS patients; (c) IS patients with secondary AKI within 48 hours of admission. According to KDIGO 2012AKI guidelines [21], patients' internal Cre level increased by more than 26.5 $\mu\text{mol/L}$ within 48 hours, or their

urine output was less than 0.5 ml/kg/h for more than 6 hours; (d) patients with primary kidney disease; (e) patients with incomplete ACR and eGFR test records 3 months before admission. (2) Disease controls (DC). 173 patients with hypertension, DM, CAD and/or hyperuricemia were randomly selected during the same period as DC. Among them, there were 98 males and 75 females, aged from 26 to 65 years old, with a median age of 49. All DC patients excluded patients with brain trauma within 1 month, patients with primary kidney disease, patients with secondary AKI, and patients with incomplete ACR and eGFR test records 3 months before admission. (3) Healthy controls (HC). During the same period, 293 volunteers with normal liver and kidney function and negative hematuria were randomly selected as healthy controls (HC). Among them, there were 183 males and 110 females, aged from 23 to 65 years old, with a median age of 48. Both their serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are <40 U/L. Sample Collection IS patients were sampled immediately upon admission, and HC and DC subjects were sampled in the morning. We used BD Vacutainer® SST II Advance tube to draw about 5 ml of venous blood, mixed well and let it stand for about 30 min. Then, it was centrifuged at a relative centrifugal force of 1017g for 15 min to separate the serum, which was used to measure urea (Ure), Cre, CysC and NGAL within 2 h. Observed Biomarkers Measurement Ure was adopted the urease method, Cre was adopted the sarcosine oxidase method, and CysC and NGAL were adopted the transmission turbidimetric method, and were measured on the LAbOSPECT 008AS automatic biochemical analyzer (Hitachi, Japan). The detection kits were provided by Sichuan Mike Biotechnology Co., Ltd. The eGFR of subjects was calculated using the CKD-EPI CysC-Cr equation applicable to the Chinese population [22]. The ACR level data of all subjects before admission were collected retrospectively, which were calculated by the ratio of urine albumin to urine creatinine, with the unit of mg/g. Urine albumin was determined by improved transmission immunoturbidimetric method and urine creatinine by sarcosine oxidase method, and were determined on the A25 automatic special protein analyzer (Biosystems, Spain). The assay kits were all purchased from Chongqing Biostec Biological Technology Co., Ltd. Statistical Analysis SPSS19.0 and MedCalc11.5 statistical software were used for data processing. Non-normally distributed measurement data was represented by M(P25, P75), and count data was represented by n(%). The difference in medical history between IS and DC patients' was compared by χ^2 test. The differences in renal function marker levels between groups and pairwise comparisons were performed by independent sample Kruskal-Wallis test and multiple comparisons of average rank. The multiple linear regression analysis was used to analyze the close degree of the relationship between the observation index and the occurrence of IS, which was expressed by the partial correlation coefficient. The larger the value, the closer the relationship. The predictive performance of renal function markers on the occurrence of IS were analyzed by receiver operating curve (ROC), and the area under curve (AUC) represents their predictive performance. Taking DC patients as a reference, cross-tabulation was used to analyze the risk of renal function markers and IS, and odds ratio (OR) and its 95% confidence interval (CI) was used to reflect the risk. When $P < 0.05$, it indicates statistical significance.

Results

Subject Clinical Information

The clinical information of all subjects is shown in Table 1. There were no significant differences in age, gender and weight among the three groups (all $P > 0.05$). There was no significant difference between the two groups of patients in terms of hypertension, DM, CAD, hyperuricemia and CKD complications (all $P > 0.05$).

