A point-by-point response to referees' issues

Manuscript NO: 62491

Title: Acute liver failure with thrombotic microangiopathy from sodium valproate toxicity:A case report

Thanks for your comments on our manuscripts. We appreciate these comments, which help us improve our work. All the new or revised information was marked in red throughout the manuscript. And we would be glad to respond to any further questions and comments that you may have.

Reviewer #1:Specific Comments to Authors: *Title and "running" title: accurately reflects the topic and content of the paper. Abstract: is appropriate, structured, quite long (238 words) – could be shortened! Key words: 6 key words, define the content of the paper. Core tip: is appropriate, 97 words. Introduction: is informative, short, 142 words, the reader is very briefly acquainted with the known facts about sodium valproat induced liver injury. Case presentation: informative, 842 words, key data about the patient are presented: chief complaints, history of present and past ilnesses, personal and family history, physical examination upon admission, laboratory data, results of imaging procedures (a welcome addition to the presentation would be histology of the liver, autopsy ?!), further diagnostic work-up and final diagnosis (including Fig. 1,2 – they should be in better resolution, which will give the reader a better insight into the chronology of treatment and the values of laboratory dara), treatment, follow-up and fatal outcome, patient died 12 days after surgery. Discussion: short, 791 words, the discussion is interesting, the authors point out the pathophysiological mechanisms that probably triggered acute liver failure in the described patient, they do not forget to mention the potential interaction with the drug that the patient was receiving also (carbapenem antibiotics) and could affect the fateful course. They also explain the mechanism of thrombotic microangiopathy (TMA), which is defined as a clinical syndrome characterized by thrombocytopenia, hemolytic anemia and multiple organ dysfunction. Drug-induced TMA (DI-TMA) is a type of acquired TMA that is caused by multiple drugs. Explained are two mechanism of injury: a non-dose-dependent immune reaction or dose- and time-dependent toxicity. The patient presented can be classified as having immune-mediated drug induced DI-TMA. Conclusion: short, 97 words, the authors summarize the key findings of the presented patient and the fatal complication. References: 37 (quite a lot), contemporary, references are appropriate. Conflict of interest: the authors declared no conflict of interest. CARE Checklist statement: the manuscript was prepared and revised according to the CARE Checklist. Informed consent statement: was obtained from the patient/family for publication of this report. Opinion of the reviewer The contribution is interesting, the authors point out an important problem with the use of sodium valproat. I suggest to accept the manuscript after language corrections, the authors should improve the figures.*

Answer: We thank the reviewer for the nice summary and advice. Following the reviewer's suggestion, revision of the report are as follows:

1. We have shortened the words of the abstract. Specific changes can be seen in the article of revise version. **(Page 3)**

2. Due to the patient's serious and rapid progress disease, liver puncture may increase the risk of haemorrhage, so physicians didn't perform a liver biopsy. Additionally, family members of the patient reject autopsy, so we didn't get the liver pathology. We described this limitation in the discussion.**(Page 14，Line 7-8)**

3. We have improved the resolution of figure 1 and figure 2 by re-uploading figures.

4. We have streamlined the amount of the reference to 26.

Reviewer #2: Specific Comments to Authors: *Thank you for submitting your interesting case report. It highlights a number of important issues, including valproic associated toxicity. While this is a known association, it is still a unique case worth highlighting. There are many excellent points throughout the article. We appreciate your clear timeline throughout the article. Our minor recommendations for ways to improve are as follows:*

We thank the reviewer for the nice summary and comments. Following the reviewer's suggestion, revision of the report are as follows:

(1) Your abstract could be shortened by not including all of the laboratory data and summarizing the highlights.

Answer: We have shortened the abstract by summarizing the laboratory data in brief words . Specific changes can be seen in the article of revise version. **(Page 3)**

(2) Additional details regarding the staging of the meningioma and pre-operative work-up would be useful to provide context to the reader.

Answer: We have added the staging of the meningioma (WHO grade II atypical meningioma)**(Page 5，Line 7-8)**, preoperative abdominal color Doppler ultrasound and Cranial MRI enhancement datas **(Page 5，Line 16-19)** in the History of present illness section. Specific changes can be seen in the article of revise version.

(3) An explanation of the antibiotic choices would be useful.

