Association between serum levels of caspase-cleaved cytokeratin-18 and early mortality in patients with severe spontaneous intracerebral hemorrhage

Leonardo Lorente1*, María M. Martín2, Antonia Pérez-Cejas3, Luis Ramos4, Mónica Argueso5, Jordi Solé-Violán6, Juan J. Cáceres7, Alejandro Jiménez8 and Victor García-Marín9

Abstract

Background: Apoptotic changes after cerebral hemorrhage in brain samples of humans have been found. Caspase-cleaved cytokeratin (CCCK)-18 could be detected in the bloodstream during apoptosis. Higher circulating CCCK-18 levels have been associated with 6-month mortality in patients with basal ganglia hemorrhage. The aim of our study was to determine whether there is an association between serum CCCK-18 levels and early mortality of spontaneous intracerebral hemorrhage (SIH) patients. We performed an observational, prospective and multicentre study. There were included patients with severe SIH defined as Glasgow Coma Scale (GCS) lower than 9. We determined serum CCCK-18 levels at the severe SIH diagnosis moment.

Results: We found that non-surviving SIH patients (n = 46) showed lower GCS, and higher serum CCCK-18 levels and APACHE-II score than survivor ones (n = 54). In ROC analysis was found that the area under the curve of serum CCCK-18 levels for 30-day mortality prediction was 90% (95% CI 82–95%; p < 0.001). In the multiple logistic regression analysis, we found an association between serum CCCK-18 levels and 30-day mortality (OR 1.034; 95% CI 1.013–1.055; p = 0.002).

Conclusions: The novel finding of our study was that there is an association between high serum CCCK-18 levels and 30-day mortality in severe SIH patients.

Keywords: Caspase-cleaved cytokeratin-18, Spontaneous intracerebral hemorrhage, Patients, Mortality

Introduction

Spontaneous intracerebral hemorrhage (SIH) results in a large amount of deaths, disabilities, and resource consumption [1–3]. Cell death by apoptosis occurs in cerebral hemorrhage [4–7]. Primary damage is caused by the effect of hematoma leading to disruption and mechanical deformation of cellular architecture. Secondary damage is induced by mitochondrial dysfunction, microglia activation and the release of neurotransmitter and inflammatory mediators; and those events lead to necrosis and to the occurrence of programmed cell death by apoptosis [4–7].

Between 1999 and 2005 in some animals models were found the presence of apoptosis after cerebral hemorrhage [8–11]. In those studies with rats and rabbits using the terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine (dUTP)-biotin nick end labeling (TUNEL) were found cells undergoing DNA fragmentation in the striatum and in deep white matter in the frontal lobe; and those cells were mostly neurons and astrocytes in the center and periphery of cerebral hemorrhage [8–11]. Afterwards, apoptotic changes after cerebral hemorrhage in human brain samples have been found in several studies from 2003, and included

*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

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TUNEL-positive cells and an increase of caspase-3 expression [12–18].

Cytokeratins (CK) family is a group of proteins distributed mainly in the epithelial tissue. Until now are known 20 types, named CK-1 to CK-20. CK-18 is cleaved by the action of caspasases during apoptosis, then caspase-cleaved cytokeratin (CCCK)-18 appears and could be detected in the bloodstream [19, 20].

Higher circulating CCCK-18 levels have been found in patients with sepsis [21–25], liver diseases [26–30], and tumoral diseases [31, 32]. Besides, higher circulating CCCK-18 levels have been associated with a poor prognosis in patients with cerebral process, such as traumatic brain injury [33], aneurysmal subarachnoid hemorrhage [34] and basal ganglia hemorrhage [35]. In the study by Gu et al. of patients with basal ganglia hemorrhage was found an association between circulating CCCK-18 levels and 6-month mortality [35]. The aim of our study was to determine whether there is an association between serum CCCK-18 levels and early mortality of SIH patients.

Methods
Design and subjects
This multicentre, observational and prospective study was carried in six Intensive Care Units from Spain after the Institutional Review Board approval of all participating hospitals and with the written consent of patient guardians. Participating hospitals were the following: H. Clínico Universitario de Valencia (Valencia), H. Universitario Nuestra Señora de Candelaria (Tenerife), H. General de La Palma (La Palma), H. Universitario de Canarias (Tenerife), H. Insular (Gran Canaria), H. Universitario Dr. Negrín (Gran Canaria).