Table 1
Clinical characteristics of all subjects

Observed indicator	HC (n = 293)	DC (n = 173)	IS (n = 498)	χ^2, P
Age (Years) ▲	48(42, 52)	49(37, 58)	49(44, 53)	5.541, 0.063
Male/Female (cases)	183/110	98/75	280/218	3.153, 0.207
Weight (kg) ▲	57.2(48.7, 64.9)	57.7(50.0, 64.2)	57.1(50.0, 64.0)	0.215, 0.898
Hypertension [n(%)]	-	30(17.3)	60(12.0)	2.658, 0.103
DM [n(%)]	-	54(31.2)	124(24.9)	2.313, 0.128
CAD [n(%)]	-	100(57.8)	251(50.4)	2.531, 0.112
Hyperuricemia [n(%)]	-	38(22.0)	112(22.5)	0.001, 0.971
CKD [n(%)]	-	37(21.4)	90(18.1)	1.222, 0.543
Note: ▲ Kruskal-Wallis test was used, and χ^2 test was used for the others. "-" means not involved. CAD, coronary artery disease; DM, diabetes mellitus; CKD, chronic kidney disease.				

Table 1 Clinical characteristics of all subjects

The Renal Function Marker Level of Subjects

The experimental results of subjects group are not normally distributed due to sampling. The data was preferred by Kruskal-Wallis test, as shown in Fig. 1. Except Ure ($\chi^2 = 3.862$, $P = 0.145$), there were statistical differences in the levels of Cre ($\chi^2 = 36.695$, $P < 0.001$), CysC ($\chi^2 = 46.0304$, $P < 0.001$), eGFR ($\chi^2 = 34.072$, $P < 0.001$) and NGAL ($\chi^2 = 153.953$, $P < 0.001$) among those three groups.. The Cre ($z = 4.482$ and 5.734 , both $P < 0.001$) and CysC ($z = 5.955$ and 5.762 , both $P < 0.001$) and NGAL ($z = 3.872$ and 12.191 , both $P < 0.001$) of DC and IS groups were higher than that in the HC group. However, the level of eGFR was decreased ($z = -4.180$ and -5.587 , both $P < 0.001$). Compared with the NGAL of DC group, that of the IS group ($z = 5.964$, $P < 0.001$) increased. However, there were no significant differences in the levels of Cre ($z = 0.085$, $P = 0.932$), CysC ($z = 1.663$, $P = 0.289$) and eGFR ($z = 0.120$, $P = 0.905$) between DC group and IS group. Among those kidney markers, only the NGAL level of the IS group were higher than that of the HC group and DC group.

Figure-1 Serum levels of kidney markers in all subjects

The Relationship between the Occurrence of IS and Kidney Injury

Multiple linear regression was used to analyze the partial correlation coefficients between the occurrence of IS and the observed indicators (including kidney function markers and clinical features). As shown in Table 2, the relationship with stroke in all IS patients was NGAL > CAD > age > hyperuricemia > eGFR > CysC > Cre > DM (all $P < 0.05$). However, the relationships of gender, Ure and hypertension with stroke were not close ($P > 0.05$). Based on the presence or absence of CKD, all IS patients were divided into IS patients with normal kidney function (NKF-IS, $n = 408$) and IS patients with CKD (CKD-IS, $n = 90$). Then, partial correlation analysis was performed, respectively. The results are shown in Table 2. The relationships that related to the occurrence of stroke in NKF-IS patients was NGAL > CAD > hyperuricemia > DM > age (all $P < 0.05$). The relationships that related to the occurrence of stroke in

CKD-IS patients is CysC > eGFR Cre > NGAL > hyperuricemia > Ure > DM > hypertension > CAD > age (all $P < 0.05$). After distinguishing the kidney function status of patients, we found that the partial correlation coefficients of NGAL and stroke in NKF-IS patients were the highest, and were significantly higher than the partial correlation coefficient of other indicators except CAD ($z = 4.812-7.622$, all $P < 0.001$). The partial correlation coefficients of CysC and stroke in CKD-IS were the highest, and were significantly higher than the partial correlation coefficient of other indicators except eGFR ($z = 3.504-6.920$, all $P < 0.001$). In patients with different kidney function states, the renal function markers closely associated with IS are different.

