Subacute Elevation of Plasma Level of Caspase-Cleaved Cytokeratin-18 is Associated with Hemorrhagic Transformation and Functional Outcome in Ischemic Stroke

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Background: Caspase-cleaved cytokeratin-18 (CCCK-18) is an apoptosis marker. Here, we analyzed the relationship between plasma level of CCCK-18 in the acute and subacute stage of ischemic stroke and early and late functional outcome. Besides, correlation among CCCK-18 and complications, such as hemorrhagic transformation (HT) were also explored. *Methods:* Plasma concentration of CCCK-18 was investigated in 54 patients at admission and poststroke 72 hours. HT was evaluated by CT scans on 24 poststroke hours. Outcome measures were assessed by modified Rankin scale at hospital discharge and 6-month later. Receiver operating characteristics (ROC) analysis was used to determine the best cut-off values of CCCK-18 as a predictor of unfavorable functional outcome. *Results:* Significantly elevated CCCK-18 level was observed at 72 hours after onset of stroke, in nonsurviving compared to surviving patients (331 ± 191 ng/L versus 251 ± 164 ng/L, $P = .01$). Based on ROC analysis, the cut-off value of plasma CCCK-18 levels >223 ng/L at 72 poststroke hours predicted 6-month unfavorable stroke outcome with a sensitivity of 84.4% and a specificity of 77.3% (area under the curve: .851, 95% confidence interval = .745-.955, $P < .001$). The rate of complications such as HT and in-hospital infection was significantly higher in patients presented with a plasma CCCK-18 level above the cut-off value. *Conclusions:* The association between high serum CCCK-18 levels and unfavorable early and late stroke outcome in an unselected study population was first described here. Besides, the apoptosis marker CCCK-18 might be a predictor of further complication such as HT and in-hospital infection.
Key Words: Caspase-cleaved cytokeratin-18—ischemic stroke—outcome—hemorrhagic transformation
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Introduction

Ischemic stroke is a leading cause of disability worldwide.[1] At the cellular level, the biochemical mechanisms involved in the ischemic brain injury vary according to the extent of cerebral ischemia. Recent research has revealed that many neurons in the ischemic penumbra or peri-infarct zone may undergo apoptosis after several hours or days, and are potentially recoverable for some time after the onset of stroke.[2] Apoptosis-related caspase activity is present in cells that lost their plasma membrane integrity involved in cerebral ischemia.[3] Detection of caspase-mediated cleavage products is considered evidence for activation of the caspase cascade.[4] CK-18 is a protein of the intermediate filament family and is present

in most epithelial and parenchymal cells,[5] and is cleaved by the action of caspases during apoptosis. Subsequently, the resulting fragments, namely caspase-cleaved cytokeratin-18 (CCCK-18), are released into the peripheral blood.[5-7] Levels of CCCK-18, as biomarker of apoptosis, have been studied in patients with hepatological disorders,[8-11] various CNS diseases,[12-15] septis processes,[16,17] myocardial injury,[18] and oncological disorders.[19,20] The aim of this study was to evaluate association between serum CCCK-18 levels measured at different time points after ischemic stroke and the outcome of ischemic stroke. We also examined association between CCCK-18 levels and hemorrhagic transformation after ischemic stroke.

Methods

Participants and Variables

The study was approved by the Reginal Ethical Committee of the University of Pecs. Written informed consent from the patients or from their legal representatives was obtained. A total of consecutive 75 patients with acute ischemic stroke were prospectively assessed. Twenty-one patients were excluded from analysis because of inadequate quality of data at admission (N = 5), unavailable biomarker measurements (n = 4), loss of follow-up (n = 8), chronic hepatic disease (n = 2), or active malignant disease (n = 2). The remaining 54 patients were included in the final analysis. Demographic features and risk factors were recorded.

On admission, serum C-reactive protein (CRP), white blood cell count, hemoglobin level, glucose, creatinine, thrombocyte count, aspartate transaminase, and alanine transaminase were measured by a central certified laboratory. Glasgow outcome scale (GCS) was calculated on admission by a trained neurologist.

Patients with active malignant disease, previous disability (modified Rankin scale [mRS] > 1), ongoing oncological treatment, or any evidence of liver disease were excluded of the study.

Atrial fibrillation (AF) was confirmed by a 12-lead electrocardiogram (Nihon Kohden Cardiofax GEM ECG-9022K). A history of hypertension was defined as the use of antihypertensive drugs or blood pressure more than 140/90 mmHg on at least 2 separate occasions. Diabetes mellitus was defined as the use of antidiabetic drugs or a fasting plasma glucose value greater than or equal to 7.0 mmol/L.

