Lorente et al. BMC Neurology (2018) 18:32
https://doi.org/10.1186/s12883-018-1038-z

BMC Neurology

RESEARCH ARTICLE Open Access

High serum levels of caspase-cleaved cytokeratin-18 are associated with malignant middle cerebral artery infarction patient mortality

Leonardo Lorente[1*], María M. Martín[2], Antonia Pérez-Cejas[3], Luis Ramos[4], Mónica Argueso[5], Jordi Solé-Violán[6], Juan J. Cáceres[7], Alejandro Jiménez[8] and Victor García-Marín[9]

Abstract

Background: There have been found apoptotic changes in brain tissue samples from humans after cerebral ischemia. Caspase-cleaved cytokeratin (CCCK)-18 could appears in blood during apoptosis. High circulating levels of CCCK-18 have been associated with a poor prognosis in patients with cerebral process, such as traumatic brain injury and spontaneous cerebral hemorrhage. However, they have not been explored in patients with ischemic stroke. Thus, the aim of this study was to determine whether there is an association between serum CCCK-18 levels and mortality in patients with severe malignant middle cerebral artery infarction (MMCAI).

Methods: This was an observational, prospective and multicentre study. We included patients with severe MMCAI. We considered MMCAI as severe when Glasgow Coma Scale (GCS) was lower than 9. We measured serum CCCK-18 levels at the diagnosis moment of the severe MMCAI.

Results: We found that non-surviving severe MMCAI patients ($n = 33$) showed lower GCS and platelet count, and higher serum CCCK-18 levels than survivor ones (n = 33). We found an area under the curve (AUC) of serum CCCK-18 levels to predict 30-day mortality of 82% (95% CI = 71%–91%; $p < 0.001$). In the multiple logistic regression analysis was found that serum CCCK-18 levels were associated with 30-day mortality (OR = 1.023; 95% CI = 1.010–1.037; $p = 0.001$) after to control for platelet count and GCS.

Conclusions: To our knowledge, this is the first series reporting data on serum CCCK-18 levels in ischemic stroke patients. The novel findings of our study were that non-surviving severe MMCAI patients had higher serum CCCK-18 levels than surviving patients, and that there is an association between high serum CCCK-18 levels and MMCAI patients mortality.

Keywords: Caspase-cleaved cytokeratin-18, Cerebral infarction, Patients, Mortality

Background

Ischemic stroke cause death, disability, and health resources consume [1]. In brain infarction appears cell death due to brain vasculature obstruction (which produces a restriction of oxygen and substrates for neurons) and due to apoptosis [2–7]. There have been found apoptotic changes in brain tissue samples from humans after cerebral ischemia [8–13].

Cytokeratins (CK) are proteins, until now named as CK-1 to CK-20, existing mainly in the intracytoplasmic cytoskeleton of epithelial tissue. During apoptosis CK-18 is cleaved at various sites by the action of caspases and appears caspase-cleaved cytokeratin (CCCK)-18, which could be released into the blood [14, 15].

Previously, there were found higher circulating CCCK-18 levels in patients with sepsis [16–20], liver diseases [21–25], and tumoral diseases [26, 27]. In addition, there

* Correspondence: lorentemartin@msn.com
[1]Intensive Care Unit, Hospital Universitario de Canarias, Ofra s/n, La Laguna, -38320 Santa Cruz de Tenerife, Spain
Full list of author information is available at the end of the article

was found an association between high circulating CCCK-18 levels and a poor prognosis of patients with different cerebral process, such as traumatic brain injury [28] and spontaneous cerebral hemorrhage [29, 30]. However, they have not been explored in patients with ischemic stroke. Thus, the aim of this study was to determine whether there is an association between serum CCCK-18 levels and mortality of patients with severe malignant middle cerebral artery infarction (MMCAI).

Methods

Design and subjects

This observational prospective multicentre study was carried with the written informed consent from patient legal guardians in 6 Intensive Care Units from Spain after the approval by the Institutional Review Board of all participanting hospitals: H. Insular from Las Palmas de Gran Canaria, H. General de La Palma from Breña Alta, H. Universitario de Canarias from La Laguna, Tenerife, H. Clínico Universitario de Valencia from Valencia, H. Universitario Dr. Negrín from Las Palmas de Gran Canaria, H.Universitario Nuestra Señora de Candelaria from Santa Cruz de Tenerife.