We included patients with severe supratentorial SIH. We used Glasgow Coma Scale (GCS) [36] to classify SIH severity; and we considered severe SIH when GCS ≤ 8. Exclusion criteria were infratentorial or traumatic hemorrhage, inflammatory or malignant disease, hemorrhagic transformation of cerebral infarction, age < 18 years, and pregnancy.

Variables recorded
The following variables were recorded for each patient: sex, site and cause of SIH, volume of SIH, presence of intraventricular hemorrhage or hydrocephalus, midline shift, evacuation of SIH, age, sodium, temperature, platelets, pressure of arterial oxygen (paO2), fraction of inspired oxygen (FiO2), international normalised ratio (INR), fibrinogen, activated partial thromboplastin time (aPTT), lactic acid, GCS, creatinine, glycaemia, Acute Physiology and Chronic Health Evaluation II (APACHE II) score [37]. The end-point study was mortality at 30 days after severe SIH diagnosis.

Blood sample collection and serum CCCK-18 analysis
We collected serum blood samples when severe SIH was diagnosed to determine serum CCCK-18 levels. We determined serum CCCK-18 levels at the Laboratory Department of the Hospital Universitario de Canarias (Tenerife, Spain) by an enzyme-linked immunosorbent assay (ELISA). The kit used was M30 Apoptosense® ELISA kit (PEVIVA AB, Bromma, Sweden), and its intra-assay coefficient of variation (CV), inter-assay CV, and detection limit assay were < 10, < 10% and 25 u/L, respectively.

Statistical methods
We reported continuous and categorical variables as medians (with interquartile ranges) and frequencies (with percentages) respectively. We compared continuous and categorical variables between groups by means of Wilcoxon–Mann–Whitney test and Chi square test respectively. We used multiple logistic regression analysis to determine the association of serum CCCK-18 levels with 30-day mortality, after controlling for GCS, midline shift, age, intraventricular hemorrhage, and volume of intracerebral hemorrhage. Odds Ratio and its 95% confidence intervals (CI) were calculated to estimate the impact of each predictor variable. A receiver operating characteristic (ROC) analysis was carried out to determine the capacity of 30 day-mortality prediction by serum CCCK-18 levels. We made 30 day-mortality Kaplan–Meier curves of SIH patients with higher/lower serum CCCK-18 levels than 156 u/L (due to that those serum CCCK-18 levels were the optimal prognostic cut-off, for sensitivity and specificity, according to Youden J index). Each p value lower than 0.05 was considered as statistically significant. We performed statistical analyses by SPSS 17.0 (SPSS Inc., Chicago, IL, USA), LogXact 4.1, (Cytel Co., Cambridge, MA), and NCSS 2000 (Kaysville, Utah).

Results
Of the 100 patients with severe SIH, a total of 54 patients were alive at 30 days and 46 patients died during the first 30 days of SIH diagnosis. We did not find statistically significant differences between patient groups (non-surviving and surviving) in sex, site of SIH, cause of SIH, intraventricular hemorrhage, hydrocephalus, SIH evacuation, sodium, temperature, sodium, PaO2/FiO2 ratio, platelets, INR, lactic acid, fibrinogen, creatinine, and aPTT. However, we found that non-surviving SIH patients showed lower GCS, and higher serum CCCK-18 levels, APACHE-II score, age, volume of SIH, midline shift, and glycemia than survivor ones (Table 1).

We found the following correlations between serum CCCK-18 levels and age (rho = 0.19; p = 0.053), SIH volume (rho = 0.26; p = 0.02), midline shift (rho = 0.19;
We found the following differences in the comparisons in the area under the curve for 30-day mortality prediction: serum CCCK-18 levels of 90% (95% CI 82–95%; p < 0.001) (Fig. 1), age of 72% (95% CI 60–82%; p = 0.004), SIH volume of 68% (95% CI 56–79%; p = 0.02), midline shift of 68% (95% CI 55–78%; p = 0.003), GCS of 78% (95% CI 66–87%; p < 0.001), APACHE-II score of 81% (95% CI 69–89%; p < 0.001).

