

Response to Reviewer 3 Comments

Thank you very much for such valuable contributions to the manuscript. I made the correspondent modifications after reviewing your suggestions.

Minor edits:

- 1) *line 52/53: grammar is odd. Would write instead: All affected genes encode proteins that participate in the TGF β signaling pathway.*

Thanks, it clearly makes the sentence more fluent and easier to read. We changed it to: "All affected genes encode proteins that participate in the transforming growth factor β (TGF- β) signaling pathway".

- 2) *In table 1: would say GDF2 is HHT-like (under phenotype). Also, the unknown gene after the GDF2 is the RASA 1 gene, which I would describe under phenotype as RASA-1 related disorders (CM-AVM). I would take out the last unknown from that table.*

Thank you for such a valuable contribution, We updated the table to actualize it with the new data.

- 3) *line 61, generate instead of generated*
- 4) *line 69: typo- HHT instead of THH*

Thank you, changes made for typos 3) and 4). Sorry for the mistakes.

- 5) *line 119-120: revise grammar.*

Thank you, We made the sentence shorter and changed to make it more understandable: "On the other hand, it has been shown that sol-eng mediates platelet adhesion through the IIb3 integrin complex present in platelets [23]. This could explain why patients with HHT, in addition to having an increased risk of bleeding, also tend to bleed longer and more profusely."

- 6) *line 148-149: What do you mean by urgent situations? Regarding targets for anemia, would describe both targeting hemoglobin and ferritin levels.*

Indeed, the sentence needed further clarification, we explained other situations and added some examples: "In patients with very symptomatic HHT (chronic uncontrolled bleeding), comorbidities (heart failure, hypoxemia from pulmonary AVM) urgent situations (such as surgery or pregnancy), maintaining hemoglobin levels above 8-9 g/dL by red blood cell transfusions can be considered to avoid low oxygen supply to peripheral tissue."

To add additional targets for anemia, we added the follow sentence: “On clinically stable patients with no cardiac or pulmonary comorbidities, iron supplementation is indicated to maintain a hemoglobin level above 7 g/dL and a ferritin level above 100 µg/L”.

We also added on Table 3 a comment on the treatment of anemia adding: “A usual dose of 35 mg elemental iron tablets daily is indicated”

7) Table 2:

- a. *I don't understand what you mean by produce recurrent bleeding in support areas. Please clarify.*

Thank you for your comment. We wanted to mean all exposed areas to the body that could harm easily if there was a telangiectasia on it. It could be better explained by “It can produce recurrent bleeding in the bearing areas of the body or those in contact with external surfaces such as the fingertips”.

- b. *Digestive avm prevalence seems low. Can you check multiple sources to confirm?*

Digestive AVM prevalence:

From the Second international HHT guideline 2020: “HHT-related GI bleeding develops in approximately 30% of HHT patients, typically manifesting in the 5th-6th decades”.

- Faughnan, M.E.; Mager, J.J.; Hetts, S.W.; Palda, V.A.; Lang-Robertson, K.; Buscarini, E.; Deslandres, E.; Kasthuri, R.S.; Lausman, A.; Poetker, D.; et al. Second International Guidelines for the Diagnosis and Management of Hereditary Hemorrhagic Telangiectasia. *Ann Intern Med* **2020**, *173*, 989–1001, doi:10.7326/M20-1443.

From another source cited on the manuscript the prevalence is between 13-30%.

“Nose bleeding is the most common symptom, followed by GI bleeding, which occurs at the site of telangiectases in 13–30% of HHT patients.”

- Canzonieri, C.; Centenara, L.; Ornati, F.; Pagella, F.; Matti, E.; Alvisi, C.; Danesino, C.; Perego, M.; Olivieri, C. Endoscopic Evaluation of Gastrointestinal Tract in Patients with Hereditary Hemorrhagic Telangiectasia and Correlation with Their Genotypes. *Genetics in Medicine* **2014**, *16*, 3–10, doi:10.1038/gim.2013.62.

Another publication has the same prevalence:

“The prevalence of GI telangiectasia ranges from 13% to 30% in the overall HHT population to more than 90% in HHT patients with anemia”.

- Mora-Luján, J.M.; Iriarte, A.; Alba, E.; Sánchez-Corral, M.Á.; Berrozpe, A.; Cerdà, P.; Cruellas, F.; Ribas, J.; Castellote, J.; Riera-Mestre, A. Gastrointestinal Bleeding in Patients with Hereditary Hemorrhagic

Telangiectasia: Risk Factors and Endoscopic Findings. JCM 2019, 9, 82, doi:10.3390/jcm9010082.