We included patients with severe malignant middle cerebral artery infarction (MMCAI). We estimated the severity of MMCAI according to Glasgow Coma Scale (GCS) [31], and we defined a MMCAI as severe when GCS ≤ 8. We excluded patients with age less than 18 years, pregnancy, inflammatory or malignant disease, intracerebral hemorrhage or subarachnoid hemorrhage.

Previously, we determined in some of those patients serum levels of biomarkers related with inflammation, coagulation and oxidation such as substance P [32], soluble CD154 [33] and malondialdehyde [34]. The aim of the current research was to determine serum levels of a biomarker related with apoptosis, such as CCCK-18, in 66 patients with severe MMCAI.

Variables recorded

We recorded the following variables in each patient: age, sex, decompressive craniectomy, sodium, temperature, leukocytes, glycemia, pressure of arterial oxygen (PaO2), fraction inspired oxygen ($FI0_2$), creatinine, bilirubin, hemoglobin, lactic acid, GCS, platelets, international normalized ratio (INR), fibrinogen, activated partial thromboplastin time (aPTT), Acute Physiology and Chronic Health Evaluation II (APACHE II) score [35]. The end-point study was 30-day mortality.

Blood sample collection and serum CCCK-18 analysis

Serum blood samples were collected at the moment of the MMCAI diagnosis to measure serum CCCK-18 levels. All determinations were performed at the Laboratory Department of the Hospital Universitario de Canarias (La Laguna, Tenerife, Spain). We determine serum CCCK-18 levels by enzyme-linked immunosorbent assay (ELISA) using M30 Apoptosense® ELISA kit (PEVIVA AB, Bromma, Sweden). The intra-assay coefficient of variation (CV), inter-assay CV, and detection limit assay were < 10%, < 10% and 25 u/L respectively.

Statistical methods

Continuous and categorical variables were reported as medians (and interquartile ranges) and frequencies (and percentages) respectively. Continuous and categorical variables were compared between groups using Wilcoxon-Mann-Whitney test and chi-square test respectively. We carried out a multiple logistic regression to anlyze the association between serum CCCK-18 levels and mortality at 30 days after to control for platelet count and GCS. We calculated Odds Ratio and its 95% confidence intervals (CI) to measure the clinical impact of predictor variables. We performed receiver operating characteristic (ROC) curve to determine the prediction capacity of serum CCCK-18 levels for mortalty at 30 days. We constructed 30-day mortality Kaplan-Meier curves of patiens with higher and lower serum CCCK-18 levels than 298 u/L. Youden J index was used for the selection of 298 u/L as the optimal prognostic cut-off value of serum CCCK-18 level. All *p*-values lower than 0.05 were considered statistically significant. We performed statistical analyses using SPSS 17.0 (SPSS Inc., Chicago, IL, USA), LogXact 4.1, (Cytel Co., Cambridge, MA), and NCSS 2000 (Kaysville, Utah).

Results

A total of 33 of 66 patients (50.0%) with severe MMCAI died within 30-day diagnosis. We did not find statistically significant differences between non-surviving and surviving patients in age, sex, descompressive craniectomy, temperature, sodium, PaO2, PaO2/FI02 ratio, leukocytes, lactic acid, INR, hemoglobin, glycemia, fibrinogen, creatinine, bilirubin, aPTT, and APACHE-II score. Although we found that non-surviving MMCAI patients showed lower GCS and platelet count, and higher serum CCCK-18 levels than survivor ones (Table 1).

We found an area under the curve of serum CCCK-18 levels to predict mortality at 30 days of 82% (95% CI = 71%–91%; $p < 0.001$) (Fig. 1). In survival analysis was found that patients with serum CCCK-18 levels higher than 298 u/L showed a higher risk of mortality at 30 days (Hazard ratio = 5.0; 95% CI = 2.35–10.64; p < 0.001) than patients showed lower levels (Fig. 2).

In the multiple logistic regression was found that serum CCCK-18 levels were associated with mortality at 30 days (OR = 1.023; 95% CI = 1.010–1.037; $p = 0.001$) after to control for platelet count and GCS (Table 2).