Severity of stroke measured by the National Institutes of Health Stroke Scale (NIHSS) and mRS at discharge and 6 months after stroke were used as outcome measures. NIHSS greater than or equal to 9 was considered as severe stroke. Unfavorable outcome was defined as mRS score 4-6, whereas favorable outcome by scores 0-3. To assess the mRS after 6 months, we either requested hospital visit or conducted a phone interview with the patient, the spouse, or the general practitioner. The interview was conducted by a trained, certified examiner blinded to the clinical information.

Neuroimaging for ischemic infarction and hemorrhagic transformation was performed on admission by Siemens SOMATOM Definition 64 Slice CT Scanner. Large artery occlusion was determined on admission by CT-angiography. Hemorrhagic transformation (HT) was defined as any level of hyperdensity within the area of low attenuation on CT. The different types of HT, that is, hemorrhagic infarction (HI, types 1 and 2) and parenchymal hemorrhage (PH, types 1 and 2) were determined on the basis of CT characteristics.[21-23] HI1 is defined as an isolated petechial staining of infarcted tissue, while HI2 is characterized by confluent patechiae in the infarcted tissue, both without associated mass effect. PH1 is characterized by a homogeneous high attenuation lesion with minimal mass effect occupying less than 30% of the infarcted area. PH2 is defined as and a lesion occupying more than 30% of the infarcted area with definite mass effect, possible extension of hemorrhage into the ventricular space, as well as any hemorrhage outside the infarcted area.[21-23] The Alberta stroke program early CT score (ASPECTS) was calculated 24 hours after admission by a trained neuroradiologist.

Sampling and Plasma CCCK-18 Analysis

EDTA-plasma samples were drawn from patients on admission within 6 hours after symptom onset, and 72 hours after onset. The samples were immediately centrifuged at 3500 r/min for 15 minutes. The supernatant was stored at −80°C until analysis. CCCK-18 concentrations were determined by using CCCK-18 enzyme-linked immunosorbent assay (Shanghai YL Biotech Co., Ltd., China). All samples were processed by the same technicians using the same equipment and blinded to all clinical data. The detection limit for the assay was 5.64 ng/L.

Statistical Analysis

Data were evaluated using SPSS (version 11.5; IBM, Armonk, NY). Categorical data were summarized by means of absolute and relative frequencies (counts and percentages). Quantitative data are presented as mean and 95% confidence interval (CI), as well as mean ± SD. The Kolmogorove-Smirnov test was applied to check for normality. The chi-square test for categorical data and Student t test for continuous data were used for analysis of demographic and clinical factors. Non-normally distributed data are presented as median and interquartile range and compared to Mann-Whitney test. The cut-off value of CCCK-18 (n = 54) to determine unfavorable outcome at 6-month follow-up was calculated by receiver operator curve (ROC) analysis. Correlation analysis was performed calculating Spearman's correlation coefficient (r). Wilcoxon signed ranks test was used for comparison of repeated measurements of CCCK-18 levels in favorable and unfavorable subgroups respectively. To find an

independent predictor of poor outcome, a binary logistic regression was used. A P value < .05 was considered statistically significant.

Results

Patients Characteristics

Comparison of demographic and clinical parameters between surviving (n = 35) and nonsurviving (n = 19) patients are summarized in Table 1. Nonsurviving patients had older age (P = .007) and higher prevalence of ischemic heart disease (P = .009). On admission, a significantly higher number of patients in the nonsurvivor group had AF (P < .001), higher NIHSS (P < .001), lower GCS (P < .001), and lower ASPECT score (P < .001). In the nonsurvivor group, we also observed a higher number of large artery occlusion (84.2% versus 37.1%, P = .001),

hemorrhagic transformation of the infarct (36.8% versus 5.7%, P = .003), and higher rate of in-hospital infections (89.5% versus 20%, P < .001). Anticoagulant treatment was more frequent in the nonsurvivor group compared to surviving patients (P = .046), but the frequency of thrombolysis was higher among survivors (74.3% versus 47.4%, P = .048). The median value of neutrophil-lymphocyte ratio (P = .011), white blood cell count (P = .015), serum glucose concentration (P = .002), and CRP levels (CRP, P < .001) were significantly higher in the nonsurvivor versus survivor group, respectively.

CCCK-18 Levels on Admission and During Follow-up, and Relation to Outcome Measured by mRS

CCCK-18 levels on admission were not different between surviving and nonsurviving patients (Table 1). However, 72 hours after stroke onset we observed

Table 1. *Demographics and clinical factors of survivors and nonsurvivors after ischemic stroke*