Table 2
Correlation between IS occurrence and kidney function/comorbidity

Observed indicator	All IS vs. no-IS(n = 964)			NKF-IS vs. no-IS(n = 874) [▲]			CKD-IS vs. no-IS(n = 556) [#]		
	r_p^*	t	P	r_p^*	t	P	r_p^*	t	P
Sex	0.052	1.604	0.109	-0.065	-1.926	0.053	0.081	1.913	0.061
Age	0.160	5.006	< 0.001	0.072	2.127	0.034	0.089	2.075	0.039
Ure	0.054	1.665	0.096	-0.010	-0.283	0.777	0.182	4.325	< 0.001
Cre	0.112	3.476	0.001	0.017	0.503	0.615	0.279	6.775	< 0.001
CysC	0.118	3.668	0.000	0.056	1.634	0.103	0.460	12.087	< 0.001
eGFR	-0.137	-4.260	< 0.001	-0.025	-0.744	0.457	-0.373	-9.383	< 0.001
NGAL	0.349	11.472	< 0.001	0.341	10.653	< 0.001	0.233	5.598	< 0.001
Hypertension	0.031	0.948	0.343	0.037	1.101	0.271	0.130	3.061	0.002
DM	0.070	2.157	0.031	0.079	2.315	0.021	0.143	3.379	0.001
CAD	0.288	9.265	< 0.0001	0.284	8.702	< 0.001	0.120	2.823	0.005
Hyperuricemia	0.148	4.624	< 0.0001	0.124	3.660	< 0.001	0.215	5.133	< 0.001

Note: IS, all IS patients (n = 498); no-IS, including health controls (n = 293) and disease controls (n = 173); NKF-IS, IS patients with normal kidney function (n = 408); CKD-IS, IS patients with chronic kidney disease (n = 90). CAD, coronary artery disease; DM, diabetes mellitus. Using health controls and disease controls (all the no-IS subjects) as control, Multiple linear regression analysis was conducted for stroke occurrence [▲] in NKF-IS patients, or [#] in CKD-IS patients. * r_p is the partial correlation coefficient. A higher r_p indicates a closer relationship, while a positive (or negative) value indicates a positive (or negative) correlation.

Table 2 Correlation between IS occurrence and kidney function/comorbidity

The Predictive Performance of Kidney Function Markers on the Occurrence of IS

HC, DC and no-IS (i.e., HC + DC) were used as references, respectively. We used ROC to analyze the predictive performance of the observed five kidney function markers on the occurrence of IS, as shown in Fig. 2 and Table 3. When HC (Fig. 2B), DC (Fig. 2C), and no-IS (Fig. 2A) were used as references, NGAL had the best predictive performance for IS, with AUC of 0.761 ($z = 15.347$, $P < 0.001$), 0.711 ($z = 10.010$, $P < 0.001$) and 0.749 ($z = 14.821$, $P < 0.001$), respectively. And the AUC of NGAL were significantly higher than that of the other four markers (HC: $z = 5.902$ to 8.271 , $P < 0.001$; DC: $z = 5.243$ to 7.096 , $P < 0.001$; no-IS: $z = 6.324$ to 7.659 , $P < 0.001$). However, comparing the AUC of NGAL in different references, there was no significant difference (HC vs. DC: $z = 1.860$, $P = 0.063$; HC vs. no-IS: $z = 0.543$, $P = 0.587$; DC vs. no-IS: $z = 1.386$, $P = 0.166$). These indicated that among the five renal function markers, NGAL had the best predictive performance for the occurrence of IS. In addition, its predictive performance analysis can be performed without distinguishing HC and DC subjects.