Table 1 Clinical and biochemical characteristics of MMCAI patients according to 30-day survival

	Survivors ($n = 33$)	Non-survivors (n = 33)	*P* value
Age (years) - median (p 25–75)	59 (47–68)	64 (54–70)	0.30
Gender female - n (%)	14 (42.4)	13 (39.4)	0.99
Arterial hypertension - n (%)	19 (57.6)	16 (48.5)	0.62
Diabetes mellitus - n (%)	4 (12.1)	9 (27.3)	0.22
Chronic renal failure - n (%)	2 (6.1)	2 (6.1)	0.99
COPD - n (%)	1 (3.0)	1 (3.0)	0.99
Heart failure - n (%)	1 (3.0)	1 (3.0)	0.99
Haemorrhagic transformation - n (%)	7 (21.2)	6 (18.2)	0.99
Decompressive craniectomy – n (%)	9 (27.3)	6 (18.2)	0.56
Temperature (°C) - median (p 25–75)	36.4 (35.8–37.0)	37.0 (36.0–37.4)	0.19
Sodium (mEq/L)- median (p 25–75)	139 (137–145)	140 (139–146)	0.41
Platelets - median×10^3/mm^3 (p 25–75)	214 (170–280)	170 (131–212)	0.008
PaO2 (mmHg) - median (p 25–75)	137 (104–207)	114 (86–153)	0.26
PaO2/$FI0_2$ ratio - median (p 25–75)	300 (197–372)	248 (184–330)	0.22
Leukocytes-median×10^3/mm^3 (p 25–75)	12.5 (9.5–17.0)	13.9 (9.3–21.4)	0.43
Lactic acid (mmol/L)-median (p 25–75)	1.30 (0.90–1.70)	1.40 (1.00–2.10)	0.25
INR - median (p 25–75)	1.09 (1.01–1.20)	1.20 (1.05–1.31)	0.10
Hemoglobin (g/dL) - median (p 25–75)	12.2 (11.4–14.4)	13.7 (11.0–15.0)	0.78
Glycemia (g/dL) - median (p 25–75)	128 (100–170)	135 (105–160)	0.99
GCS score - median (p 25–75)	7 (6–8)	6 (3–7)	0.01
Fibrinogen (mg/dl) - median (p 25–75)	440 (335–494)	419 (311–631)	0.83
Creatinine (mg/dl) - median (p 25–75)	0.80 (0.60–1.15)	1.00 (0.76–1.28)	0.12
CCCK-18 (u/L) - median (p 25–75)	238 (160–290)	321 (279–351)	< 0.001
Bilirubin (mg/dl) - median (p 25–75)	0.70 (0.40–0.95)	0.70 (0.33–1.10)	0.86
aPTT (seconds) - median (p 25–75)	28 (26–30)	27 (26–32)	0.77
APACHE-II score - median (p 25–75)	20 (16–25)	22 (19–27)	0.09

COPD Chronic Obstructive Pulmonary Disease, *P 25–75* Percentile 25th–75th, PaO_2 Pressure of arterial oxygen/fraction inspired oxygen, FIO_2 Pressure of arterial oxygen/fraction inspired oxygen, *INR* International normalized ratio, *GCS* Glasgow Coma Scale *aPTT* activated partial thromboplastin time, *APACHE II* Acute Physiology and Chronic Health Evaluation, *CCCK* Caspase-cleaved cytokeratin

Discussion

To our knowledge, this is the first series reporting data on serum CCCK-18 levels in ischemic stroke patients. The novel findings of our study were that non-surviving severe MMCAI patients had higher serum CCCK-18 levels than surviving patients, and that there is an association between high serum CCCK-18 levels and MMCAI patients mortality.

Previously there has been found apoptotic changes in brain tissue samples from humans after cerebral ischemia [8–13]. However, the association between high serum CCCK-18 levels and MMCAI patients mortality found in our study is a novel finding. Those findings are in consonance with those of previous studies, due to that there is be found an association between high serum CCCK-18 levels and poor prognosis of patients with traumatic brain injury [28], acute spontaneous intracerebral haemorrhage [29] and aneurysmal subarachnoid hemorrhage [30].