	Nonsurvivors (N = 19)	Survivors (N = 35)	P value
Male	9 (47.4)	23 (65.7)	.197
Age (y)	71.2 ± 9.8	64.8 ± 9.4	**.007**
Smoking	6 (31.6)	15 (42.9)	.417
Diabetes mellitus	7 (36.8)	8 (22.9)	.273
Hypertension	18 (94.7)	27 (77.1)	.098
COPD	4 (21.1)	2 (5.7)	.087
IHD	17 (89.5)	19 (54.3)	**.009**
Alcohol	1 (5.3)	2 (5.7)	.945
Atrial fibrillation	14 (73.7)	5 (14.3)	**<.001**
NIHSS on admission	15 (12-18)	9 (6-12)	**<.001**
GCS on admission	10 (5-13)	15 (0)	**<.001**
ASPECT score	4 (3-6)	9 (7-10)	**<.000**
Occlusio on admission CTA	16 (84.2)	13 (37.1)	**.001**
Thrombolysis	9 (47.4)	26 (74.3)	.048
Thrombectomy	5 (26.3)	7 (20)	.594
Haemorrhagic transformation	7 (36.8)	2 (5.7)	**.003**
Infection, in-hospital	17 (89.5)	7 (20)	**<.001**
Anticoagulation	8 (42.1)	6 (17.1)	**.046**
Heart rate on admission	92 ± 27	83 ± 16	.293
SBP on admission (mmHg)	168 ± 32	158 ± 35	.256
DBP on admission (mmHg)	89 ± 16	88 ± 13	.677
PLR	156 (122-212)	127 (103-163)	.069
NLR	5.93 (3.4-7.5)	3.16 (2-6)	**.011**
WBC (G/L)	10.1 (8.3-12.3)	7.9 (6.5-10.2)	**.015**
Hemoglobin (G/L)	125 (112-156)	142 (134-150)	**.049**
C-reactive protein (mg/L)	10.6 (5.8-38.7)	3.8 (1.9-7.4)	**<.001**
Platelets (G/L)	206 (159-307)	248 (197-300)	.356
Glucose (mmol/L)	8.05 (7.4-9.9)	6.64 (5.8-7.9)	**.002**
Creatinine (μmol/L)	83 (57-114)	82 (67-96)	.935
CCCK-18 acute (within 6 h)	279 ± 213	220 ± 122	.751
CCCK-18 on 72 h	331 ± 191	251 ± 164	**.01**

Abbreviations: COPD, chronic obstructive pulmonary disease; IHD, ischemic heart disease; NIHSS, National Institue of Health Stroke Scale; GCS, Glasgow coma scale; ASPECT, Alberta stroke program early CT; CTA, computer tomography angiography; SBP, systolic blood pressure; DBP, diastolic blood pressure; PLR, platelet-lymphocyte ratio; NLR, neutrophil-lymphocyte ratio; WBC, White blood count; CCCK-18, caspase-cleaved cytokeratin 18 (ng/L).

The categorial variables are presented as frequency and percentage, and the continous are presented as mean ± standard deviation or median (percentile 25-75). Statistics: chi-square test, Fisher exact test, Student's t or Mann-Whitney U test.
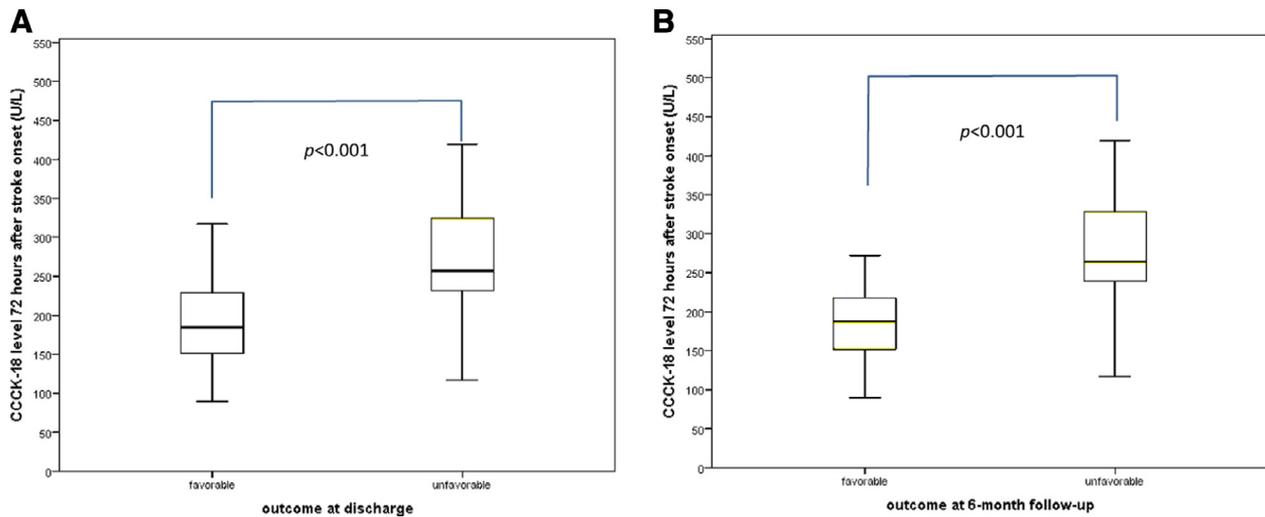
A