Answer: The patient developed fever on postoperative day 1, and intracranial infection needed to be firstly considered. According to experience, the surgeon selected ceftriaxone plus

vancomycin. And then, reexamination revealed elevated CSF leukocytes on postoperative day 5, so it was adjusted to biapenem plus linezolid. We have added this detail in the History of present illness section. **(Page 6，Line 4-5)**

(4) You state that since he did not have active alcohol use or Hepatitis B, it was presumed to be secondary to Valproic acid, but were there any other findings that suggested this. While you state there is no correlation between serum level and hepatotoxicity, it would still be worthwhile to note.

Answer: Sodium valproate is an antiepileptic drug known to have hepatotoxicity. the current diagnosis of Drug-induced liver injury (DILI) is primarily governed by exclusive method[1]. After excluding other causes of liver damage, we considered his acute liver failure to be attributed to the drug. As shown in the second paragraph of the discussion, according to the onset time of the patient and the characteristics of liver injury, we conjecture that his liver injury was induced by sodium valproate. Here, our diagnostic tool is the RUCAM score,which is recognized as the primary DILI causality assessment tool[2].

We discussed the correlation between serum level and hepatotoxicity in the DISUSSION part. As the literature shown[3,4], some research suppose that hepatotoxicity is independent of the serum while many others hold the opinion that high serum of sodium valproate is more likely to induce liver injury. **(Page 11，Line 14 - Page 12，Line 1)**

(5) Any details regarding imaging during this post-operative period of the liver or abdomen would be useful (such as CT scans).

Answer:We have added the imaging examination by describing the results of abdominal CT scans **(Page 8，Line 8-13)** and uploading the related pictures.

(6) It would be worth clarifying when the patient developed renal failure and was this simply shock liver or secondary to another cause.

Answer:

1,It can be seen in the history of present illness, although the patient's Scr was normal (80 μmol/L) in the morning on postoperative day 9, but he had developed soy-sauce-colored and oliguria. On postoperative day 10, he gradually became anuria and his Scr elevated to 255

μmol/L. According to those clinical manifestations and laboratory datas, we considered that kidney damage began to develop on postoperative day 9.

2,The pathophysiology of acute kidney injury secondary to liver failure contains prerenal azotemia, acute tubular necrosis and hepatorenal syndrome[5]. As we know, patient with AKI usually present with oliguria, anuria, and increased serum creatinine while hemolysis was rare. However, our patient developed hemoglobinuria which indicate hemolysis, so we diagnosed that his renal failure was associated with TMA rather than AKI secondary to liver failure.

(7) Discussion: I recommend clarifying the sentence "Carbapenem antibiotics can reduce the plasma concentration of sodium valproate by inhibiting its transmembrane transport in erythrocytes[37]; therefore, sodium valproate in combination with carbapenem antibiotics may increase the risk of hemolysis." It is not clear how inhibiting the transmembrane transport of valproate impacts a reduction in the plasma concentrations of the drug.

Answer: We conjectured that sodium valproate in combination with carbapenem antibiotics may increase the risk of hemolysis according to our case investigation.The animal experiment showed that carbapenem antibiotics can reduce the plasma concentration of sodium valproate by inhibiting multidrug resistance-associated proteins which can efflux sodium valproate back to the plasma from erythrocytes[6].It has been reported that sodium valproate can destroy erythrocytes by changing the membrane fluidity and receptor protein on the membrane[7] . However, the correlation between blood concentration of sodium valproate and hemolysis is not clear. Based on the studies above, we consider that the effect of carbapenem antibiotics in combination with sodium valproate on hemolysis needs further research. we have changed the sentence in the DISUSSION part in the revised version. **(Page 13, Line 15 - Page 14，Line 4)**

(8) Two additional relevant articles to review include are: -Thrombocytopenic purpura and anemia in a breast-fed infant whose mother was treated with valproic acid. Stahl MM, Neiderud J, Vinge E. J Pediatr. 1997 Jun;130(6):1001-3. doi: 0.1016/s0022-3476(97)70292-0. PMID:9202628 -Thrombopenia and erythroblastopenia in a 1-month-old infant treated with valproate.Nathan D, Guillon JL, Chevallier B, Gallet JP. Ann Pediatr (Paris). 1987 Feb;34(2):149-50. PMID:3107456.

Answer: Thanks for your recommendation. We have review the literatures which advanced our knowledge about TMA due to sodium valproate.