The interpretation of all those findings is uncertain. Cytokeratin-18 exists mainly in the intracytoplasmic cytoskeleton of epithelial tissue and during apoptosis citokeratin-18 is cleaved by caspases and appears as CCCK-18 into the blood [14, 15]. Then the question about the origen of CCCK-18 in patients with traumatic brain injury [28], spontaneous cerebral hemorrhage [29, 30], and cerebral infarction (our current study) arise now. There is two posible splanations for that question. First, that there is cytokeratin-18 in brain; and this has been found in a study of patients with pituitary adenomas [36], and in a study of rats with glioma [37]. In the study by Luiciani et al. was found CCCK-18 in cell extracts of patients with pituitary adenomas, and the use of octreotide induced apoptosis in cells of growth hormone-secreting tumors assessed by the increased of CCCK-18 in cell extracts [36]. In the study by Adri et al was found

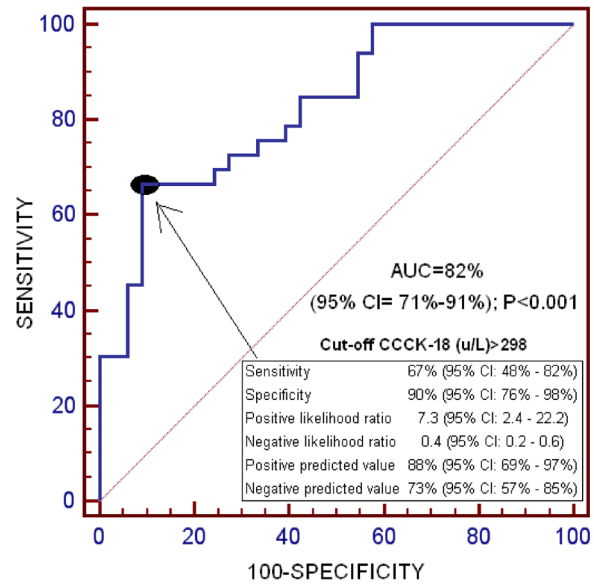

Fig. 1 Receiver operation characteristic analysis using serum caspase-cleaved cytokeratin (CCCK)-18 levels as predictor of mortality at 30 days

Table 2 Multiple logistic regression analysis to predict 30-day mortality

Variable	Odds Ratio	95% Confidence Interval	*P*
Serum CCCK-18 levels (u/L)	1.023	1.010–1.037	0.001
Glasgow Coma Scale (points)	0.769	0.534–1.105	0.16
Platelet count (each 1000/mm^3)	0.987	0.975–0.998	0.02

CCCK Caspase-cleaved cytokeratin

CCCK-18 in cell extracts of glioma from rats, and the use of *Parmelia sulcata* Taylor (one of the most common lichens that lives mainly in the bark of the trees) induced apoptosis in cell tumors assessed by the increased of CCCK-18 in cell extracts [37]. Second, that MMCAI may cause a systemic inflammatory response syndrome (SIRS), and this could activate sistemic cellular apoptosis. In fact, there are studies reporting SIRS after cerebral infarction [38–40], and in SIRS appears different pro-inflammatory cytokines [41] that could activate apoptosis [2–7].

The administration of some antiapoptotic agents in ischemic cerebral animal models have reduced brain apoptosis degree and functional deficits [42–44].

Some limitations of our study should be recognized. First, data about the evolution of circulating CCCK-18 concentrations during the evolution of non-surviving and surviving patients were not reproted. Second, data about serum CCCK-18 levels in healthy controls were not reported; although, the objective of our study was to determine whether there is an association between serum CCCK-18 levels and mortality in MMCAI patients and was not to determine whether there is an increase of serum CCCK-18 levels in MMCAI patients. Third, we have not explored apoptosis in cerebral samples; although, the objective of our study was to determine whether apoptosis is associated with mortality of MMCAI patients using a technique easily reproducible by other researchers. Fourth, we have not data about how many patients were excluded from the study and the exclusion motivation.