B

Figure 1. *Box plots demonstrating median plasma CCCK-18 levels in patients with favorable versus unfavorable outcome at discharge (A), and at 6-month follow-up (B). CCCK-18 measured at 72 hours after onset of stroke. Data are presented as median and 25th and 75th percentiles. Abbreviation: CCCK-18, caspase-cleaved cytokeratin-18.*

significantly elevated CCCK-18 levels in nonsurviving compared to surviving patients (331 ± 191 ng/L versus 251 ± 164 ng/L, P = .01). CCCK-18 levels in patients with an unfavorable outcome on discharge (mRS 4-6: 327 ± 200 U/L) were also higher compared to patients with favorable outcome (mRS 0-3: 191 ± 60.3 ng/L; P < .001). Similar results were found at 6-month follow-up comparing patients with unfavorable and favorable outcome (340 ± 204 ng/L versus 191 ± 56 ng/L, P < .001; Fig 1). The change in CCCK-18 levels between admission and 72 hours follow-up was significantly higher in patients with unfavorable outcome compared to those with favorable outcome on discharge (59 ± 118 versus −2 ± 47.7, P = .036). This difference was even more pronounced 6 months after the onset of stroke (67.9 ± 120 versus −6 ± 45.6, P = .003).

The area under the curve for plasma CCCK-18 levels as a predictor of unfavorable outcome at 6 months after the onset of stroke was .851 (95% CI = .745-.955; P < .001). Based on ROC analysis, plasma CCCK-18 levels greater than 223 ng/L predicted 6-month unfavorable outcome of patients with a sensitivity of 84.4% and a specificity of 77.3% (Fig 2).

Clinical Variables Related to CCCK-18 Level at 72 Hours After Stroke Onset

By using the cut-off value to define patients with high (CCCK-18 > 223, ng/L) and low CCCK-18 levels (CCCK-18 < 223, ng/L), we next examined differences in stroke severity, present of large artery occlusion on admission imaging, in-hospital infection rate, unfavorable outcome at 6-month follow-up (mRS score > 3), and hemorrhagic transformation on follow-up imaging. Among patients with high plasma CCCK-18 concentration at 72 hours after stroke, we found higher percentage of large artery

occlusion, in-hospital infection, more severe stroke (NIHSS scores >9 on admission, and worse outcome measured by mRS greater than 3 at discharge (Fig 3). Higher plasma levels of CCCK-18 levels at 72 hours were associated with higher NIHSS and lower ASPECT scores measured on admission (Fig 4, A, B).

We also aimed to explore deeper relationships among severity of stroke (on admission NIHSS), neuroimaging (ASPECT score), and CCCK-18 at 72 poststroke hours. Using binary logistic regression analysis including multiple variables (e.g., age, gender, NIHSS, GCS, smoking,
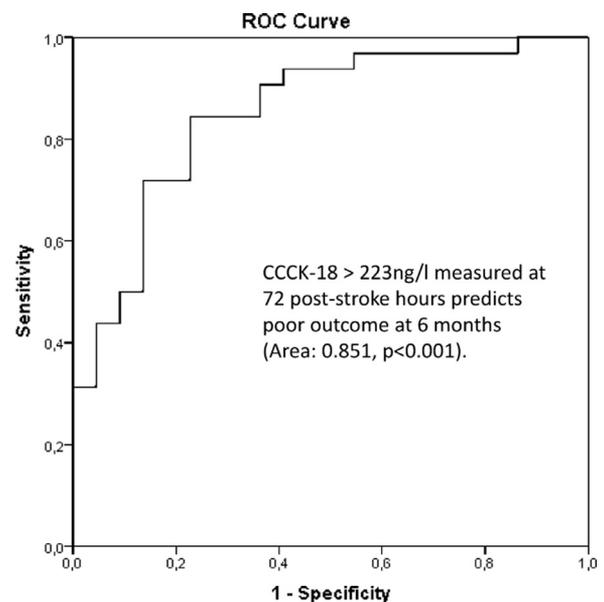

