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A literature review of microvascular proliferation in arteriovenous malformations of skin and soft tissue

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# Contributed equally

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A SYSTEMATIC REVIEW OF ANGIOGENESIS IN VASCULAR MALFORMATIONS OF SOFT-TISSUE AND SKIN
Journal of Clinical and Translational Research

Dear Mrs Utami,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below. Please ensure that the review is better organized and structured for optimal legibility - a point that several reviewers noted.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that

the track changes function is switched on when implementing the revisions.
This enables the reviewers to rapidly verify all changes made.

Your revision is due by Jan 09, 2021.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: This was a study of the literature in regards to angiogenesis in AVM. The methodology seems adequate. It is novel and fairly well written. Overall it could definitely add value to the existing literature. However, I have some major comments that require addressing before being able to recommend publication.

Major comments:
1. The title ('vascular malformations'), introduction, abstract, and search description in the Methods suggest a much broader scope than what is represented in the methods, search, inclusion criteria and results, which is limited to AVM.
If it was in fact your goal to review the role of angiogenesis in the wider field of 'vascular malformations' there is important missing data: there is, for example, plenty of evidence implicating a role for angiogenesis in capillary malformations as well.

2. A hermeneutic methodology seems to be an appropriate approach for the study's goals. However, I belief the authors should re-consider calling this a 'systematic review' and use the term 'literature review' instead.
The use of the word 'systematic' suggests adherence to a strict set of standards. It is currently not completely clear what exact hermeneutic methodology was used. More importantly, there are (as far as I know) no clear standards for a hermeneutic systematic review.

Either way, any review but a systematic review in particular requires extensive reporting so that the results can be reproduced and I find the Methods section a bit too limited in that regard and recommend expansion. E.g., was quality assessment performed by only one author, date of last searches, etc.
I am also missing a Table with the included studies (sample) characteristics (e.g., lesion location, (previous) treatment, age (mean and SD/IQR), Schöbinger stage, etc.). I'd be good to add another Table with this information (Table 3 is too limited).
Also, I would add the Supplemental flow chart to the main text.

3. Why did the authors chose not to include clinical trials that employed MMP/angiogenesis/inflammation-modifying drugs? These could prove supporting evidence for the involvement of specific pathways in AVM progression. E.g. https://pubmed.ncbi.nlm.nih.gov/30326494/ and https://pubmed.ncbi.nlm.nih.gov/19293678/ .

4. Why did the authors chose to exclude animal studies? Animal models could provide valuable information. For example: https://pubmed.ncbi.nlm.nih.gov/24957885/ .

5. Why did the authors not include AVM angiogenetic signaling pathway aberrations? E.g., https://pubmed.ncbi.nlm.nih.gov/32703450/ .

Minor comments:

I highly suggest extensive textual editing. A few examples, and other points, are listed below:
The abbreviations require attention (e.g., the abbreviation "AVM" is inconsistently used throughout the manuscript, AGGF1 is not defined, VEGF is defined multiple times, high molecular weight is abbreviated but only used once, undefined abbreviations in the Tables, etc.)
The graphical abstract lacks information/is unclear. How does it explain the content of the study? What is MVP?
p.4, line 23. "over" is redundant.
p. 6, lines 48. The [Methyl-3H] thymidine incorporation test needs some explaining: what does this purport to measure?
p. 7, lines 50-52: what 'systematic angiogenic factor' was tested?
p. 8, lines 6-10: How can GH receptor be upregulated and simultaneously have similar density?
p. 8, lines 11-16. Can you confirm that this is the expression of the hormones, not the corresponding receptors?
p. 8-9, Table 3. Can you confirm that all studies coincidentally received the same one-star rating for all three factors? This seems highly unlikely. Can you share your Newcastle-Ottawa quality scale scores as supplemental data to make it publicly available (Open data initiative)?
p. 10, line 24. "Tie-2 was also increased threefold." What is meant by this? (In the previous sentence it was increased tenfold…)
p. 10, line 47. Which defense mechanisms?
p.11, line 25. Do you mean expression of the corresponding genes?
p. 12, line 18. "pro-inflammatory have been proposed […].". What is meant by this?
p. 12, line 29. "125-kd is a complex of […]." What is meant by this? 125 kd merely refers to the molecular weight.
p.12, line 36-37. "Since there was no cure yet and recurrence rate after treatment was high for AVM, […]". Why is this (partially) written in past time?
Table 3. What does the asterisk (*) after NOS (header) refer to?

