

**Manuscript ID:** 80845

**Manuscript Title:** Elevated sFas blood concentrations in patients dying from spontaneous intracerebral hemorrhage

**Response to Editor comments:**

Thank you very much for offering to reconsider a revised version of our manuscript. Thank you for your comments and those from reviewers which have helped us to improve our manuscript. The points that we have modified in the manuscript are written in red. We enclosed the answer to editor and reviewers, and the new version of the manuscript.

**Response to Referee 03976790 comments:**

**Comments about the manuscript: “Elevated sFas blood concentrations in patients dying from spontaneous intracerebral hemorrhage.”** Brain samples from hematomas evacuated by patients with spontaneous intracerebral hemorrhage (SIH) show changes of an apoptotic nature. However, there does not seem to be any data on blood concentrations of sFas involved in apoptosis in patients with SIH. The aim of this work was to investigate whether an association existed between blood concentrations of sFas and mortality in patients with SIH. This work is of some interest. Conclusions open up a new field of investigation concerning a particularly important health problem. However, the manuscript requires some improvements before considering its possible publication.

**Remarks Page 4 “When the binding between Fas and FasL occurs, then a death signal appear and the extrinsic pathway is activated.”: replace “appear” with “appears”.**

We have replaced this point as was suggested by the reviewer

**Page 5. “We recruited patients with severe and supratentorial SIH.”: How many patients? How many males, females? (In table 1, only female percentage is given: why not males?)**

As was suggested by the reviewer, we have included the following information in page 5: **“We recruited 75 patients (29 females and 46 males) with severe and supratentorial SIH”.**

In addition, also as was suggested by the reviewer, we have included male percentage in table 1.

**Page 6. “We taken serum samples at moment of severe SIH diagnosis and we frozen the samples at -80°C”. I suggest: “We collected serum samples at the time of severe HIS diagnosis and frozen the samples at -80°C”.**

We have replaced this point as was suggested by the reviewer.

**Page 6. “We determined all sFas at the same time with Human Fas ELISA Kit”: Describe the method and explain the technique**

We have added the following information to describe the method and technique: “**We determined all sFas at the same time with Human Fas ELISA Kit (Elabscience, Houston, Texas, United States), which had 19 pg/mL as detection limit and <6% as intra and inter-assay variation coefficients. This kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human Fas. Standards or samples are added to appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibodies specific for Human Fas and Avidin-Horseradish Peroxidase (HRP) conjugate are added to each micro plate well successively and incubated. After incubation, free components are washed away. Then the Substrate Reagent is added to each well, only those wells that contain Human Fas, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction will be terminated by adding Stop Solution and appears yellow in color. The optical density (OD) can be measured with spectrophotometry at a wavelength of 450 nm  $\pm$  2 nm. The OD value is proportional to the concentration of Human Fas. The concentration of Human Fas in samples can be calculated by comparing the OD of the samples with the standard curve.**”

**Page 6 (statistical methods). “We constructed Kaplan-Meier curves of 30-day mortality in patiens with serum sFas concentrations”: write “patients “instead of “patiens”.**

We have replaced this point as was suggested by the reviewer.

**Page 7, discussion. “Thus, our study report novel findings”: write “reports” instead of “report”.**

We have replaced this point as was suggested by the reviewer.

**Page 8. “However, a limitation...”: I appreciate the caution and honesty of the authors. Their idea leads to a new research path.**

We are pleased with its opinion about our manuscript.

**References: check that all the bibliographical references are presented according to the standards of the journal.**

We have checked the bibliographical references as was suggested by the reviewer.

**Response to Referee 06073015 comments:**

We are pleased with its opinion about our manuscript.