- c. *Under pulmonary HTN comments-would say under comments:
Can be caused by different mechanisms including hereditary
group 1 PAH, or due to high cardiac output in the setting of liver
avms (mostly associated with ACVRL1 mutation).*

On the pulmonary hypertension comment, your suggestion clearly helps to better understand it, thank you. We have made the corresponding changes on the table.

- 8) *Line 160/161: I would state that most pulmonary avms are asymptomatic, but can rarely cause hypoxemia and/or digital clubbing. Other rare complications include hemoptysis or hemothorax.*

It seems correct, thank you.

- 9) *Line 181-181: Would change to: The most frequent clinical manifestations of liver avms are symptoms related to high cardiac output heart failure, including dyspnea and edema. Other patients can present with abdominal pain from biliary ischemia or anicteric cholestasis.*

Updated, thank you.

- 10) *Line 190- please specify which genotype*

We clarified the genotype: "An increase in these AVMs has been observed with age and patient genotype ENG".

- 11) *Line 194-seizures (instead of epilepsies)*

Updated, thank you.

- 12) *Line 220-normal or low cardiac output.*

Updated thank you.

- 13) *Line 244-247: In all patients with HHT, an echo with bubble study is recommended every 5 years. Only if bubble study is positive, should a CT scan be done. In patients who continue to have positive bubble studies despite pulmonary avm embolization, a CT scan can be done every 5 years.*

Thank you very much for the clarification, it adds a lot to the review.

- 14) *Line 249-250: Most HHT experts recommend brain imaging (1 MRI with and without contrast, and repeat as an adult if first one done as a child) for screening. I'm not sure I would say that this is debatable in adults, but*

you can say that it is somewhat controversial due to some data suggesting low risk of complications.

Thank you, we agree that it can be difficult to affirm that sentence. We have made an update in the paragraph pointing out what most clinicians perform, making a small emphasis on the lack of data.

“For cerebral AVMs, MRI is recommended in children and some reviews suggest at the time of diagnosis [25,32]. Most HHT experts recommend brain imaging (1 MRI with and without contrast and repeat as an adult if first one done as a child) for screening. However, there is controversial data regarding imaging on adults, since some data suggest low risk of complications. Indeed, follow-up imaging tests are not recommended when the MRI done in adulthood is negative”.

15)Line 253-254: "the study should be extended", should be changed to: additional imaging can be considered with the use of MRI...

Updated, thank you

16)Table 3: I think this table is messy and includes a lot of treatments that are not approved for HHT or have been shown to be ineffective in clinical trials. This should be noted somewhere (for instance, all the topical treatments were negative in the NOSE trial). I would revise the epistaxis and GI bleeding section. Would take out the comment about oral beta blockers, tacrolimus, and octreotide could be considered. There is no data to support this statement. Under cerebral abscess: avoid administering air bubbles when cannulating veins (not airways). Would refer to the new HHT guidelines to improve this table and the recommendations (hhtguidelines.org).

We have updated the table considering your comments. We deleted the topical treatment that has no evidence and we added a sentence on the comments as on the manuscript to cite the NOSE trial: “There is no evidence showing better results comparing topical therapy to humidification for preventing epistaxis [46].”

We also clarify the statement of local tranexamic acid on the “antifibrinolytic section: “Antifibrinolytics are placed in the first line of treatment in HHT, aminocaproic acid and tranexamic acid the principal ones. The role of local tranexamic acid in the management of epistaxis has conflicting data results, but its systemic use has clearly benefits [46,47]”.

We added the NOSE trial onto the references:

- Gaillard, S.; Dupuis-Girod, S.; Boutitie, F.; Rivière, S.; Morinière, S.; Hatron, P.-Y.; Manfredi, G.; Kaminsky, P.; Capitaine, A.-L.; Roy, P.; et al. Tranexamic Acid for Epistaxis in Hereditary Hemorrhagic Telangiectasia Patients: A European Cross-over Controlled Trial in a Rare Disease. *J Thromb Haemost* **2014**, *12*, 1494–1502, doi:10.1111/jth.12654.

We also deleted the statement of the oral beta blockers, tacrolimus, and octreotide on the table.

We edit the table taking into account the Second International Guidelines for the Diagnosis and Management of Hereditary Hemorrhagic Telangiectasia.

Finally, we corrected the typo mistakes.

17)Line 290: would mention that sclerotherapy requires expertise and is not a procedure that is readily available in most areas. Only done in a few centers around the US.