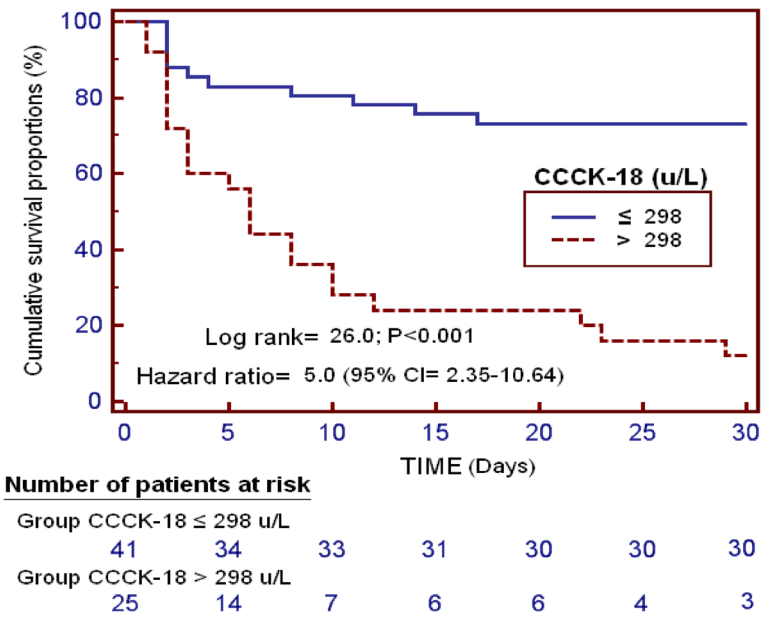

Fig. 2 Survival curves at 30 days using serum levels of caspase-cleaved cytokeratin (CCCK)-18 higher or lower than 298 u/L

Conclusions

To our knowledge, this is the first series reporting data on serum CCCK-18 levels in ischemic stroke patients. The novel findings of our study were that non-surviving severe MMCAI patients had higher serum CCCK-18 levels than surviving patients, and that there is an association between high serum CCCK-18 levels and MMCAI patients mortality.

Abbreviations

APACHE: Acute Physiology and Chronic Health Evaluation; CCCK: Caspase-cleaved cytokeratin; FIO_2: Fraction inspired of oxygen; GCS: Glasgow Coma Scale; ICU: Intensive Care Unit; INR: International normalized ratio; ISS: Injury Severity Score; PaO_2: Pressure of arterial oxygen

Acknowledgments

Not applicable.

Funding

This study was supported by a grant from Instituto de Salud Carlos III (INT16/00165) (Madrid, Spain) co-financed by Fondo Europeo de Desarrollo Regional (FEDER), and by a grant from Grupo de Expertos Neurológicos de Canarias (GEN-Canarias. Santa Cruz de Tenerife. Spain). Fundings did not influence in the study design, the collection, analysis, and interpretation of data, the manuscript writing, and the decision to submit it for publication.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LL conceived, designed and coordinated the study, participated in acquisition and interpretation of data, and drafted the manuscript. MMM, MA, LR, JSV, JJC, VGM participated in acquisition of data. APC participated in blood determination levels. AJ participated in the interpretation of data. All authors revised the manuscript critically for important intellectual content, made the final approval of the version to be published, and were agree to be accountable for all aspects of the work.

Ethics approval and consent to participate

The study was approved by the local ethics committees of the 6 hospitals participating in the study: Insular of Las Palmas de Gran Canaria, Universitario Nuestra Señora de Candelaria of Santa Cruz de Tenerife, General of La Palma, Universitario Dr. Negrín of Las Palmas de Gran Canaria, Clínico Universitario of Valencia, and Universitario de Canarias of La Laguna. Legal guardians of the patients signed informed consent to participate in the study. The study adheres to the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Author details

[1]Intensive Care Unit, Hospital Universitario de Canarias, Ofra s/n, La Laguna, -38320 Santa Cruz de Tenerife, Spain. [2]Intensive Care Unit, Hospital Universitario Nuestra Señora de Candelaria, Crta del Rosario s/n, -38010 Santa Cruz de Tenerife, Spain. [3]Laboratory Deparment, Hospital Universitario de Canarias, Ofra, s/n., La Laguna -, 38320 Tenerife, Spain. [4]Intensive Care Unit, Hospital General La Palma, Buenavista de Arriba s/n, -38713 Breña Alta, La Palma, Spain. [5]Intensive Care Unit, Hospital Clínico Universitario de Valencia, Avda Blasco Ibáñez n°17-19, -46004 Valencia, Spain. [6]Intensive Care Unit, Hospital Universitario Dr. Negrín, CIBERES, Barranco de la Ballena s/n, -35010 Las Palmas de Gran Canaria, Spain. [7]Intensive Care Unit, Hospital Insular, Plaza Dr. Pasteur s/n, 35016 Las Palmas de Gran Canaria, Spain. [8]Research Unit, Hospital Universitario de Canarias, Ofra s/n. La Laguna, -38320 Santa Cruz de Tenerife, Spain. [9]Deparment of Neurosurgery, Hospital Universitario de Canarias, Ofra, s/n. La Laguna, 38320 Santa Cruz de Tenerife, Spain.