The discussion is very thorough but also contains a lot of repetitions from the results.

I would also recommend to draw some more in-depth parallels (not only VEGF) in the discussions between your results and what we already know about angiogenesis in cerebral AVM, considering the fact that these lesions are likely to be biologically identical.

Finally, I would recommend adding a deeper (translational) outlook to the discussion on what the results imply for future treatment.

Reviewer #2: This review is well intended, trying to capture a conundrum in the field of vascular malformations as these lesions are classified as non-proliferative lesions but in particular the AVM types show an important vascular hyperproliferative component. Hence, the review is timely and needed. However, there are many aspects which need a careful revision. Overall, it is current form is very immature.

1-the graphical abstract is not a graphical abstract.

2-The review is not well organized, there are many aspects which are discussed in one section and introduced several sections after. Also, there are some sections which are difficult to follow, as there is not clear rational. It often seems a bunch of interesting observations with no clear link/rational.

3-A better description of the subtypes of vascular malformations should be included in the introduction.

4-A rational of focus on AVM should be provided

5-What is a hermeneutic approach? I have never heard of. a better description of what it is, and the intention should be provided.

Reviewer #3: This is a nice and well-written review on angiogenesis and factors involved in AVM expansion. I suggest to include more emphasis on the genetics of AVM and an update on the non-inherited somatic mutations identified in AVM such as KRAS and MAP2K1 which are not mentioned in the text.

Reviewer #4:
This is a review of angiogenesis in vascular malformations.
It is really a review of angiogenesis in arteriovenous malformations and I would make this clear by adjusting the title and abstract and throughout the paper.
Angiogenic mediators have been reported in other malformations that are not discussed in this review.
I do think the review is of interest.
Additional comments:
Introduction:
ISSVA has had several updated classifications. You should provide information on the most recent.
Results 3.1
The following sentence should be capitalized: Studies reported presence of vasoproliferative...
Discussion:
4.4
Words seems to be missing from the following sentence: However, pro inflammatory have been proposed to play a role...

Reviewer #5: The paper is a review about the role of angiogenesis and its factors in arteriovenous malformations (AVM). The authors conclude that the reviewed data support occurrence of angiogenesis in AVM. A causal relationship between proliferation and clinical signs of expansion cannot be drawn. Further studies are required to understand proliferation in AVM.

Major comments:
1. Inclusion criteria of the reviewed articles are "patients with congenital arteriovenous malformations" although the authors state that "the primary objective is to evaluate whether angiogenesis occurs in (all) congenital vascular malformations" . Due to the inclusion criteria the article should be clarified/modified throughout and focus in AVMs only.
2. Angiogenic potential of AVMs is an interesting topic and have been suggested previously. Change accordingly the aims as it now states: "the review aims to determine whether angiogenesis is involved in AVM expansion in skin and soft tissue, and secondly, to identify which factors are involved" (p4). This review collects the data together and is thus of value to draw conclusions.
3. Mention in introduction the two inherited disorders HHT and CM-AVM.
4. Add number of patients to table 3 from all studies, and if possible also to the text. If number of patient is small, state this clearly in text in order to enable the reader to assess the meaning of the finding. State in the text and tables what is the control tissue.
5. Table of the angiogenic factors found in the studies, number of patients and control tissue used would highly improve the review (section 4.1). Discuss also the data in context of the patient number used in the studies e.g. which findings require additional data, which is supported by many studies and have been detected in a high number of patients.
6. TGF-beta signaling is involved in vessel maturation (recruitment of mural cells) and crosstalks with VEGFR2 signaling. VEGF also increases upregulation of multiple other growth factors e.g. BMPs, TGFβs and PDGFs (e.g. PMID: 33021694). Add discussion to section 4.1.
7. Add data of studies of
D. Rothbart, I.A. Awad, J. Lee, J. Kim, R. Harbaugh, G.R. Criscuolo Expression of angiogenic factors and structural proteins in central nervous system vascular malformations Neurosurgery, 38 (1996), pp. 915-924
T. Kiliç, M.N. Pamir, S. Küllü, F. Eren, M.M. Ozek, P.M. Black Expression of structural proteins and angiogenic factors in cerebrovascular anomalies. Neurosurgery, 46 (2000), pp. 1179-1191
8. Add studies about the role of genetic mutations in sporadic AVMs and CM-AVM e.g MAPK21, KRAS, EPHB4 and discuss in context of angiogenesis in AVM (section 4.1) . These all have effects to endothelium function. Add what is known about the role of these mutations in human endothelial cell proliferation/migration. A table of genetic mutations in AVMs can be found e.g. PMID: 30342234. Also Nguyen, Boon Vikkula et al. 2020. The Genetic Basis of Vascular Anomalies.
9. AGGF1 has been shown to be an anti-inflammatory factor and also to regulate PIK3CA function in angiogenesis (e.g. pmid:27522498). Modify the text accordingly.

