Peer Review File

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Reviewer A

The authors describe a study focused on acquired hypofibrinogenemia and administration of batroxobin for hemoptysis. Acquired hypofibrinogenemia is most frequently caused by hemodilution and consumption of clotting factors. Batroxobin acts on fibrinogen to produce a fibrin monomer that can be converted by thrombin to a fibrin clot. It is used as a haemostatic.
The methodological part is processed very thoroughly.
However, some parts need to be corrected as recommended.

Comment 1. Page 4 lines 33-34. TXA is used in surgery but also in various other bleeding manifestations in congenital coagulopathies, such as fibrinogen deficiency. Authors should cite the manuscript in which it was described: „Diagnostics 2021, 11(11), 2140; https://doi.org/10.3390/diagnostics11112140".
Reply 1. Thank you for your comment. In the reference you recommended, we found no evidence of tranexamic acid. We found other reference and cited it instead.
Changes in the text: Tranexamic acid is used to prevent bleeding associated with traumatic injury, surgery, and congenital fibrinogen deficiency (See line 34-35 on page 5.)

Comment 2. Page 6 lines 70-75. In this section, the authors should specify in more detail that. On the basis of the level of fibrinogen concentration, hypofibrinogenemia is classified as severe, moderate, or mild: J. Clin. Med. 2022, 11(4), 1083; https://doi.org/10.3390/jcm11041083"
Reply 2. Thank you for your suggestions. We added the classification of hypofibrinogenemia in the method section as you recommend. We also added the incidence of mild, and moderate hypofibrinogenemia in the result section.
Changes in the text: We also classified the hypofibrinogenemina as mild (200 ~ 100 mg/dL), moderate (100 ~ 50 mg/dL), and severe (50 ~ 10 mg/dL). (See line 81-82 on page 7.) Mild hypofibrinogenemia occurred in 28 patients (15.3%) and moderate

hypofibrinogenemia in 60 patients (32.8%) after batroxobin use. There were no cases of severe hypofibrinogenemia. (See line 123-124 on page 10)

Comment 3. In addition, in this section, more authors should state that minimum hemostatic fibrinogen level is 0.5 g / l at bleeding. For unprovoked hemorrhage, suggested fibrinogen concentrations are >1 g/L until hemostasis is normalized and >0.5 g/L until the bleeding surface is entirely restored. Authors should cite the manuscripts in which it were described: Diagnostics 2021, 11(11), 2140; https://doi.org/10.3390/diagnostics11112140".

Reply 3. Thank your comments. We added your advice to the discussion section.

Changes in the text: The minimum hemostatic fibrinogen level is 50 mg/dL at bleeding. For unprovoked hemorrhage, suggested fibrinogen concentrations are >100 mg/dL until hemostasis is normalized and >50 mg/dL until the bleeding surface is entirely restored. (See line 230-233 on page 15)

Comment 4. Page 13 lines 202-205. In addition, it is important to note that oerioperative management in patients with congenital hypofibrinogenemia is also a significant progranostic factor. Difficulty of management, because in addition to bleeding there is also a risk of thrombotic treatment, a balance in treatment is necessary. It is appropriate to make the following relevant reference: ,, Thrombosis Research, 2020, vol. 188, p. 1-4. doi: 10.1016/j.thromres.2020.01.024"

Reply 4. Thank your comments. We added your advice to the discussion section.

Changes in the text: In hypofibrinogenemic patients, thrombotic events can occur when thrombotic factor risks are present, so bleeding risk and thrombotic risk must be managed jointly. (See line 236-238 on page 16)

Comment 5. Figures and tables in the text are very clearly written.

Reply 5. Thank you.

Comment 6. I have to say that with these 28 references of which only half of the references are in the last 5 years. In the manuscript, new references should be added.

Reply 6. Thank you for your comments. We added additional references as your recommendation.

Changes in the text: See page 20-23 (References)

Reviewer B

In this study, the authors investigated the risk factors and prognosis of acquired hypofibrinogenemia in hemoptysis patients treated systemically with batroxobin. And this study showed high-dose or long-term batroxobin use was associated with the development of hypofibrinogenemia. Furthermore, hypofibrinogenemia was associated with poorer outcomes and higher 30-day mortality in patients. This report is very interesting. However, several problems should be resolved for the manuscript to be accepted for publication in Journal of Thoracic Disease.

<Major points>

Comment 1. What criteria were used for the decision on whether to treat with batroxobin in this study? Did the judgment of a particular clinician affect the patient's inclusion?

Reply 1. Thank you for your comments. Regardless of bronchial artery embolization, batroxobin was administered according to the clinician's judgment. Patient inclusion was not affected by the judgment of a specific clinician in this retrospective study. We added the modification to the method section.