Reviewer #3: Specific Comments to Authors: *Dear authors I have reviewed your manuscript dealing about a male patient after neurosurgical procedure and valproic acid induced lethal liver failure. Although it is an interesting report I have some relevant comments.*

You postulate a novel pathway of valproic acid and drug-induced liver failure: thrombotic microangiopathy. But there is no imaging or further diagnostic tools mentioned which support your hypothesis. The characteristic features of thrombotic microangiopathy as you mentioned are not different to parameters of liver failure (anemia, thrombocytopenie, organ dysfunction). You do not discuss other causes or pathways for liver failure (e.g. rhabdomyolisis which has been previously postulated, this would be an explanation for the increase of creatine kinase) in this setting. What makes you sure that this is the pathway of liver failure.

Answer: The current diagnosis of Drug-induced liver injury(DILI) is primarily governed by exclusive method. First, according to the patient's history of present illness, history of past illness, and medication history, it is know to us that there were no common causes for rhabdomyolisis such as trauma, exertion, hyperthermia, infections et al and genetic causes[8].Second, though he was treated by various drugs during hospitalization, the major adverse reactions of those drugs do not include rhabdomyolisis. Third, the patient didn't develop classic symptom like muscle pain. On the other hand, we excluded hepatic virus which is the most common cause of liver failure in china. After excluding the above possible causes of liver failure, combining with his onset time and clinical manifestation, we consider his liver failure was caused by treatment of sodium valproate.

Did you measure other lab parameters (e.g. ammonia) or did you perform a liver biopsy to reveal histological changes.

Answer:

1.We didn't detect ammonia because the patient can be diagnosed with hepatic encephalopathy based on his clinical history of acute liver failure, mental status change and flapping tremors.

2. Due to the patient's serious and rapid progress disease, liver puncture may increase the risk of haemorrhage, so physicians didn't perform a liver biopsy. Additionally, family members of the patient reject autopsy, so we didn't get the liver pathology. We described this limitation in the discussion.**(Page 14，Line 7-8)**

Did you treat your patient with N-acetylcysteine or other symptomatic or supportive treatment? There are some language and editorial issues which should be revised (delete all dates within the text: e.g. November 9, 2019....).

Answer: Yes, we used N-acetylcysteine for liver protection. Additionally, vitamin, electrolyte and enteral nutritional supplementation are also be used for symptomatic or supportive treatment. Drug therapy of the patient was described in the Tretment part in the revise version. **(Page 9，Line 6-8)**

Reviewer #4: Specific Comments to Authors: *The authors described the newly diagnosis of TMA due to sodium valproate. Case presentation was written well, however this manuscript included several problems.*

First, *the basis of TMA diagnosis was described not enough. In the diagnosis of TMA, fragmented erythrocytes in the peripheral blood smear was needed (https://www.uptodate.com/contents/drug-induced-thrombotic-microangiopathy?topicRef=88648&source=see_link). However, the authors described "but there were no fragmented erythrocytes on the peripheral blood smear" in Laboratory examinations section. Similarly, severe thrombocytopenia was needed. Why didn't the authors explained the data on ADAMTS13 activities and ADAMTS13 inhibitors? I considered that this patient couldn't diagnosed with TMA.*

Answer: Thanks for the reviewer for the questions. TMA syndromes are incited by microvascular endothelial injury leading to arteriolar and capillary thrombosis and subsequent organ injury. Evidences consistent with TMA are as follows:

1, microthrombosis: D-D exceeded 35.2 mg/L, thrombocytopenia(109 g/L on postoperative day 9 to 39 g/L on postoperative day 11)

2, microangiopathic hemolysis: anemia(Hgb dropped from 132 g/L before surgery to 61 g/L on postoperative day 10) with reticulocyte count of 4.5%, LDH was up to 21962.0 U/L, significantly elevated indirect bilirubin, hemoglobinuria, proteinuria and positive urobilinogen.

3, acute kidney injury: anuria and increased creatinine level.

4, response to PE and methylprednisolone: The patient's hemolysis was controlled, the color of his separated plasma gradually changed from red-brown to dark yellow, and the hemoglobin level did not decline after PE and methylprednisolone therapy.

About fragmented erythrocytes in the peripheral blood smear: We have detect the patient's peripheral blood smear twice, One was before PE therapy (on postoperative day 10) and another was after PE therapy (on postoperative day 11), unfortunatelly, both peripheral blood smears result were negative. We consider the second negative result could be attributed to plasmapheresis which can eliminate erythrocyte fragments[9,10].