Minor comments:
-The authors use abbreviation VM to both vascular malformation (e.g. abstract) and venous malformation (Figure 1). Please, modify.
-For clarity, use abbreviations that are used by ISSVA e.g. CM-AVM instead of CAVM.
-Differentiate clearly in text intracranial and extracranial AVMs.

-Current ISSVA classification is from y. 2018, add reference to the website.
-Add figure text to graphical abstract
-Row 38-41 add reference.
-For readability explain e.g. what are p21 and p27 (p7, rows 41 and 42) as these may not be familiar to all the readers of the journal.
-Change The VEGF family is one of the most important factors (p10, row 10)
-Add pg/ml to p8 row 29.
-Change to stage III and and to stage II (p11, rows 29-30)
-"1.8 to 6.4" is this a fold change? (p13 row 4)
-"AVMs occur mostly intracranial", add a reference
-"In contrast, pyogenic granuloma, diffuse dermal angiomatosis and acro-angiodermatitis are all reactive capillary lesions and have similar morphologic and immunophenotypic features than AVM? which could indicate AVM to be a reactive lesion as well[4]". Please clarify the sentence and what is considered to be a reactive lesion. (p13)
-Another study of colon related to intussusception and mucosal prolapse believed that AVM in the intestinal walls were also of a reactive process as a result from the mechanic forces during those conditions trigger the angiogenesis. Modify or omit the sentence. (p13)

Authors' response

REBUTTAL LETTERS:

We thank the reviewers for the positive comments and suggestions for improving our manuscript. All comments are addressed below point by point (*in italic)* for each reviewer. Appropriate extensive changes in the text of the article following reviewers comments are indicated in **bold** and *italic,* and kept with track changes in the manuscript.

PS: extensive language editing was also performed, but which is not kept with the track change module.

Reviewer #1:

The methodology seems adequate. It is novel and fairly well written. Overall it could definitely add value to the existing literature. However, I have some major comments that require addressing before being able to recommend publication.

Major comments:

1. The title ('vascular malformations'), introduction, abstract, and search description in the Methods suggest a much broader scope than what is represented in the methods, search, inclusion criteria and results, which is limited to AVM.
If it was in fact your goal to review the role of angiogenesis in the wider field of 'vascular malformations' there is important missing data: there is, for example, plenty of evidence implicating a role for angiogenesis in capillary malformations as well.

Answer:

At the initial point, we started the literature search for microvascular proliferation (MVP) on one subtype of vascular malformations, which is AVM. Then, we proceeded to search on other subtypes, but it resulted in no further cases of MVP. Therefore this review is focussed on AVM.

We agree with your comment, and changed the title, also based on the comments of other reviewers, as follows:. ***A Literature Review of Microvascular Proliferation in Arteriovenous Malformations of Skin and Soft-Tissue***

Second, following your comment we consulted again the current version of the ISSVA classification of vascular anomalies (2018, in which congenital capillary malformations (CVM), such as port-wine stains, are defined as quiescent lesions composed of dilated mature vessels, with no tendency to regression, conform the standing concept of congenital vascular malformations.

2. A hermeneutic methodology seems to be an appropriate approach for the study's goals. However, I belief the authors should re-consider calling this a 'systematic review' and use the term 'literature review' instead.
The use of the word 'systematic' suggests adherence to a strict set of standards. It is currently not completely clear what exact hermeneutic methodology was used. More importantly, there are (as far as I know) no clear standards for a hermeneutic systematic review.
Either way, any review but a systematic review in particular requires extensive reporting so that the results can be reproduced and I find the Methods section a bit too limited in that regard and recommend expansion. E.g., was quality assessment performed by only one author, date of last searches, etc. I am also missing a Table with the included studies (sample) characteristics (e.g., lesion location, (previous) treatment, age (mean and SD/IQR), Schöbinger stage, etc.). I'd be good to add another Table with this information (Table 3 is too limited).
Also, I would add the Supplemental flow chart to the main text.
Answer:
At first we tried the original systematic review methodology and it resulted in very few suitable articles which could be included. Then we explained and discussed the topic with a librarian on how we could set up this study with systematic review approach, but finally the hermeneutic methodology was suggested as the best method for this review due to multifaceted nature of this subject.
Following your comment we expanded in more detail on the methodology of the hermeneutic approach:

This iterative process aims to deepen understanding of the subject. Searching is systematic but versatile, allowing relevant articles to be critically interpreted and ideas to be understood in context of the subject. This process of understanding should be seen as open ended and circular in nature. A conventional systematic review has a highly structured search strategy and consequently downplays the importance of interaction between the literature and reader. This interaction is of high value as it leads to creative ideas, seeking originality rather than reproducibility. Searching together with reading interchangeably encircled relevant articles which provided valuable information. Retrieving small sets of highly relevant publications is therefore preferred over a single extensive search to discover relevant factors involved in AVM expansion. Database searching, citation tracking and snowballing have been used to gather high value articles to answer the research questions.*(Boell & Cecez-Kecmanovic, 2010)* ***Inclusion and exclusion criteria for the study were mentioned in Table 2.***

The quality assessment for all the included articles was performed by 2 investigators (AMU and SA) together using Newcastle-Ottawa Scale (NOS).

Missing data as far as available from the literature are now included in the table in ***Supplementary Data****.*

3.Why did the authors chose not to include clinical trials that employed MMP/angiogenesis/inflammation-modifying drugs? These could prove supporting evidence for the involvement of specific pathways in AVM progression. E.g. https://pubmed.ncbi.nlm.nih.gov/30326494/ and https://pubmed.ncbi.nlm.nih.gov/19293678/

Answer:

We do agree on this comment, however our research question and literature searches were focused on occurrence of microvascular proliferation (MVP) in arteriovenous malformation (AVM) lesions in humans. Articles mentioned above could provide circumstantial support, but as reported, no evidence for actual presence of angiogenesis / histopathological proven vasoproliferative processes. Therefore, they were not included in the search

4. Why did the authors chose to exclude animal studies? Animal models could provide valuable

information. For example: https://pubmed.ncbi.nlm.nih.gov/24957885/

Answer:

*Our research question focused on angiogenesis in AVM of skin and soft tissue in humans. We must agree that these findings are interesting however do not fit the scope of our research. The article refers to AVM in hereditary hemorrhagic telangiectasia (HHT) which are mostly located in the organs rather than skin and soft-tissue. To our knowledge there are no proven experimental animal models available resembling the situation of congenital vascular malformations in the skin or soft tissues of humans. The role of ALK-1 mutations in the development of AVM is addressed in manuscript under the headline '****Genetic involvement'****.*

5. Why did the authors not include AVM angiogenetic signaling pathway aberrations? E.g., https://pubmed.ncbi.nlm.nih.gov/32703450/ ***Answer:***

*We expanded the text on this information under the headline '****Genetic involvement'*** *as follow:*

Mutation of the MAP2K1 gene on endothelial cells (EC) was assumed to affect EC function and initiation pathological arteriovenous shunting through signalling activation of RAS/MAPK. This aberration was also presumed to promote angiogenesis.[66]

Minor comments:

1. I highly suggest extensive textual editing. A few examples, and other points, are listed below: The abbreviations require attention (e.g., the abbreviation "AVM" is inconsistently used throughout the manuscript, AGGF1 is not defined, VEGF is defined multiple times, high molecular weight is abbreviated but only used once, undefined abbreviations in the Tables, etc.)

Answer:

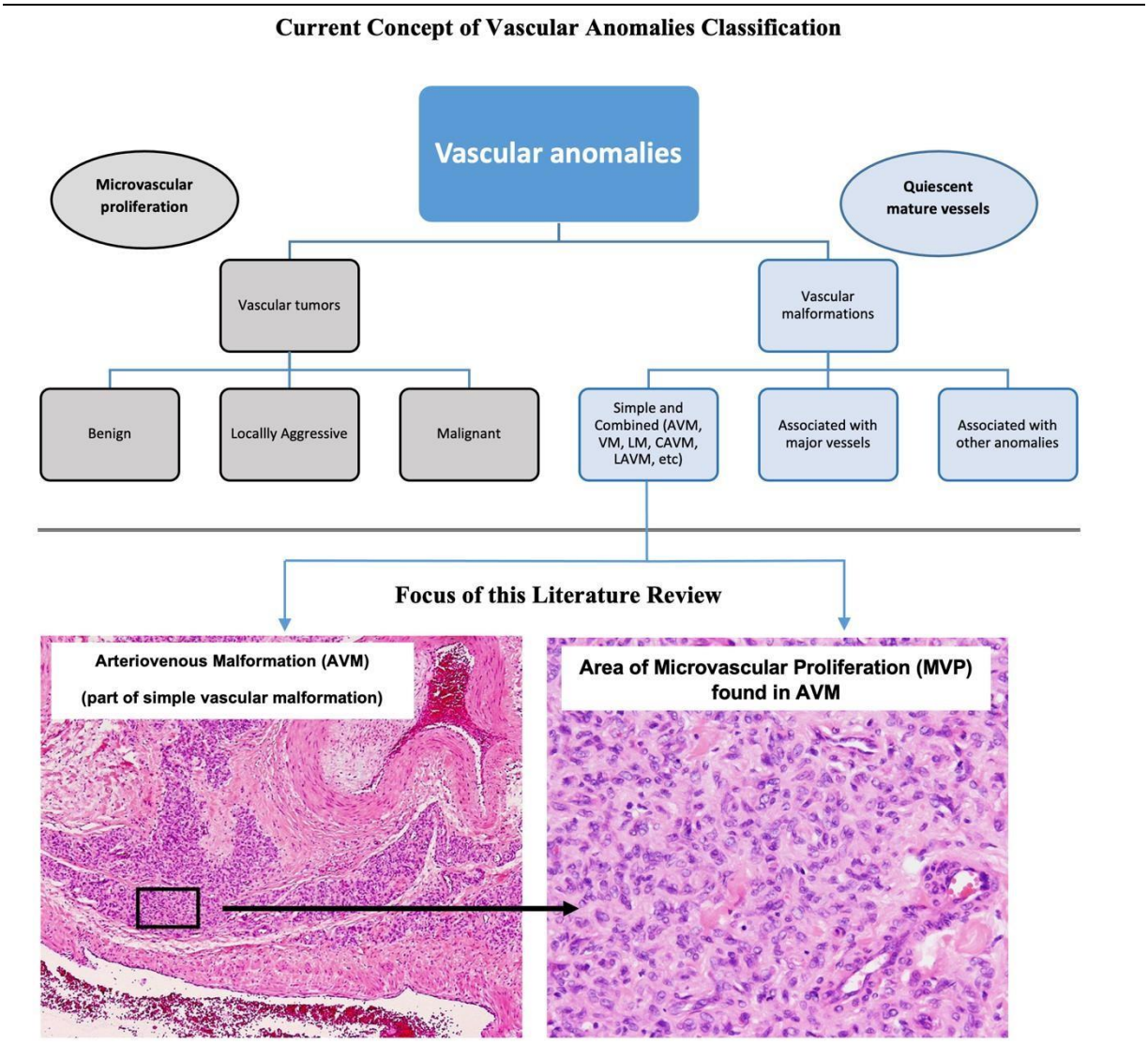
We have revised all the mixed and undefined abbreviations throughout the paper, and additional language editing took place.

2. The graphical abstract lacks information/is unclear. How does it explain the content of the study?

What is MVP?

Answer:

We agree with the reviewer that the graphical abstract indeed needs more explanation, in order to clarify the histological images of the abstract. The images represent the central illustration for the scope of this study, which is the presence of immature microvascular proliferations, to be interpreted as angiogenesis, amidst the large mature vessels of a congenital AVM. We added a scheme adapted from ISSVA 2018 classification to present the dichotomous classification, and provided more textual information, which resulted in a new graphical abstract as follows:



3. p.4, line 23. "over" is redundant.

Answer: *The word 'over' was deleted.*

4. p. 6, lines 48. The [Methyl-3H] thymidine incorporation test needs some explaining: what does this purport to measure?

Answer:

We added a sentence to explain the use of [Methyl-3H] thymidine incorporation test:

Quantitative measurement of [Methyl-^{3}H] thymidine incorporation in cultured cells is widely used as an indicator of cell proliferation.

5. p. 7, lines 50-52: what 'systematic angiogenic factor' was tested?