Changes in the text: Batroxobin was administered according to the clinician's judgment regardless of bronchial artery embolization. (See line 91-92 on page 8)

Comment 2. From this study, it is unclear whether batroxobin should be given to patients with hypofibrinolysis. In other words, compared with no batroxobin administration, does batroxobin improve the prognosis even if patients with hypofibrinolysis? The authors should show the result of the mortality in hemoptysis patients who were not treated with batroxobin. If it is difficult, the author should discuss this topic in the discussion section.

Reply 2. Thank you for your comments. Batroxobin was administered according to the clinician's judgment regardless of bronchial artery embolization. However, most hemoptysis patients in our institution receive batroxobin. Therefore, we could not evaluate hypofibrinogenemia and prognosis based on batroxobin use. We added this

limitation to the limitation part of discussion section.

Changes in the text: Fourth, because most hemoptysis patients in our institution receive batroxobin, we could not evaluate hypofibrinogenemia and prognosis based on batroxobin use. Additional studies are needed to investigate the hemostatic effect, hypofibrinogenemia occurrence, and prognosis of batroxobin in hemoptysis patients. (See line 250-254 on page 16)

Comment 3. The results of Tables 2-4 are very interesting. However, in multivariate analysis, selecting the independent variable from the statistically significant factors in the univariate analysis may not be appropriate (PMID: 8699212). In addition to the current model, the authors should create a multivariate analysis model that selects the independent variable from the factors already reported to be associated with the dependent variable. If it is difficult, the author should discuss this topic in the discussion section.

Reply 3. Thank you for your valuable comments. Multivariate regression models have the limitation of selecting only statistically significant variables after univariate analysis. To reduce the possibility of missing variables in the univariate analysis, we performed multivariate analysis after selecting variables with a P value within 0.25. DIC had a P value of 0.392 in the univariate analysis, but it is associated with hypofibrinogenemia. Multivariate analysis also included DIC. Consequently, table, 2, 3, and 4 have been changed. The statistical part of the method, the results, tables 2,3,4 and limitations have been changed.

Changes in the text: Subsequent multivariate logistic regression analyses included variables with P values of < 0.25 in the univariate analysis using a forward method. Although DIC and chronic liver disease had P values greater than 0.25 in the univariate analysis, we included both variables in the multivariate logistic regression analysis as they were associated with acquired hypofibrinogenemia. (See line 104-108 on page 8) Factors associated with acquired hypofibrinogenemia were identified using a Cox-regression analysis that included variables with P values of < 25 in the univariate analysis using the forward method. (See line 111-113 on page 8.) Fifth, to avoid missing important variables in the multivariate analysis, we selected variables with a P value within 0.25 in the univariate analysis. However, the possibility of missing some important variables cannot be completely ruled out. (See line 254-256 on page 16). See

also table 2, 3, and 4.

<Minor points>

Comment 1. Did the authors confirm the distribution of the value? Characteristics with a non-normal distribution should be indicated by median (IQR) rather than mean + SD.

Reply 1. Thank you for your comments. We changed values as median (IQR).

Changes in the text: All data were expressed as the median (interquartile range) or number (percentage). Demographic and clinical variables were compared between the hypofibrinogenemia group and non-hypofibrinogenemia group using the chi-square test (for categorical variables) or the Mann-Whitney U test (for continuous variables). (See line 97-100 on page 8). See also table 1.

Comment 2. In table 1, the authors should add the information about respiratory parameters (e.g., P/F ratio)

Reply 2. Thank you for your comment. We added respiratory parameter such respiratory rate, PF ratio, pH, and pCO2.

Changes in the text: See table 1.

Respiratory rate

20 (20–20)

20 (20–20)

0.216

PaO2/FiO2 ratio

335 (263–403)

313 (195–426)

0.312

pH

7.43 (7.39–7.45)

7.42 (7.39–7.44)

0.168

pCO2

38.9 (35.0–43.8)

40.0 (33.7–45.0)
0.875

Reviewer C

This is an interesting and well written review paper. However, the value can still be improved by addressing the following comments.

Introduction:
Comment 1. Line 51. Plasma fibrinogen level of inferior (<) 150 ug/dl. It is an exclusion factor. Should be changed to understand the sentence.
Reply 1. Thank you for your comment. We changed the text as your advice.
Changes in the text: Patients with a baseline plasma fibrinogen level of <150 mg/dL, those without plasma fibrinogen measurements, or those aged <18 years were excluded. (See line 52 on page 6.)