The diagnostic criteria and classic teaching has revealed that evidence of microangiopathic hemolytic anemia is a sine qua non for the diagnosis of TMA[11]. However, Cases of TMA without presence of fragmented erythrocytes on the peripheral blood smear have been reported previously[12-14]. In the meantime, this patient responded to PE and methylprednisolone. Therefore, we supposed that sometimes the presence of fragmented erythrocytes may not be absolutely essential for the diagnosis of TMA.We have described this issue in the DISCUSSION in the revise version.**(Page 12，Line 4-18)**

About detection of ADAMTS13 activity: Although sometimes it was difficult to distinguish between TTP and HUS because both HUS and TTP have similar clinical manifestations such as thrombocytopenia, microangiopathic hemolysis, and renal insufficiency. A severe deficiency of ADAMTS13 activity (<10%) in classic form of TTP while high activity of ADAMTS13 (>10%) in HUS may offer some help in differential diagnosis[15,16]. Due to our unit had not carried out this detecting item, we didn't detect ADAMTS13 activities or ADAMTS13 inhibitors in the early stage of the onset of our patient, which was a limitation in our study.We have described the limitation of this case report in the DISCUSSION in the revise version.**(Page 14，Line 8-12)**

Second, *because this manuscript was based on wrong diagnosis, there were no novelty. For*

example, "therefore, sodium valproate in combination with carbapenem antibiotics may increase the risk of hemolysis" was written in Discussion section, this consideration was syllogism.

Answer: Thanks for the reviewer for the questions.

We conjectured that sodium valproate in combination with carbapenem antibiotics may increase the risk of hemolysis according to our case investigation.The animal experiment showed that carbapenem antibiotics can reduce the plasma concentration of sodium valproate by inhibiting multidrug resistance-associated proteins which can efflux sodium valproate back to the plasma from erythrocytes[6].It has been reported that sodium valproate can destroy erythrocytes by changing the membrane fluidity and receptor protein on the membrane[7]. However, the correlation between blood concentration of sodium valproate and hemolysis is not clear. Based on the studies above, we consider that the effect of carbapenem antibiotics in combination with sodium valproate on hemolysis needs further research. we have changed the sentence in the DISUSSION part in the revised version. **(Page 13, Line 15 - Page 14，Line 4)**

Third, *limitations of this study was out of my hands.*

Answer: We have added the limitations of our study in the revised version. **(Page 14, Line 6-12)**

Minor revision The author used the abbreviations at the wrong time. The abbreviation was used when the words were first used in your manuscript. For example, in line 12, Introduction section, "thrombotic microangiopathy" was not used the abbreviation.

Answer: We have made corrections in the revised version by following the guideline for writting abbreviations in the article.

Reviewer #5: Specific Comments to Authors: *This case is very interesting and worthy of publication, but I have some comments:*

1) the normal value of laboratory indicators should be given, especially such as D-dimer; different clinics use different methods, which makes it difficult to evaluate the data;

2) if the authors have already used abbreviations, then they should apply them further in the text, and not write the words completely.

Answer: We would like to thank the reviewer for the advice. Following the reviewer's advice, we have

1) added the normal value of laboratory indicators in the article. Specific details can be seen in the revised version. **(Page 5, Line 11-16)、(Page 7，Line 4 and Line 18 and Line 20)**

2) made corrections in the revised version by following the guideline for writting abbreviations in the article.

Reference

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Thanks for your comments on our manuscripts. We appreciate these comments, which help us improve our work. All the new or revised information was marked in red throughout the manuscript. And we would be glad to respond to any further questions and comments that you may have.

Reviewer's（code: 02549939）**:** *Dear authors I have reviewed your revised manuscript again. Thank you for your comments and answers regarding my concerns. I think the manuscript has improved and you could demonstrate why this case is probably interesting for other clinicians. Unfortunately, you did not include all your answers within the revised manuscript. I would include these comments and references, because other readers will probably have the some questions regarding this case. In addition you should revise the manuscript again regarding language and editorial issues (e.g. spelling errors). Best regards.*

Answer: We thank the reviewer for the nice summary and advice. Following the reviewer's suggestion, revision of the report are as follows:

1.We have written all our answers within the revised manuscript by adding explanations for why the patient's liver failure is not caused by rhabdomyolysis in the discussion. **(Page 11，Line 10-16)**

2. We have improved the language and modified the spelling errors in the revise version.