Figure 2. *Determination of predicted cutoff level for CCCK-18 by receiver operator curve analysis. Concentration of plasma CCCK-18 >223ng/L measured at 72 poststroke hours predicts poor outcome at 6 months (Area: .851, P< .001). Abbreviation: CCCK-18, caspase-cleaved cytokeratin-18.*
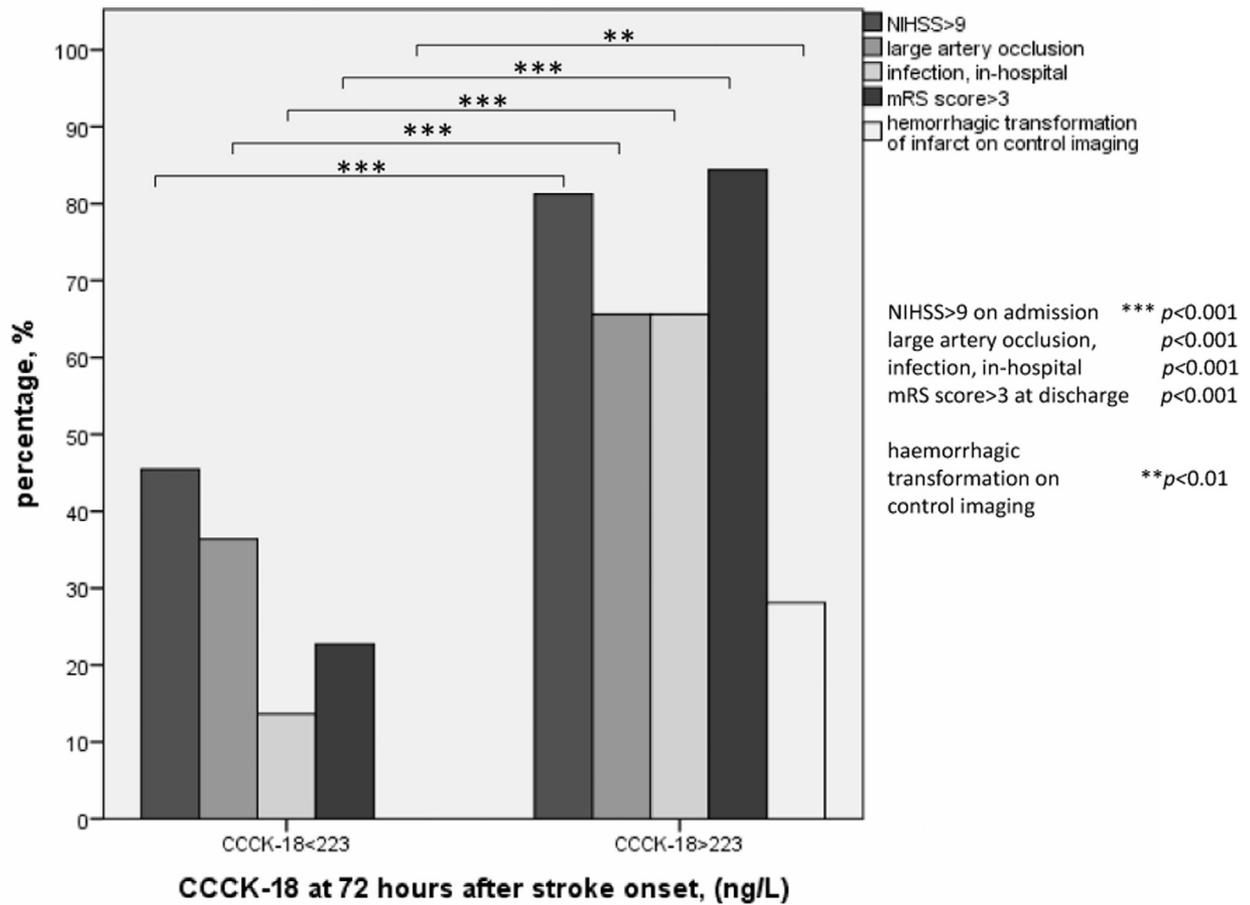
Figure 3. *Bar graphs demonstrating the percentage of patients with high NIHSS at admission (>9), large artery occlusion, in-hospital infection, mRS score >3 at discharge and haemorrhagic transformation of stroke on control imaging in high CCCK-18 group (>223 ng/L) versus low CCCK-18 group (<223 ng/L). This cutoff value was determined by ROC analysis. Significant differences are indicated with ** (P < .01), *** (P < .001). Abbreviations: CCCK-18, caspase-cleaved cytokeratin-18; mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale.*
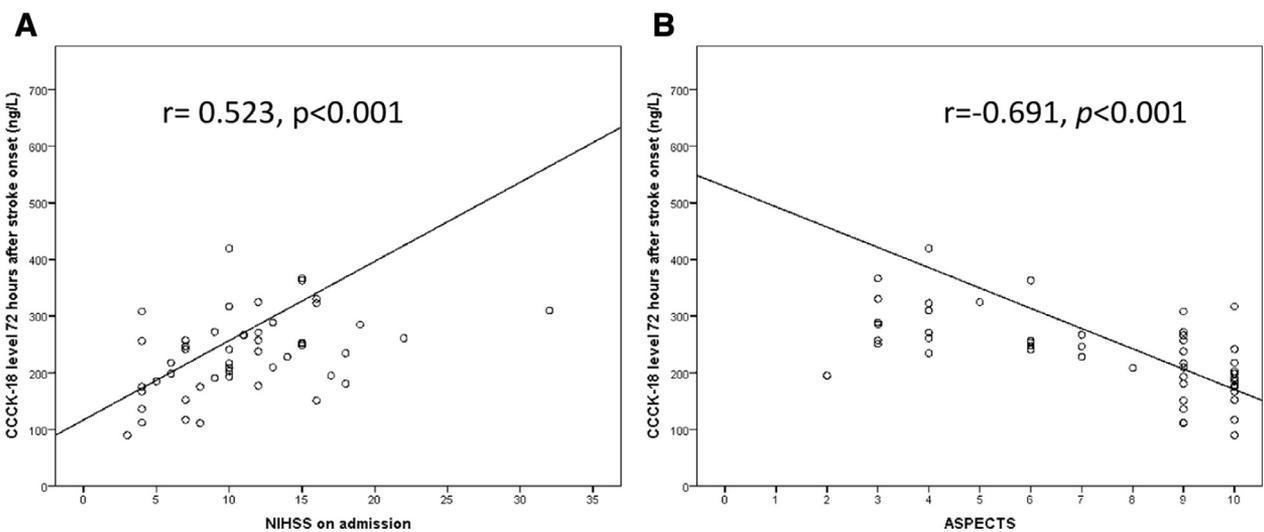