We found the following differences in the comparisons between the study by Gu et al. [35] and our study. In the study by Gu et al. patients were included with basal ganglia hemorrhage [35]. However, the association between serum CCCK-18 levels and basal ganglia hemorrhage [35]. In our study, patients with SIH and GCS ≤ 8 were included, and in the study by Gu et al., the clinical condition of severity was not used for the inclusion of patients. In the study by Gu et al. the end-point study was late mortality (at 6 months), and in our study our end-point was early mortality (at 30 days). The findings of our study are in consonance with those of other previous studies by our team, of patients with traumatic intraventricular haemorrhage, and age (Table 2).

### Table 1 Clinical and biochemical characteristics of 30-day surviving and non-surviving patients with spontaneous intracerebral hemorrhage (SIH)

| Characteristic                        | Survivors (n = 54) | Non-survivors (n = 46) | P value |
|---------------------------------------|------------------|------------------------|---------|
| Gender female—n (%)                  | 17 (31.5)        | 17 (37.0)              | 0.67    |
| Site of SIH—n (%)                    | 0.81             |                        |         |
| Lobar                                 | 41 (75.9)        | 33 (71.7)              |         |
| Basal ganglia                         | 3 (5.6)          | 4 (8.7)                |         |
| Thalamus                              | 5 (9.3)          | 3 (6.5)                |         |
| Periventricular                       | 5 (9.3)          | 6 (13.0)               |         |
| Cause of SIH—n (%)                   | 0.07             |                        |         |
| Hypertension                          | 37 (68.5)        | 30 (65.2)              |         |
| Amyloid angiopathy                    | 2 (3.7)          | 4 (8.7)                |         |
| Aneurysm                              | 3 (5.6)          |                        |         |
| Arteriovenous malformation            | 5 (9.3)          |                        |         |
| OAT in therapeutic range              | 3 (5.6)          | 6 (13.0)               |         |
| OAT out of therapeutic range          | 3 (5.6)          | 6 (13.0)               |         |
| Fibrinolytic treatment                | 1 (1.9)          |                        |         |
| Intraventricular hemorrhage—n (%)    | 17 (31.5)        | 23 (50.0)              | 0.07    |
| Hydrocephalus—n (%)                  | 21 (38.9)        | 26 (56.5)              | 0.11    |
| Evacuation SIH—n (%)                 | 18 (33.3)        | 9 (19.6)               | 0.18    |
| Age (years)—median (p 25–75)         | 59 (52–67)       | 68 (57–74)             | 0.006   |
| Volume of SIH (cc)—median (p 25–75)  | 38 (17–62)       | 68 (29–99)             | 0.02    |
| Midline shift (mm)—median (p 25–75)  | 1 (0–7)          | 5 (0–11)               | 0.005   |
| Sodium (mEq/L)—median (p 25–75)      | 139 (137–142)    | 139 (135–143)          | 0.93    |
| Temperature (°C)—median (p 25–75)    | 36.9 (36.0–37.4) | 36.5 (35.0–37.0)       | 0.10    |
| PaO2/FIO2 ratio—median (p 25–75)      | 270 (189–350)    | 289 (215–397)          | 0.40    |
| Platelets—median*10⁹/mm³ (p 25–75)   | 193 (145–252)    | 198 (159–270)          | 0.57    |
| INR—median (p 25–75)                 | 1.10 (1.00–1.31) | 1.14 (1.02–1.87)       | 0.34    |
| Lactic acid (mmol/L)—median (p 25–75)| 1.70 (1.00–2.51) | 1.80 (1.30–2.55)       | 0.23    |
| GCS score—median (p 25–75)           | 8 (6–8)          | 4 (3–6)                | <0.001  |
| Glycemia (g/dL)—median (p 25–75)     | 141 (118–190)    | 170 (141–216)          | 0.01    |
| aPTT (s)—median (p 25–75)            | 29 (27–32)       | 30 (24–34)             | 0.68    |
| Fibrinogen (mg/dl)—median (p 25–75)  | 390 (280–493)    | 382 (350–510)          | 0.34    |
| APACHE-II score—median (p 25–75)     | 18 (14–20)       | 24 (20–26)             | <0.001  |
| Creatinine (mg/dl)—median (p 25–75)  | 0.77 (0.68–0.90) | 0.80 (0.60–1.01)       | 0.36    |
| CCC-18 (u/L)—median (p 25–75)        | 129 (112–177)    | 291 (212–341)          | <0.001  |

P 25–75 25th–75th percentile, OAT oral anticoagulant treatment, PaO2 pressure of arterial oxygen/fraction inspired oxygen, FIO2, 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

The novel finding of our study was that there is an association between high serum CCCK-18 levels and 30-day mortality in severe SIH patients.