Received: 1 November 2017 Accepted: 15 March 2018
Published online: 24 March 2018

References

1. Adams HP Jr, del Zoppo G, Alberts MJ, Bhatt DL, Brass L, Furlan A, Grubb RL, Higashida RT, Jauch EC, Kidwell C, Lyden PD, Morgenstern LB, Qureshi AI, Rosenwasser RH, Scott PA, Wijdicks EF, American Heart Association; American Stroke Association Stroke Council; Clinical Cardiology Council; Cardiovascular Radiology and Intervention Council; Atherosclerotic Peripheral Vascular Disease and Quality of Care Outcomes in Research Interdisciplinary Working Groups. Guidelines for the early management of adults with ischemic stroke: a guideline from the American Heart Association/American Stroke Association stroke council, clinical cardiology council, cardiovascular radiology and intervention council, and the atherosclerotic peripheral vascular disease and quality of care outcomes in research interdisciplinary working groups: the American Academy of Neurology affirms the value of this guideline as an educational tool for neurologists. Stroke. 2007;38:1655–71.
2. Radak D, Katsiki N, Resanovic I, Jovanovic A, Sudar-Milovanovic E, Zafirovic S, Mousad SA, Isenovic ER. Apoptosis and acute brain ischemia in ischemic stroke. Curr Vasc Pharmacol. 2017;15:115–22.
3. Khoshnam SE, Winlow W, Farzaneh M, Farbood Y, Moghaddam HF. Pathogenic mechanisms following ischemic stroke. Neurol Sci. 2017;38:1167–86.
4. Chelluboina B, Klopfenstein JD, Gujrati M, Rao JS, Veeravalli KK. Temporal regulation of apoptotic and anti-apoptotic molecules after middle cerebral artery occlusion followed by reperfusion. Mol Neurobiol. 2014;49:50–65.
5. Akpan N, Troy CM. Caspase inhibitors: prospective therapies for stroke. Neuroscientist. 2013;19:129–36.
6. Chen SD, Yang DI, Lin TK, Shaw FZ, Liou CW, Chuang YC. Roles of oxidative stress, apoptosis, PGC-1α and mitochondrial biogenesis in cerebral ischemia. Int J Mol Sci. 2011;12:7199–215.
7. Broughton BR, Reutens DC, Sobey CG. Apoptotic mechanisms after cerebral ischemia. Stroke. 2009;40:e331–9.
8. Duan SR, Wang JX, Wang J, Xu R, Zhao JK, Wang DS. Ischemia induces endoplasmic reticulum stress and cell apoptosis in human brain. Neurosci Lett. 2010;475:132–5.
9. Lorberboym M, Blankenberg FG, Sadeh M, Lampl Y. In vivo imaging of apoptosis in patients with acute stroke: correlation with blood-brain barrier permeability. Brain Res. 2006;1103:13–9.
10. Qi JP, Wu AP, Wang DS, Wang LF, Li SX, Xu FL. Correlation between neuronal injury and Caspase-3 after focal ischemia in human hippocampus. Chin Med J. 2004;117:1507–12.
11. Love S, Barber R, Wilcock GK. Neuronal death in brain infarcts in man. Neuropathol Appl Neurobiol. 2000;26:55–66.
12. Rami A, Sims J, Botez G, Winckler J. Spatial resolution of phospholipid scramblase 1 (PLSCR1), caspase-3 activation and DNA-fragmentation in the human hippocampus after cerebral ischemia. Neurochem Int. 2003;43:79–87.
13. Mitsios N, Gaffney J, Krupinski J, Mathias R, Wang Q, Hayward S, Rubio F, Kumar P, Kumar S, Slevin M. Expression of signaling molecules associated with apoptosis in human ischemic stroke tissue. Cell Biochem Biophys. 2007;47:73–86.
14. Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. Histopathology. 2002;40:403–39.
15. Caulín C, Salvesen GS, Oshima RG. Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. J Cell Biol. 1997;138:1379–94.
16. Roth GA, Krenn C, Brunner M, Moser B, Ploder M, Spittler A, Pelinka L, Sautner T, Wolner E, Boltz-Nitulescu G, Ankersmit HJ. Elevated serum levels of epithelial cell apoptosis-specific cytokeratin 18 neoepitope m30 in critically ill patients. Shock. 2004;22:218–20.
17. Moore DJ, Greystoke A, Butt F, Wurthner J, Growcott J, Hughes A, Dive C. A pilot study assessing the prognostic value of CK18 and nDNA biomarkers in severe sepsis patients. Clin Drug Investig. 2012;32:179–87.