Yes, indeed there are few centers that appley this technique. In our case we know of some centers also in Europe. We add a reference to justify this sentence.

We added a new sentence on the paragraph: "Sclerotherapy requires expertise and is not a procedure that is readily available in most areas. Only done in a few centers around the US and Europe [55]."

- Marcos, S.; Botella, L.M.; Albiñana, V.; Arbia, A.; de Rosales, A.M. Sclerotherapy on Demand with Polidocanol to Treat HHT Nosebleeds. *JCM* **2021**, 10, 3845, doi:10.3390/jcm10173845.

18)Line 331: would talk about the complications/morbidity of septal dermoplasty and Youngs procedure, and how with the advent of medical therapies, these procedures have mostly fallen out of favor.

Thank you, we added some new info to update it:

"Complications of these techniques include partial reopening or dehiscence and worsening sinus infections. In addition, about 25% of patients will need to continue with other small procedures despite the initial surgery. It is noted that the frequency of these techniques has decreased with the advent of medical therapies [30,57]".

19)Line 315-318: Mention that embolization is done primarily to prevent strokes and cerebral abscess. Also would mention that patients with pulmonary avms are recommended to use filtered IVs and to avoid scuba diving.

Thank you, we added these two points onto the paragraph:

"Embolization is done primarily to prevent strokes and cerebral abscess. Treatment of pulmonary AVMs by embolization is recommended when the afferent artery of the malformation is at least 1 to 3 mm depending the expertise of the radiologist [25,28]. A high percentage of these malformations can be recanalized (18%), so it is recommended that these lesions be followed up and embolized if necessary. On the other hand, it is important that patients with diagnosed pulmonary AVMs use antibiotic prophylaxis prior to any dental

intervention. In refractory cases or diffuse pulmonary AVMs, lung transplantation can be considered as a therapeutic option [37,48,49]. Patients with pulmonary AVMs are recommended to use filtered IVs and to avoid scuba diving”.

20) Lines 328-330: Would emphasize that the data for most of these drugs is scant and that larger trials are currently ongoing (pomalidomide, pazopanib, and doxy) that may help elucidate the safety and efficacy of these drugs in HHT related bleeding. Plasma coagulation is only recommended as a temporizing measure, until a patient can get better control with medical therapy. Repeated argon plasma coagulation for recurrent avms in the GI tract, as a main treatment strategy, is not recommended.

We updated the paragraph on the approach to gastrointestinal AVMs to add your relevant suggestions:

“The technique of choice is endoscopic argon plasma coagulation of digestive AVMs. However, repeated argon plasma coagulation for recurrent AVMs in the GI tract, as a main treatment strategy, is not recommended [30]. Therefore, Oral antifibrinolytics are also recommended, and in particular tranexamic acid, which have been shown to reduce the number of endoscopic studies in these patients due to suspected gastrointestinal bleeding [60]. As in the treatment of epistaxis, if these patients persist with anemia despite previous treatment, initiation of systemic bevacizumab is recommended [30,31]. As in vascular ectasia on the intestinal wall bleeding, octreotide has been applied to HHT with some efficacy proven [61]. Other pharmacological treatments have also been used in these severe cases with less evidence. There is evidence on the use of other anti-angiogenic drugs such as estrogen receptor modulators such as tamoxifen, raloxifene or bazedoxifene is described in some cases. as pazopanib, pomalidomide or doxycycline and estrogen receptor modulators such as tamoxifen, raloxifene or bazedoxifene. Other therapies are under investigation and include pazopanib, pomalidomide and doxycycline. Data for most of these drugs is scant and that larger trials are currently ongoing that may help elucidate the safety and efficacy of these drugs in HHT related bleeding [63-67]”.

21) Line 332: not sure surgical resection is recommended over embolization or ablation. There is clinical equipoise here and lack of data comparing these different strategies. One should rely on the expertise and recommendation of their local HHT center.

According to the little evidence, we updated the paragraph to emphasize it:

“There is clinical uncertainty about how to manage these complications. Most decisions require multidisciplinary consent and will depend on the experience and recommendations of the local HHT center. Different techniques (surgical resection, interventional embolization and radiosurgical ablation) can be performed depending on the size and location of the lesions. If the lesions are approachable and of adequate size, surgical resection is the preferred treatment option. Interventional embolization and radiosurgical ablations are also considered. Other studies suggest following up-up with imaging tests in asymptomatic patients as long as they do not present changes in size or a high

risk of rupture [68]. There is little evidence on which is the best option, so the decision should often be made by a multidisciplinary committee and the patient should be involved in the decision-making process [30,37]"