Table 3
Diagnostic performance of serum kidney markers for IS occurrence

Observed indicator	AUC	Cut-off	Se(%)	Sp(%)	YI	z	P
IS vs. no-IS (n = 964)							
Ure	0.529(0.492, 0.565)	4.31	74.6(72.2–76.5)	33.7(30.8–37.0)	0.083	1.543	0.123
Cre	0.561(0.525, 0.598)	80.0	27.9(24.0–32.1)	88.2(84.9–91.0)	0.161	3.299	0.001
CysC	0.576(0.540, 0.612)	1.04	22.9(19.3–26.8)	95.1(92.7–96.8)	0.180	4.128	< 0.001
eGFR	0.576(0.540, 0.612)	85.0	25.1(21.3–29.2)	92.7(90.0–94.9)	0.178	4.107	< 0.001
NGAL	0.749(0.717, 0.782)	147.1	49.0(44.5–53.5)	92.9(90.2–95.1)	0.419	14.821	< 0.001
IS vs. HC (n = 791)							
Ure	0.538(0.497, 0.578) ▲	3.80	94.5(91.1–97.6)	11.4(8.0–15.7)	0.059	1.801	0.072
Cre	0.616(0.577, 0.654) ▲	80.0	27.9(19.3–26.8)	92.7(89.1–95.4)	0.207	5.879	< 0.001
CysC	0.621(0.582, 0.660) ▲	0.96	28.9(25.0–33.1)	92.7(89.1–95.4)	0.216	6.061	< 0.001
eGFR	0.618(0.578, 0.656) ▲	94.2	36.1(31.9–40.5)	85.1(80.5–89.0)	0.212	5.882	< 0.001
NGAL	0.761(0.728, 0.794) ▲	132.1	58.4(54.0–62.8)	87.5(83.2–91.1)	0.459	15.347	< 0.001
IS vs. DC (n = 671)							
Ure	0.508(0.460, 0.556) ▲	3.87	83.3(79.8–86.5)	5.4(2.5–10.0)	0.113	0.331	0.741
Cre	0.504(0.456, 0.552) ▲	93.3	13.9(10.9–17.2)	95.2(90.8–97.9)	0.091	0.150	0.880
CysC	0.531(0.487, 0.576) ▲	1.04	28.5(24.6–32.7)	89.8(84.2–94.0)	0.181	1.384	0.166
eGFR	0.509(0.462, 0.557) ▲	85.0	25.1(21.3–29.2)	86.8(80.7–91.6)	0.119	0.389	0.697
NGAL	0.711(0.671, 0.752) ▲	145.6	54.0(49.5–58.5)	93.4(88.5–96.7)	0.474	10.010	< 0.001

Note: no-IS, health controls (n = 293) and disease controls (n = 173); HC, health controls; DC, disease controls; NKF-IS, IS patients with normal kidney function (n = 408); CKD-IS, IS patients with chronic kidney disease (n = 90). ▲Compared with no-IS as control, $P > 0.05$. # Compared with NKF-IS vs. no-IS, $P < 0.05$.

Observed indicator	AUC	Cut-off	Se(%)	Sp(%)	YI	z	P
NKF-IS vs. no-IS (n = 874)							
Ure	0.504(0.465, 0.543)	3.80	90.8(87.8–93.2)	17.9(14.3–22.0)	0.087	0.186	0.852
Cre	0.519(0.480, 0.557)	61.7	59.8(54.9–64.6)	49.4(44.8–54.1)	0.092	0.823	0.410
CysC	0.532(0.493, 0.570)	1.04	15.2(12.1–18.9)	94.6(92.2–96.5)	0.098	1.144	0.252
eGFR	0.523(0.484, 0.562)	93.4	52.2(47.3–57.2)	57.3(52.7–61.8)	0.095	0.946	0.344
NGAL	0.734(0.698, 0.769)	147.1	47.3(42.4–52.3)	92.3(89.5–94.5)	0.396	12.928	< 0.001
CKD-IS vs. no-IS (n = 556)							
Ure	0.802(0.750, 0.854) [#]	5.34	90.0(81.9–95.3)	60.3(55.7–64.8)	0.503	10.190	< 0.001
Cre	0.817(0.752, 0.882) [#]	81.6	71.1(60.6–80.2)	92.1(89.2–94.3)	0.632	10.777	< 0.001
CysC	0.835(0.774, 0.896) [#]	1.14	74.4(64.2–83.1)	91.8(89.0–94.2)	0.662	11.343	< 0.001
eGFR	0.822(0.760, 0.884) [#]	85.0	68.9(58.3–78.2)	95.1(92.7–96.8)	0.640	11.261	< 0.001
NGAL	0.754(0.679, 0.830)	156.7	59.9(49.0–70.2)	96.1(94.0–97.7)	0.560	6.317	< 0.001
Note: no-IS, health controls (n = 293) and disease controls (n = 173); HC, health controls; DC, disease controls; NKF-IS, IS patients with normal kidney function (n = 408); CKD-IS, IS patients with chronic kidney disease (n = 90). [▲] Compared with no-IS as control, $P > 0.05$. [#] Compared with NKF-IS vs. no-IS, $P < 0.05$.							