18. Hofer S, Brenner T, Bopp C, Steppan J, Lichtenstern C, Weitz J, Bruckner T, Martin E, Hoffmann U, Weigand MA. Cell death serum biomarkers are early predictors for survival in severe septic patients with hepatic dysfunction. Crit Care. 2009;13:R93.
19. Lorente L, Martín MM, González-Rivero AF, Ferreres J, Solé-Violán J, Labarta L, Díaz C, Jiménez A, Borreguero-León JM. Serum levels of Caspase-cleaved Cytokeratin-18 and mortality are associated in severe septic patients: pilot study. PLoS One. 2014;e109618:9.
20. Lorente L, Martín MM, Pérez-Cejas A, López RO, Ferreres J, Solé-Violán J, Labarta L, Díaz C, Palmero S, Buitrago M, Jiménez A, Borreguero-León JM. Higher serum caspase-cleaved cytokeratin-18 levels during the first week of sepsis diagnosis in non-survivor patients. Clin Chem Lab Med. 2017;55:1621–9.
21. Bantel H, Lügering A, Heidemann J, Volkmann X, Poremba C, Strassburg CP, Manns MP, Schulze-Osthoff K. Detection of apoptotic caspase activation in sera from patients with chronic HCV infection is associated with fibrotic liver injury. Hepatology. 2004;40:1078–87.
22. Sgier C, Müllhaupt B, Gerlach T, Moradpour D, Negro F, Malé PJ, Heim MH, Malinverni R, Cerny A, Dufour JF. Effect of antiviral therapy on circulating cytokeratin-18 fragments in patients with chronic hepatitis C. J Viral Hepat. 2010;17:845–50.
23. Sumer S, Aktug Demir N, Kölgelier S, Cagkan Inkaya A, Arpaci A, Saltuk Demir L, Ural O. The clinical significance of serum apoptotic cytokeratin 18 Neoepitope M30 (CK-18 M30) and matrix metalloproteinase 2 (MMP-2) levels in chronic hepatitis B patients with cirrhosis. Hepat Mon. 2013;e10106:13.
24. Parfieniuk-Kowerda A, Lapinski TW, Rogalska-Plonska M, Swiderska M, Panasiuk A, Jaroszewicz J, Flisiak R. Serum cytochrome c and m30-neoepitope of cytokeratin-18 in chronic hepatitis C. Liver Int. 2014;34:544–50.
25. Lorente L, Rodriguez ST, Sanz P, Pérez-Cejas A, Padilla J, Díaz D, González A, Martín MM, Jiménez A, Barrera MA. Prognostic Value of Serum Caspase-Cleaved Cytokeratin-18 Levels before Liver Transplantation for One-Year Survival of Patients with Hepatocellular Carcinoma. Int J Mol Sci. 2016;17
26. Ueno T, Toi M, Bivén K, Bando H, Ogawa T, Linder S. Measurement of an apoptotic product in the sera of breast cancer patients. Eur J Cancer. 2003; 39:769–74.
27. Greystoke A, O'Connor JP, Linton K, Taylor MB, Cummings J, Ward T, Maders F, Hughes A, Ranson M, Illidge TM, Radford J, Dive C. Assessment of circulating biomarkers for potential pharmacodynamic utility in patients with lymphoma. Br J Cancer. 2011;104:719–25.
28. Lorente L, Martín MM, González-Rivero AF, Argueso M, Ramos L, Solé-Violán J, Cáceres JJ, Jiménez A, Borreguero-León JM. Serum levels of caspase-cleaved cytokeratin-18 in patients with severe traumatic brain injury are associated with mortality: a pilot study. PLoS One. 2015;10:e0121739.
29. SJ G, Lu M, Xuan HF, Chen XZ, Dong WF, Yan XF, Si Y, Gao GL, Hu DX, Miao JQ. Predictive value of serum caspase-cleaved cytokeratin-18 concentrations after acute intracerebral hemorrhage. Clin Chim Acta. 2016;452:124–8.