Figure 4. *Correlation of CCCK-18 measured at 72 hours after stroke onset and NIHSS on admission. (A) and ASPECT score on admission imaging (B). Abbreviation: CCCK-18, caspase-cleaved cytokeratin-18; NIHSS, National Institutes of Health Stroke Scale.*
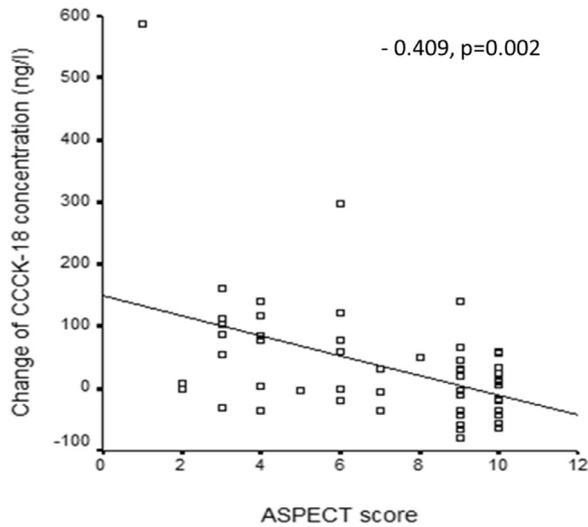
Figure 5. *Correlation between the ASPECT score and the change in plasma concentration of CCCK-18 between admission and 72 hours after-stroke onset. Abbreviation: CCCK-18, caspase-cleaved cytokeratin-18.*

ASPECT score, and biomarkers), only ASPECT score greater than 7 with an odds ratio:2.5 (P = .03), a sensitivity of 92% and a specificity of 75% were found as an independent variable to predict favorable outcome based on mRS at 6 months follow-up (ROC analysis; area: .863, 95%CI: .767-.960, P < .001).

We also found a significant negative correlation between the ASPECT score and the change of CCCK-18

concentration between admission level and 72 hours post-stroke (r = −.409, P = .002), which was more pronounced in patients developing poststroke infection (Fig 5). It suggests that larger infarct is associated with more release of apoptosis marker CCCK-18 into the systemic circulation, and change of CCCK-18 may also select a subgroup of patients who are more susceptible to infectious complications and subsequent poor clinical outcome.

In our cohort, two-thirds of the patients with hemorrhagic transformation had PH1 or PH2 parenchymal hemorrhage. In patients with hemorrhagic transformation, CCCK-18 concentration was higher in the plasma, and all patients had CCCK-18 less than 223 ng/L associated with worse outcome (Table 2). ASPECT score and GCS were significantly lower in patients with HT, while NIHSS, neutrophil-lymphocyte ratio, serum glucose concentration, CRP levels, and the rate of in-hospital infection were higher in patients with hemorrhagic transformation. Interestingly, neither the thrombolysis nor the frequency of thrombectomy differed significantly between the 2 groups (Table 2).