Figure-2 Diagnostic performance of kidney markers for the IS occurrence under different controls

The IS patients were divided into NKF-IS patients and CKD-IS patients. The difference in the predictive performance of five kidney function markers between those two groups of IS patients was observed. As shown in Fig. 3 and Table 3, the analysis results of NKF-IS patients and overall IS patients were similar (Fig. 3A). NGAL had the best predictive performance AUC = 0.734 ($z = 12.928$, $P < 0.001$), which was significantly higher than the others four markers ($z = 7.574$ to 8.537 , $P < 0.001$). In CKD-IS patients, CysC had the best predictive performance AUC = 0.835 ($z = 11.343$, $P < 0.001$), and NGAL had the lowest AUC = 0.754 ($z = 6.317$, $P < 0.001$) (Fig. 3B). Therefore, the ability of kidney function markers to predict the occurrence of IS was different in patients with different kidney function states.

Figure-3 Diagnostic performance of kidney markers for the IS occurrence under different kidney function states

In addition, comparing the Cut-off value of the five kidney function markers at the maximum Youden index (YI) with their reference upper limit (or medical decision level), only the Cut-off values of NGAL and CysC in CKD-IS

patients (156.7µg/L and 1.14mg/L) were higher than the upper limit of the reference values. The Cut-off values in NKF-IS patients (147.1µg/L and 1.04mg/L, respectively) were close to the upper limit of the reference values. The Cut-off values of Ure and Cre were far lower than the upper limit of the reference values, and the Cut-off value of eGFR was far higher than the medical decision level. Therefore, among those five kidney function markers, NGAL and CysC may be more suitable for the prediction of occurrence of IS.

Table 3 Diagnostic performance of serum kidney markers for IS occurrence

Kidney Function Impairment and IS Risk

According to the above ROC analysis results, CysC and NGAL selected the cut-off values obtained in IS patients with corresponding kidney function status. Cut-off values of Ure, Cre, and eGFR were difficult to be clinically accepted. Thus, the reference upper limit (Ure and Cre) or the medical decision level (eGFR) recommended by KIDGO was selected as the cut-off value. The cross-tabulation was used to analyze the kidney function markers and the risk of occurrence of IS. As shown in Table 4, the results showed that the increase of NGAL [OR(95%CI) = 4.08(2.68,6.19)], CysC [OR(95%CI) = 1.98(1.22,3.22)] and eGFR [OR(95%CI) = 2.41(1.07, 5.45)] was a risk factor for the occurrence of IS in all patients (IS and DC patients). After grouping by the kidney function status of patient, only the increase of NGAL [OR(95%CI) = 6.54(3.75, 11.41)] is a risk factor of IS for NKF-IS patients. The increase of CysC [OR(95%CI) = 5.97(2.45, 14.56)] and eGFR [OR(95%CI) = 3.28(1.24, 8.69)] were risk factors of IS for CKD-IS patients. Therefore, NGAL (when renal function is normal) or CysC and eGFR (when renal function is reduced) should be paid attention to in patients with IS risk diseases with different kidney function status.

Table 4

Predictive performance of serum kidney markers for IS occurrence in patients with different kidney function states