Answer:

An assay was used to search for a circulating angiogenic factor. This same assay successfully found a circulating angiogenic factor in diabetics with proliferative retinopathy, however failed to find a circulating angiogenic factor in 14 patients with large AVM.

We revised the sentences as follow:

The presence of a circulating systemic angiogenic factor in the sera of 14 patients was tested using an assay which successfully demonstrated such activity in diabetics with proliferative retinopathy. However, the assay failed to detect a circulating systemic factor in the AVM group.[23].

6. p. 8, lines 6-10: How can GH receptor be upregulated and simultaneously have similar density?

Answer:

In the reference article:

Growth hormone receptor was more commonly found in AVM specimens (72.7 %) as compared with controls (25.8%) (p = 0.01) (Tables 1 and 2 and Fig. 1); however, when present, receptor density was not different between arteriovenous malformation (2+ staining, 100 %) and controls (2+ staining, 63%) (p = 0.2). (Kulungowski et al., 2012) We added a sentence as follow:

When present, receptor density was similar between AVM and control. Patients with a clincal Schobinger stage III AVM had increased GHR compared to stage II lesions (p=0.05). Age, sex and location had no effect on GHR expression (p=0.8).

7. p. 8, lines 11-16. Can you confirm that this is the expression of the hormones, not the corresponding receptors?

Answer:

We thank the reviewer for this correction: the study was referring to the corresponding receptor, not the hormones.

We revised the sentence as follow:

Expression of ER, AR, and PGR, which assumed to be responsible in AVM expansion, did not differ compared to control (p=0.2).

8. p. 8-9, Table 3. Can you confirm that all studies coincidentally received the same one-star rating for all three factors? This seems highly unlikely. Can you share your Newcastle-Ottawa quality scale scores as supplemental data to make it publicly available (Open data initiative)?

Answer:

It received the same one-star rating for all three factors. This was a qualitative rating scored by 2 authors independently (see above). The table of included articles followed by the scoring of NewcastleOttawa quality scale is now presented in the supplementary data.

9. *p. 10, line 24. "Tie-2 was also increased threefold." What is meant by this? (In the previous sentence it was increased tenfold…)* ***Answer:***

We removed the word "also".
The previous sentence was referred to Ang-2, not the Tie-2.

10. p. 10, line 47. Which defence mechanisms? ***Answer:***

In cultured AVM endothelial cells exposed to IL-1β, IFN-γ,TGF-β and TNF-α, growth was not inhibited. ICAM expression was highly increased. However, E-selectin and VCAM were not. The lack of leukocyte adhesion molecules expression may pose a barrier to leukocyte infiltration and constitute a resistance to the defense mechanisms. We revised sentences as follow:

However, E-selectin and vascular cell adhesion molecule (VCAM) expression was not increased. The lack of leukocyte adhesion molecules expression may pose a barrier to leukocyte infiltration and constitute a resistance to the defense mechanisms mediated by leukocytes[22]

11. p.11, line 25. Do you mean expression of the corresponding genes?

Answer:

The sentence was revised to:

The expression of vasculogenic factors stromal derived factor (SDF-1α) and hypoxia inducible factor
(HIF-α) were determined by quantitative real-time reverse-transcriptase PCR (qRT-PCR).

12. p. 12, line 18. "pro-inflammatory have been proposed […].". What is meant by this? **Answer:**

We thank you for the correction: unfortunately, there was a missing part in this chapter. We have revised the text as follows:

However, this could also imply that inflammation is not a fundamental factor in the onset of expansion in AVM. Embolization treatment creates a hypoxic, pro-inflammatory environment with production of pro-angiogenic features. This could at least partially explain why embolization results in increased re-expansion rates compared to excision (with or without embolization).

13. p. 12, line 29. "125-kd is a complex of […]." What is meant by this? 125 kd merely refers to the molecular weight.

Answer:

High molecular weight (hMW MMP) represents MMP with125-kDa (or kD) molecular weight. The hMW form of MMP is an MMP-9 complex together with neutrophil gelatinase- associated lipocalin (NGAL) or the MMP-9-NGAL complex.

The sentence revised as follow:

MMP levels were found to be elevated in patients with AVM, in the form of hMW MMP (125 kDa). This MMP form is a complex of MMP-9 and NGAL.

14. p.12, line 36-37. "Since there was no cure yet and recurrence rate after treatment was high for AVM, […]". Why is this (partially) written in past time? **Answer:**

In page 13, line 36-37.