Discussion

In this prospective study, we examined CCCK-18 levels in an unselected patient population, that is, in consecutively enrolled patients with acute ischemic stroke. We found that higher CCCK-18 level measured 72 hours after stroke was associated with (1) unfavorable outcome both

Table 2. *Hemorrhagic transformation versus no transformation of infarcts*

	Non-HT (n = 45)	HT (n = 9)	*P* value
Male	29 (64.4)	3 (33.3)	.086
Age (y)	66 ± 10	71 ± 7.8	.226
GCS	15 (13-15)	11 (4.5-14)	**.009**
ASPECT score	9 (5.5-10)	4 (2.5-6)	**<.001**
NIHSS on admission	10 (7-15)	16 (13.5-20.5)	**<.001**
NLR	3.7 (2.2-6.1)	6.8 (3.4-19.9)	**.027**
hsCRP	4.6 (2.1-9)	19 (6.9-46.9)	**.008**
Glucose	6.8 (6-8)	9.2 (7.5-10.2)	**.014**
Creatinine	83 (67-103)	62 (59-101)	.370
INR	1.04 (1-1.15)	1.19 (1.1-2)	**.007**
PLT count	245 (193-307)	159 (138-273)	.133
Thrombolysis	28 (62.2)	7 (77.8)	.382
Thrombectomy	10 (22.2)	2 (22.2)	1.000
Atrial fibrillation	12 (26.7)	7 (77.8)	**.003**
Anticoagulation	11 (24.4)	3 (33.3)	.587
Infection	16 (35.6)	8 (88.9)	**.003**
CCCK-18 at 72 h	234 (179-271)	285 (247-580)	**.025**
CCCK-18 > 223	23 (51.1)	9 (100)	**.006**
Non-survivor	12 (26.7)	7 (77.8)	**.003**
Unfavorable outcome*	26 (57.8)	9 (100)	**.015**

Abbreviations: HT, haemorrhagic transformation; n indicates number of patients; GCS, Glasgow coma scale; ASPECT score, Alberta stroke program early CT score; NIHSS, National Institute of Health Stroke Scale; NLR, Neutrophil-lymphocyte ratio; hsCRP, high-sensitivity C-reactive protein (mg/L); INR, international normalized ratio; PLT, platelet (G6/l); CCCK-18, caspase-cleaved cytokeratin 18 (ng/L).

The variables were presented as counts (percentage), mean ± SD or median (interquartile range 25−75).

*Assessed at discharge from hospital, and defined as mRS score 4-6.

on discharge and 6-month after stroke onset; (2) death; (3) large artery occlusion and more severe stroke; (4) higher chance of hemorrhagic transformation; (5) and higher rate of in-hospital infection. Similar to our findings, a positive association was recently found between high serum CCCK-18 levels and patients mortality caused by malignant medial cerebral artery infarct.[13] High serum CCCK-18 levels were also associated with poor prognosis in traumatic brain injury,[12] acute spontaneous intracerebral hemorrhage,[15] and aneurysmal subarachnoid hemorrhage.[14]

Although we found that CCCK-18 levels measured 72 hours after ischemic stroke predicted worse prognosis in several outcome measures in contrast to CCCK-18 concentration measured on admission. In the case of subarachnoid hemorrhage and spontaneous intracerebral hemorrhage, blood samples taken within 6 hours after the stroke onset were examined.[14,15] In these hemorrhagic strokes, primary injury is evoked by the physical destruction due to rapid hematoma formation, and apoptosis correlates with the hematoma volume.[24-26] In case of acute ischemia, irreversible injury and subsequent necrotic cell death occur in the ischemic core just minutes after ischemic attack, but apoptosis within the ischemic penumbra may occur after several hours or days.[27,28] Therefore, it is likely that a more robust mechanical injury-related apoptosis elicits higher release of CCCK-18 into the peripheral blood in hemorrhagic stroke, and is associated with more extensive vascular and blood-brain barrier damage. In contrast, the apoptosis related to the ischemic penumbra injury may result in a slower accumulation of CCK-18 release. This is also supported by our findings that indicate that hemorrhagic transformation is associated with higher levels of CCCK-18 in the plasma. In addition, thrombolysis/thrombectomy may also influence the extent of ischemic penumbra and the magnitude of the core ischemia in the acute phase.

Our study indicated that plasma CCCK-18 concentrations 72 hours after ischemic stroke were significantly higher in patients with unfavorable short- and long-term outcome compared to favorable outcomes. Unfavorable outcome was also highly associated with severe stroke, large artery occlusion, and larger infarct size indicated by lower ASPECT score. Large-vessel occlusions not responsive to intravenous thrombolysis have been shown to be associated with a poor prognosis.[29] With persistent vessel occlusion, fewer patients achieve functional independence, and mortality rates are higher.[10] After focal cerebral ischemia, most of the cells in the ischemic core undergo necrosis and cell death, while in the ischemic penumbra is considered an active process that gradually leads to apoptosis.[28] Therefore, it is plausible that more extensive infarct accompanied by more extensive apoptosis resulting in increased release of CCCK-18 into the systemic circulation.

In addition, nonsurviving patients showed higher serum CCCK-18 levels than survivors.[12] Higher hematoma volumes in nonsurviving patients with hemorrhagic stroke also showed higher serum CCCK-18 levels.[16] Nonsurviving patients with severe malignant middle cerebral artery infarction had also higher serum CCCK-18 levels compared to surviving patients.[13] In our study, we examined consecutively enrolled patients with acute ischemic stroke and showed that CCCK-18 levels are also predictive in an unselected patient population.