Observed indicator	DC patients [n(%)]	IS patients [n(%)]	OR(95%CI)	z	P
All patients	(n = 173)	(n = 498)			
increased Ure	12(6.9)	35(7.0)	1.01(0.51, 2.00)	0.041	0.968
increased Cre	15(8.7)	67(13.5)	1.64(1.08, 2.95)	1.642	0.101
increased CysC	23(13.3)	116(23.3)	1.98(1.22, 3.22)	2.758	0.006
decreased eGFR	7(4.0)	46(9.2)	2.41(1.07, 5.45)	2.119	0.034
increased NGAL	33(19.1)	244(49.0)	4.08(2.68, 6.19)	6.588	< 0.001
NKF patients	(n = 136)	(n = 408)			
increased Ure	6(4.4)	11(2.7)	0.60(0.22, 1.66)	0.986	0.324
increased Cre	6(4.4)	29(7.1)	1.66(0.67, 4.08)	1.099	0.272
increased CysC	13(9.6)	54(13.2)	1.44(0.76, 2.74)	1.125	0.261
decreased eGFR	1(0.7)	11(2.7)	3.74(0.48, 29.24)	1.257	0.209
increased NGAL	16(11.8)	190(46.6)	6.54(3.75, 11.41)	6.991	< 0.001
CKD patients	(n = 37)	(n = 90)			
increased Ure	6(16.2)	24(26.7)	2.32(0.81, 6.66)	1.574	0.116
increased Cre	9(24.3)	38(42.2)	2.27(0.96, 5.37)	1.873	0.061
increased CysC	8(21.6)	56(62.6)	5.97(2.45, 14.56)	3.930	< 0.001
decreased eGFR	6(16.2)	35(38.9)	3.28(1.24, 8.69)	2.401	0.016
increased NGAL	15(40.5)	51(56.7)	1.91(0.88, 4.17)	1.642	0.101
Note: NKF patients, patients with normal kidney function; CKD patients, patients with chronic kidney disease. For predicting the occurrence of IS, NGAL is a risk factor in the patients with normal renal function, while CysC and eGFR are risk factors in the patients with abnormal renal function.					

Discussion

There are many studies about the relationship between kidney injury and the occurrence of IS [22–26]. Few scholars believe that only IS can induce kidney injury [23, 24]. Many scholars have confirmed that not only IS can induce kidney injury, but also kidney injury can induce IS [25–27]. The structure and function of the microvascular system of the brain are similar to that of the kidney. Therefore, the factors that cause kidney impairment may also damage the brain tissue. It is speculated that kidney function damage may be accompanied by brain tissue damage [7]. Therefore, some clinicians used traditional kidney function markers to predict the occurrence of IS in CKD patients. Many studies have confirmed that the decrease in eGFR and the increase in ACR are risk factors for the occurrence of non-fatal IS [7]. Other scholars have reported that the traditional kidney function markers that can be used to predict the occurrence of IS are not serological kidney function markers (including eGFR), but proteinuria-related indicators (such as ACR or 24h urinary excretion rate, etc.) [8,28]. Therefore, it is controversial to use traditional renal function markers as predictors for judging the occurrence of IS. For the new kidney function

markers, such as CysC and NGAL, research reports have shown that the increase in CysC is a risk factor for IS [10, 11], and it is also a protective factor for patients with neurodegenerative changes [29]. There is no research showing that NGAL can predict the onset of IS. However, it has been reported that NGAL does not have the ability to predict the recurrence of IS during the recovery period for patients with IS [30]. Therefore, it is unclear which kidney function damage is a risk factor for IS, and which serum kidney function markers reflecting renal function impairment can be used to predict the occurrence of IS.

This study included four IS-related chronic diseases (hypertension, DM, CAD and hyperuricemia) and five common serum kidney function markers (Ure, Cr, CysC, NGAL and eGFR) to study whether renal function impairment can be used to predict the onset of IS, and which kidney function markers have the ability to predict the occurrence of IS. In IS patients with CKD, the five kidney function markers are more closely related to the occurrence of IS than the above diseases. This indicates that the relationship between CKD and the occurrence of IS is closer than that between the above diseases with the occurrence of IS. These results indicate that renal function impairment can predict and even participate in the development of IS.

This study further analyzed the predictive performance of five renal function markers on the occurrence of IS. In addition to the above four diseases, IS can also be seen in patients with occult atrial fibrillation and patent foramen ovale [31]. However, patients with such diseases are often regarded as healthy without obvious physical signs. Therefore, this study first conducted ROC analysis with HC, DC or no-IS (i.e., HC + DC) as controls. Compared with subjects with no-IS (HC + DC) as controls, in subjects with HC or DC alone as controls, there was no significant difference in the ability of the five renal function markers to predict the occurrence of IS. Therefore, the results of ROC analysis with no-IS as the control may be more representative. In this case, only NGAL has a good predictive ability for the occurrence of IS. However, dividing IS patients into NKF-IS and CKD-IS, we used ROC analysis to find out that only NGAL has a certain predictive value in NKF-IS patients, and CysC has the best predictive ability in CKD-IS patients. The other three kidney function markers are not outstanding in predictive ability. When the YI is maximum, the cut-off values of Ure and Cre are far lower than the upper limit of the reference values, and the cut-off value of eGFR is far higher than the medical decision. Under this cut-off value, the patient's Ure, Cre and eGFR may still be at normal levels. These three markers are not suitable for reflecting and evaluating the causal relationship between renal function impairment and IS. Therefore, NGAL and CysC can be used as kidney function markers to reflect and evaluate renal function impairment and IS, and their applicabilities are different.