30. Yuan ZG, Wang JL, Jin GL, Yu XB, Li JQ, Qiu TL, Dai RX. Serum caspase-cleaved cytokeratin-18 levels and outcomes after aneurysmal subarachnoid hemorrhage. J Neurol Sci. 2015;359:298–304.
31. Teasdale G, Jennett B. Assessement of coma and impaired conciousness. A practical scale. Lancet. 1974;2:81–4.
32. Lorente L, Martín MM, Almeida T, Pérez-Cejas A, Ramos L, Argueso M, Riaño-Ruiz M, Solé-Violán J, Hernández M. Serum Levels of Substance P and Mortality in Patients with a Severe Acute Ischemic Stroke. Int J Mol Sci. 2016;17.
33. Lorente L, Martín MM, González-Rivero AF, Ramos L, Argueso M, Cáceres JJ, Solé-Violán J, Jiménez A, Borreguero-León JM. Association between Serum Soluble CD154 Levels and Mortality in Patients with Malignant Middle Cerebral Artery Infarction. Int J Mol Sci. 2015;16:12147–58.
34. Lorente L, Martín MM, Abreu-González P, Ramos L, Argueso M, Solé-Violán J, Riaño-Ruiz M, Jiménez A. Serum malondialdehyde levels in patients with malignant middle cerebral artery infarction are associated with mortality. PLoS One. 2015;10:e0125893.
35. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med. 1985;13:818–29.
36. Luciani P, Gelmini S, Ferrante E, Lania A, Benvenuti S, Baglioni S, Mantovani G, Cellai I, Ammannati F, Spada A, Serio M, Peri A. Expression of the antiapoptotic gene seladin-1 and octreotide-induced apoptosis in growth hormone-secreting and nonfunctioning pituitary adenomas. J Clin Endocrinol Metab. 2005;90:6156–61.
37. Ari F, Aztopal N, Oran S, Bozdemir S, Celikler S, Ozturk S, Ulukaya E. Parmelia sulcata Taylor and Usnea filipendula Stirt induce apoptosis-like cell death and DNA damage in cancer cells. Cell Prolif. 2014;47:457–64.
38. Kalita J, Bastia J, Bhoi SK, Misra UK. Systemic inflammatory response syndrome predicts severity of stroke and outcome. J Stroke Cerebrovasc Dis. 2015;24:1640–8.
39. Xue YY, Xu XY, Li G, Wang Y. A prospective study for systemic inflammatory response syndrome (SIRS) after cerebral infarction. Zhonghua Nei Ke Za Zhi. 2008;47:988–90.
40. Boehme AK, Kapoor N, Albright KC, Lyerly MJ, Rawal PV, Bavarsad Shahripour R, Alvi M, JT H, Sisson A, Beasley TM, Alexandrov AW, Alexandrov AV, Miller DW. Predictors of systemic inflammatory response syndrome in ischemic stroke undergoing systemic thrombolysis with intravenous tissue plasminogen activator. J Stroke Cerebrovasc Dis. 2014;23:e271–6.
41. Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med. 2013;369:840–51.
42. Chen B, Wang G, Li W, Liu W, Lin R, Tao J, Jiang M, Chen L, Wang Y. Memantine attenuates cell apoptosis by suppressing the calpain-caspase-3 pathway in an experimental model of ischemic stroke. Exp Cell Res. 2017; 351:163–72.
43. Cho YS, Shin MS, Ko IG, Kim SE, Kim CJ, Sung YH, Yoon HS, Lee BJ. Ulinastatin inhibits cerebral ischemia-induced apoptosis in the hippocampus of gerbils. Mol Med Rep. 2015;12:1796–802.
44. Liu DM, Wang ZH, Liu L, Zhang XM, Lou FL. Acetylpuerarin increases cell viability and reduces apoptosis in rat hippocampal neurons following oxygen-glucose deprivation/reperfusion. Mol Med Rep. 2013;8:1453–9.