Definitions:
Comment 2. Line 62. Definition of hemoptysis 100ml/24h??? You should explain your definition.
Reply 2. Thank you for your comment. Unfortunately, the definition of massive has not been established completely. In previous studies, the cut-off volume for massive hemoptysis ranged from 100 to 1000 mL/24 hours or 200 to 600 mL. We set the massive hemoptysis at 100 mL/24hr since it was the smallest amount reported. [Eur Respir J . 2008 Oct;32(4):1131-2. doi: 10.1183/09031936.00080108. PMID: 18827169] We modified manuscript.
Changes in the text: “presence of massive hemoptysis (≥ 100 mL/24 hour of hemoptysis)” (See line 70 on page 7.) Massive hemoptysis was defined as hemoptysis of more than 100 mL/24 h (6,24). (See line 86-87 on page 7.)

Comment 3. Line 69. You should explain the analytic method used to determine fibrinogen levels.
Reply 3. Thank you for your comment. The Fibrinogen Clauss assay was used to measure plasma fibrinogen concentration. We modified text of method section.

Changes in the text: The Fibrinogen Clauss assay was used to measure plasma fibrinogen concentration. (See line 82-83 on page 7.)

Comment 4. Also you have to include the current doses of batroxobin that have been used in this study and the timing during the 3 or 4 days.

Reply 4. Thank you for your comments. It is unknown what dosage of batroxobin is appropriate for patients with hemoptysis. Batroxobin at 10–20 NIHU was diluted with 500 mL of normal saline and administered intravenously for 24 hours. After hemoptysis had been confirmed, batroxobin was administered. Batroxobin administration was stopped after hemoptysis improved clinically. We modified the manuscript in the method section.

Changes in the text: Batroxobin was administered according to the clinician's judgment regardless of bronchial artery embolization. Batroxobin at 10–20 NIHU was diluted with 500 mL of normal saline and administered intravenously for 24 hours. Batroxobin administration was stopped after hemoptysis improved clinically. (See line 91-94 on page 8.)

Discussion:

Comment 5. Line 198. You would have to explain how and when you have to determine fibrinogen levels and the timing of batroxobin administration.

Reply 5. Thank you for your comments. We modified the manuscript in the discussion section as your advice.

Changes in the text: Therefore, plasma fibrinogen levels should be measured before administration of batroxobin, and patients with low hypofibrinogen levels or on long-term or high-doses of batroxobin should have their plasma fibrinogen levels monitored closely. (See line 217-219 on page 14-15.)

Comment 6. Line 215. Take into account that hemodilution it is a very important parameter. You should reference if those patients who develop hypofibringonemia received more fluids. It could be a confusion factor.

Reply 6. Thank you for your comments. The medical charts in this study were thoroughly reviewed, and no hemodilution conditions were found. However, hemodilution could not be completely excluded from hydration because of the

retrospective study design. We added this limitation in the limitation part of discussion section.

Changes in the text: The medical charts in this study were thoroughly reviewed, and no hemodilution conditions were found. However, hemodilution could not be completely excluded from hydration because of the retrospective study design. (See line 246-248 on page 16.)

Comment 7. Line 218. More references to disseminated intravascular coagulation and hypofibrinogenemia. How you defined this situation?

Reply 7. Thank you for your comment. DIC was diagnosed following the algorithm of the International Society on Thrombosis and Haemostasis. There is decreased platelet count and prolonged PT and aPTT in patients with DIC, as well as hypofibrinogenemia in patients with DIC. We modified the manuscript in the method section.

Changes in the text: Disseminated intravascular coagulation (DIC) was diagnosed following the algorithm of the International Society on Thrombosis and Haemostasis. (25) (See line 87-88 on page 7.)

Conclusions

Comment 8. You need to mention the point of care methods to determine fibrinogen level as ROTEM or TEG because they are quicker that analytic methods and nowadays we have strong evidence for using it in an emergency situation as a hemoptysis.

Reply 8. Thank you for your valuable comments. We added your advice in the discussion section.

Changes in the text: There has been a recent increase in the use of functional fibrinogen assays such as thromboelastography and rotational thromboelastometry, allowing for the identification of fibrinogen deficiency and transfusion more quickly in trauma and surgical bleeding cases. (30) Further research is needed on the effectiveness of measuring baseline and follow-up fibrinogen levels with these functional fibrinogen assays in patients receiving batroxobin for hemoptysis. (See line 219-224 on page 15.)

Comment 9. Also it is important to propose future studies taking into account the fibrinogen levels (monitoring the patients with point of care devices as ROTEM or TEG) just arriving to the emergency room department to start the treatment with batroxobin

and maybe improving at the same time fibrinogen levels with concentrate of fibrinogen.

Reply 9. Thank you for your valuable comments. We added your advice in the discussion section.

Changes in the text: There has been a recent increase in the use of functional fibrinogen assays such as thromboelastography and rotational thromboelastometry, allowing for the identification of fibrinogen deficiency and transfusion more quickly in trauma and surgical bleeding cases. (30) Further research is needed on the effectiveness of measuring baseline and follow-up fibrinogen levels with these functional fibrinogen assays in patients receiving batroxobin for hemoptysis. (See line 219-224 on page 15.)