NGAL has been accepted by many nephrologists as a biomarker of early acute and chronic kidney injury, and some scholars also regard it as a neuroinflammatory marker [15, 16, 32]. This study found that the level of NGAL in IS patients was higher than that of HC and DC, while the levels of the other four renal function markers were only higher than HC. It indicates that serum NGAL level may be increased in both kidney function impairment and IS occurrence. This may also indicate that the risk of IS in patients with kidney injury is extremely high, and the patients with IS may have renal function impairment for a long time. For subjects with normal renal function, among the five serum kidney function markers, only NGAL has the good predictive performance and risk assessment ability for stroke occurrence, while other markers have no or extremely low predictive value. We need to know the increase in NGAL levels in these patients is dominated by neuroinflammation, or is caused by NGAL being more sensitive to kidney damage than other markers. However, recent animal research reports have shown that in the early stage of IS, even during transient ischemic attacks, there may be potential inflammation or stress and other pathological damage in the brain tissue. This leads to an increase in its NGAL level [32]. Therefore, in IS patients with normal renal function, the influence of neuroinflammation on the serum NGAL level cannot be ignored, and it may be even more significant than the influence of kidney injury. In short, for patients with IS risk

diseases with normal kidney function, as a marker of kidney injury or neuroinflammation, NGAL may be a risk factor for IS independent of DM, hypertension, CAD, and hyperuricemia.

CysC is an ideal marker for the evaluation of glomerular filtration rate published by the US Food and Drug Administration in 2002, and has the ability to reflect kidney injury more sensitively than Cre. So far, there have been many reports on the predictive ability of CysC for IS [10, 11, 33]. However, most of them are based on cardiovascular disease as the research object [11, 33]. In the process of cerebral ischemia/reperfusion injury, the increase of endogenous CysC can maintain the integrity of the lysosomal membrane, thereby playing a neuroprotective role [29]. Therefore, the occurrence of IS is usually accompanied by changes in CysC levels. This study showed that CysC may be more suitable for predicting the occurrence of IS in CKD patients, and the threshold value for the maximum YI is 1.14 mg/L. However, under this threshold, it needs to be further verified that the clinical prediction of IS occurrence is consistent with the actual results, or whether it is more reliable to predict IS occurrence by the increasing multiple of the CysC baseline level.

In contrast, when IS patients are accompanied with CKD, NGAL's ability to predict the occurrence of IS is significantly lower than other kidney function markers, and it does not have the advantage of predicting the occurrence of IS. The reason may be that NGAL is more sensitive to kidney injury than other markers. Although the increase of NGAL in CKD-IS patients is the result of both kidney injury and neuroinflammation, it is more caused by renal function impairment in patients with CKD because NGAL is extremely sensitive to renal function impairment. The influence of renal function impairment on NGAL in CKD patients largely masks the influence of neuroinflammation, which ultimately leads to the weakening of NGAL's ability to predict the occurrence of IS.

In summary, there are significant differences in the ability to predict the occurrence of IS between NGAL and CysC. CysC is suitable for predicting the occurrence of IS in CKD patients. The application of CysC to predict the occurrence of IS in CKD patients needs more in-depth exploration. The predictive value of NGAL for the occurrence of IS is more prominent in patients who have not yet significant kidney injury. In recent years, a few research reports have found that NGAL can be used to predict the recurrence of transient ischemia or stroke [34, 35]. However, how to better use NGAL to assess the risk of IS requires further research, especially in patients with stroke-related high-risk diseases and transient ischemic attacks who have no significant kidney injury. Long-term follow-up are needed to verify the level of NGAL that can be used as a warning threshold for IS occurrence.