In this study, we found a strong positive correlation between the severity of stroke measured by NIHSS and CCCK-18 levels at 72 poststroke hours. Also, ASPECT score was negatively associated with levels of CCCK-18 at 72 poststroke hours. Systemic CCCK-18 concentrations were also positively associated with NIHSS scores in patients with intracerebral hemorrhage.[15] In addition, serum CCCK-18 level was positively associated with the severity of Subarachnoideal hemorrhage (SAH) indicated by World Federation of Neurosurgical Societies (WFNS) score, and also with modified Fisher scores indicating the extent of hemorrhage. Moreover, the predictive value of CCCK-18 was similar to WFNS scores and modified Fisher scores.[14] These results collectively suggest that CCCK-18 could have high prognostic significance and reflect the extent of both tissue damage and the severity of ischemic/hemorrhagic stroke.

We also calculated cut-off value of CCCK-18 concentration predicting poor outcome, and found high CCCK-18 level in all patients with hemorrhagic transformation on control neuroimaging. When stroke patients with and without hemorrhagic transformation were compared, CCCK-18 levels were higher in stroke with hemorrhagic transformation. The main mechanism of HT in acute ischemic stroke is still unclear, and more than 1 mechanism might operate under different circumstances. Augmented collateral circulation into the ischemic zone[30] and disruption of the basal lamina, which is connected by fibronectin and laminin to the endothelium, might play a part in the pathogenesis of HT.[31] Patient age and therapeutic intervention (thrombolysis versus thrombectomy) did not differ significantly between patients with and without HT in our study. The role of age as a risk factor is controversial: age was not a significant predictor of symptomatic intracerebral hemorrhage after thrombolysis,[32] while in a meta-analysis of 55 studies older age was associated with increased risk of postalteplase intracerebral hemorrhage.[33] In our cohort, AF was closely related to HT, which is consistent with previously reported data. Cardioembolism has been linked to the highest frequency of HT,[34] and 15% of embolic strokes show hemorrhagic infarction on CT several days after ischemic stroke.[35] In the context of treatment with intravenous alteplase, AF is a strong risk factor for intracranial hemorrhage.[36] We found that parenchymla damage indicated by the lower ASPECT score was associated with higher prevalence of HT. In the PROACT II and ECASS II study, the rates of symptomatic intracerebral hemorrhage were also high in

patients with an Alberta stroke program early CT score less than or equal to 7.[37,38] The presence of focal hypodensity on CT was associated with subsequent HT in 77% of cases in patients not treated with alteplase.[39] In alteplase-treated patients, the presence of early signs of ischemia on baseline CT is also predictive of HT.[40] In our cohort, higher INR values also correlated with HT, but anticoagulant treatment alone was not a risk factor for HT. A meta-analysis indicated that early anticoagulation was associated with an increased intracranial bleeding in patients with acute cardioembolic stroke.[41] Patients with acute ischemic stroke treated with oral anticoagulants had a 10 times greater risk of symptomatic intracerebral hemorrhage in the setting of treatment with intravenous alteplase than those without anticoagulation.[42]

Our data also indicated that, in-hospital infection and CRP level measured 24 hours after admission were associated with higher level of plasma CCCK-18. Nonsurvivor septic patients showed higher serum CCCK-18 levels during the first week than survivor patients, and it was associated with sepsis severity and mortality.[16] Serum CCCK-18 levels may be of great pathophysiological significance in septic patients, and apoptosis could contribute to multiple organ failure and death of septic patients.[17]

There are some limitations of our study: (1) low number of enrolled patients decreased the power of this study and precluded detailed multivariate analysis; (2) lack of follow-up imaging in all patients tackled us to explore more precise relationship between penumbral or hypoperfused brain area and markers; and (3) although this was a prospective study, the determination of infarct volume was not feasible for all patients, so only the ASPECT score could be calculated from the 24-hour control imaging.

In summary, higher circulating CCCK-18 level was previously reported to be associated with 30-day mortality in patients with malignant medial cerebral artery infarct.[13] However, the association between high serum CCCK-18 levels and unfavorable outcome both on discharge and 6-month after stroke onset in this unselected study population is a novel finding. Besides, the apopotosis marker CCCK-18 might be a predictor of further complication such as hemorrhagic transformation and in-hospital infection. Based on these findings, further studies are required.

Authors Contribution

P.C., T.M., K.B., J.J., and A.S. conceived, designed, and coordinated the study, participated in acquisition and interpretation of data. P.C., T.M., and Z.I. drafted the manuscript. L.S. participated in acquisition of data. T.B. participated in blood determination levels. P.C. and T.M. participated in the statistical analysis. All authors read and approved the manuscript.

Competing Interests

The authors declare that they have no competing interests.

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