Previously, higher circulating CCCK-18 levels have been associated with 6-month mortality in patients with basal ganglia hemorrhage [35]. However, the association between high serum CCCK-18 levels and early SIH mortality found in our study is a novel finding. There are some differences between the study by Gu et al. [35] and our study. In the study by Gu et al. patients were included with basal ganglia hemorrhage [35], and our study included patients with supratentorial SIH at different locations (lobar, basal ganglia, thalamus and periventricular). In our study, patients with SIH and GCS ≤ 8 were included, and in the study by Gu et al., the clinical condition of severity was not used for the inclusion of patients. In the study by Gu et al. the end-point study was late mortality (at 6 months), and in our study our end-point was early mortality (at 30 days). The findings of our study are in consonance with those of other previous studies by our team, of patients with traumatic
Our study presents some limitations. First, we do not report serum CCCK-18 concentrations in healthy controls; however, our objective study was to determine whether there is an association between serum CCCK-18 levels and SIH patient mortality and was not to determine whether SIH patients showed increased serum CCCK-18 levels. Second, there was not reported data about apoptosis in cerebral samples; however, our aim was to use an easy technique to facilitate the reproduction by other researchers. Third, data about the evolution of circulating CCCK-18 concentrations in non-surviving and surviving patients during the evolution were not reported.

CCCK-18 is not a specific biomarker of brain apoptosis; thus, higher circulating CCCK-18 levels have been found in patients with sepsis [21–25], liver diseases [26–30], and tumoral diseases [31, 32]. However, serum CCCK-18 levels have been associated with mortality in patients with SIH in the study by Gu et al. [35] and in our study. The clinical utility of serum CCCK-18 levels for mortality prediction should be taken with caution; however, we think that could be considered as an additional biomarker in the prognostic prediction of ICH patients to other markers (as GCS, age, midline shift, volume of SIH, or intraventricular hemorrhage). In addition, we found that serum CCCK-18 levels could be a better prognostic biomarker in ICH patients that age, SIH volume, midline shift, GCS, APACHE-II score according the area under the curve for 30-day mortality prediction of those variables.

Finally, the administration of antiapoptotic agents in SIH animal models have reduced cerebral apoptosis degree and functional deficits [9, 38–43]. Thus, all these findings could foster interest about research on apoptosis in SIH patients.

Conclusions
The novel finding of our study was that there is an association between high serum CCCK-18 levels and 30-day mortality in severe SIH patients.
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. All authors read and approved the final manuscript.

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 Department, Hospital Universitario de Canarias, Ofra, s/n, La Laguna, 38320 Santa Cruz de Tenerife, Spain. 4 Intensive Care Unit, Hospital General La Palma, Buenavista de Arriba s/n, Breña Alta, 38713 La Palma, Spain. 5 Intensive Care Unit, Hospital Clinico Universitario de Valencia, Avda. Blasco Ibáñez 16, 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 Department of Neurosurgery, Hospital Universitario de Canarias, Ofra, s/n, La Laguna, 38320 Santa Cruz de Tenerife, Spain.

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Not applicable.

Competing interests
The authors declare that they have no competing interests.

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

Consent for publication
Not applicable.

Ethics approval and consent to participate
This multicentre, observational and prospective study was carried in 6 Intensive Care Units from Spain after the Institutional Review Board approval of all participating hospitals and with the written consent of patient guardians. Participating hospitals were the following: H. Clinico Universitario de Valencia (Valencia), H. Universitario Nuestra Señora de Candelaria (Tenerife), H. General La Palma (La Palma), H. Universitario de Canarias (Tenerife), H. Insular (Gran Canaria), H. Universitario Dr. Negrín (Gran Canaria).

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