The limitations of this study are as follows. (1) In this study, patients with AKI were excluded through the changes in Cre and urine volume within 48 h after admission. However, due to the lack of Cre baseline level in patients, AKI patients whose Cre level rises more than 1.5 times the baseline level within 7 days cannot be excluded. (2) Testing was carried out on the second day after the patient was admitted. It is impossible to eliminate the effect of the patient taking drugs for treatment of diseases within 7 d before admission. Therefore, there are confounding factors in the experimental results. However, these experimental results suggest that NGAL may be a risk factor for the occurrence of IS in patients with normal kidney function, and CysC is a risk factor for the occurrence of IS in CKD patients. If there is an increase in NGAL levels in patients with IS-related risk diseases, and there is no clear evidence of kidney injury, we should be alert to the occurrence of IS.

Abbreviations

ACR: Albumin to creatinine ratio; AHA: American Heart Association; AKI, acute kidney injury; ASA: American Stroke Association; AUC: area under curve; CAD: coronary artery disease; CKD: chronic kidney disease; CKD-IS, IS patients

with CKD; Cre, creatinine; CysC: cystatin C; DC, Disease controls; DM: diabetes mellitus; DWMRI: diffusion-weighted magnetic resonance imaging; eGFR: estimated glomerular filtration rate; GFR: glomerular filtration rate; HC, Healthy controls; HS: hemorrhagic stroke; IS: ischemic stroke; KIDGO: Kidney Disease Improving Global Outcomes; NGAL: neutrophil gelatinase-associated lipocalin; NKF-IS, IS patients with normal kidney function; OR: odds ratio; ROC: receiver operating curve; Ure, urea; YI: Youden index.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Mianyang Central Hospital (approval no. S2014048 and S2018085, see Appendix S1 and S2). All subjects signed informed consent to participate.

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Competing interests

The authors declare that they have no competing interests.

Authors' Contributions

Conceptualization, YY and JF; Funding acquisition, YY and JF; Investigation, LP and XC and YF; Writing – original draft, YY and LP; Writing – review & editing, JF. All authors critically reviewed and approved the final manuscript.

Availability of data and materials

All data, models, and code generated or used during the study appear in the submitted article.

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This appendix certifies that the manuscript was edited by one or more of the highly qualified English-speaking editors at SCI Editing (See Appendix S3).

Consent for publication

Not applicable.

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Figures

Figure 1

Serum levels of kidney markers in all subjects Note—Levels of Ure, Cre, CysC, eGFR and NGAL were measured in units of mmol/L, μ mol/L, mg/L, ml/min/1.73m² and μ g/L, respectively. HC, health controls; DC, disease controls; IS, patients with ischemic stroke. $\blacktriangle$ Compared with HC, $P < 0.001$; $\triangle$ Compared with DC group, $P < 0.001$. Among the 5 kidney markers, only NGAL level in the IS group was higher than that both in the HC group ($P < 0.001$) and in the DC group ($P < 0.001$).

Figure 2

Diagnostic performance of kidney markers for the IS occurrence under different controls Note: IS, patients with ischemic stroke (n=498); no-IS, health controls (n=293) and disease controls (n=173); HC, health controls (n=293); DC, disease controls (n=173). When no-IS (Figure-2A) and HC (Figure-2B) and DC (Figure-2C) as the controls, respectively, NGAL had the highest diagnostic performance for the IS occurrence. However, there was no significant among the AUCs of NGAL when IS vs. no-IS, HC and DC, respectively ($P>0.05$).

Figure 3

Diagnostic performance of kidney markers for the IS occurrence under different kidney function states Note: NKF-IS, IS patients with normal kidney function (n=408); CKD-IS, IS patients with chronic kidney disease (n=90); no-IS, health controls (n=293) and disease controls (n=173). After grouping patients according to their kidney function, NGAL had the highest diagnostic performance in the NKF-IS patients (Figure-3A), while CysC in CKD-IS patients (Figure-3B).

Supplementary Files

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