Thank you for the correction: we deleted the "past time" in the text, and added some more information in the conclusion as follow:

Since there is no cure yet and recurrence rate after treatment is high for AVM, further research is therefore required to better understand the angiogenic microproliferative processes in AVM. This could potentially serve to develop novel anti-angiogenic pharmacotherapy. Targeted pharmacotherapy directed to any of the pathways outlined in this review, may theoretically have therapeutic potential. This review is limited by the scarce amount of existing literature regarding angiogenesis in AVM of skin and soft tissue. In vitro and in vivo studies are necessary to further unravel which angiogenic mechanisms are involved and whether anti-angiogenic agents could have a significant role to inhibit vasoproliferation in AVM of skin and soft tissue. A large prospective cohort study is proposed to provide more insights in the relationship between vasoproliferation and clinical symptoms.

15. Table 3. What does the asterisk (*) after NOS (header) refer to?

Answer:

The asterisk (*) after NOS (header) was initially referred to the NOS scoring as it was reported in the old version of the article. We now deleted it, because we have placed the NOS scoring with details of each article included in the supplementary data.

16. The discussion is very thorough but also contains a lot of repetitions from the results.

Answer:

*We have **r**evised the results and the discussion for its structure and contents, and removed repetitions as much as possible. However some overlap appeared inevitable to our opinion for a proper dicussion of the outcome of the literature review, so we hope this is acceptable.*

17. I would also recommend to draw some more in-depth parallels (not only VEGF) in the discussions between your results and what we already know about angiogenesis in cerebral AVM, considering the fact that these lesions are likely to be biologically identical.

Answer:

We added some explanation in discussion regarding the pathomehcanism of angiogenesis of AVM skin and soft-tissue. One of the main patomechanism explained is Ang/Tie-2 and MMP-9 pathway. We also mentioned cerebral AVM, although in the literature and in ISSVA classification they are considered as distinct diseases and aeras of research; Cerebral AVM were not incorporated in our literature searches.

18. Finally, I would recommend adding a deeper (translational) outlook to the discussion on what the results imply for future treatment.

Answer:

We expandend the discussion with the following paragraph:

Since VEGF is recognized for its important role in the pathology of skin and soft tissue AVMs, inhibition of VEGF could be used as an adjuvant therapy after primary therapy (surgery or radiosurgery). This approach might be able to suppress the proliferation process and accelerate the process of decreasing microvessels density. In addition, several other anti-angiogenesis-based may be useful in the treatment of AVM. A currently common anti-angiogenic therapy in oncology is the application of bevacizumab, a monoclonal antibody that binds to VEGF-A.[75] Mutations in the KRAS and MAPK-ERK pathways which can occur in AVM of skin and soft-tissue as stated earlier, could serve as a target for treatment

using the currently available MEK inhibitors, Trametinib or Conimetinib. A successful study reported that Trametinib therapy was able to reduce the volume of AVM after 6 months.[76]

Reviewer #2:

This review is well intended, trying to capture a conundrum in the field of vascular malformations as these lesions are classified as non-proliferative lesions but in particular the AVM types show an important vascular hyperproliferative component. Hence, the review is timely and needed. However, there are many aspects which need a careful revision. Overall, it is current form is very immature.

Comments: **:**

1. the graphical abstract is not a graphical abstract.

Answer:

We agree with the reviewer that the abstract needed a further workout and better explanation of the central figure showing microvascular proliferation AVM. We revised the graphical abstract also following the comment of reviewer1 (see the answer reviewer #1, minor comment no.2)

2.The review is not well organized, there are many aspects which are discussed in one section and introduced several sections after. Also, there are some sections which are difficult to follow, as there is not clear rational. It often seems a bunch of interesting observations with no clear link/rational.

Answer:

We profoundly revised the text of the article, particularly the results and discussion section, for a better structure and organization of data

3. A better description of the subtypes of vascular malformations should be included in the introduction. ***Answer:***

Our description of the pathology was mainly focused on the subtype AVM, since the literature search revealed occurrence of angiogenesis / microvascular proliferation to occur exclusively in AVM. In addition to the simplified ISSVA classification of vascular malformations shown in the graphical abstract and figure, we have now added the following text to the introduction:

AVM have an absent capillary bed between the arterial and venous component of the lesion, which results in so called 'high-flow lesions' that may affect skin, soft tissue and viscera. Arteriovenous (and other types of malformations) occur also in the brains, but these are not included in the ISSVA classification. Compared with purely venous, lymphatic or capillary malformations, AVM are potentially the most dangerous type of vascular malformations clinically, and are most difficult to treat.[5] Collateralization, thickening of adjacent vessels and dilatation of the vessels are mechanisms considered to explain the enlargement of AVM.[6] Progression of clinical symptoms of AVM can be evaluated in the Schobinger's clinical classification of AVM symptomatology (Table 1).[7]

4. A rational of focus on AVM should be provided **Answer:**

We agree with the reviewer that this aspect was outlined insufficiently in our initial text. We explained this also in our answer to the comment 1 of reviewer#1, and revised the article accordingly.

5. What is a hermeneutic approach? I have never heard of. a better description of what it is, and the intention should be provided.

Answer:

See also our reply to comment 2 by reviewer #1. The hermeneutic approach is now also explained and outlined in more detail in the text of the article, and is illustrated in a figure.

Reviewer #3:

This is a nice and well-written review on angiogenesis and factors involved in AVM expansion. I suggest to include more emphasis on the genetics of AVM and an update on the non-inherited somatic mutations identified in AVM such as KRAS and MAP2K1 which are not mentioned in the text.

Answer:

Thank you for your feedback on our manuscript.

We agree that currently the genetics of vascular malformations, including AVM, are under intense investigation.. Unfortunately, there are up yo now no studies reported on genetic mutations in AVM which are characterized by presence of microvascular proliferations. Because of the interest for genetics in current research of vascular malformations, we did make adjustments , including KRAS and MAKP2K1 under the heading "Genetic Involvement". (see also comment 18 by reviewer #1)

Reviewer #4:

1. This is a review of angiogenesis in vascular malformations. It is really a review of angiogenesis in arteriovenous malformations and I would make this clear by adjusting the title and abstract and throughout the paper.

Angiogenic mediators have been reported in other malformations that are not discussed in this review.

I do think the review is of interest.

Answer:

Thank you for your feedback on our manuscript. We would like to provide you answers to your questions and some of the changes we made based on your and others reviewers feedback.

We revised the title, abstract an throughout the paper accordingly specific on MVP in AVM.

2. Additional comments:

Introduction:

ISSVA has had several updated classifications. You should provide information on the most recent.

Answer:

We agree with the reviewer, this was our mistake. We mixed up and put 2016 ISSVA classification instead of the latest on 2018.. The paragraph is revised as follows:

In 2014, a revised ISSVA classification was established, and more recently updated in 2016 and 2018 [8,9] due to ongoing advances in knowledge on the biological behavior, histopathology and underlying genetics of vascular anomalies. Although this classification still uses the dichotomous discrimination between vascular tumors and vascular malformations, subcategories are added to the group of vascular malformations: (1) simple, (2) combined, (3) associated with major vessels and (4) associated with other anomalies (syndromal lesions). According to the ISSVA classification AVM can manifest as sporadic lesions, or occur in patients with hereditary hemorrhagic teleangiectasia (HHT) or with capillary malformation-arteriovenous malformations (CM-AVM) associated with RASA-1 mutation. Moreover, AVM can occur also in combination with other types of vascular malformations [10]. Lesions in which a clear diagnosis cannot (yet) be made are categorized as 'provisionally unclassified vascular anomalies'.[8]. However, the involvement of mass forming microvascular proliferation is not considered in the expansion of AVM, and vascular malformations are still described by definition as: 'A heterogeneous group of lesions that demonstrate cellular turnover without true proliferation, generally growing commensurate with the patient'.[8] A simplified version, adapted from ISSVA 2018 classification is shown in Figure 1.

3. Results 3.1
The following sentence should be capitalized: Studies reported presence of vasoproliferative...
Answer:
We thank you for the correction. In fact, we deleted this sentence completely, since its a repeat of the first sentence of this chapter (3.1), and was erroneously not deleted earlier.

4. Discussion:
4.4
Words seems to be missing from the following sentence: However, pro inflammatory have been proposed to play a role...
Answer:
We thank you for the correction: we have revised the text (see also comment 12 of reviewer#1)

Reviewer #5

The paper is a review about the role of angiogenesis and its factors in arteriovenous malformations (AVM). The authors conclude that the reviewed data support occurrence of angiogenesis in AVM. A causal relationship between proliferation and clinical signs of expansion cannot be drawn. Further studies are required to understand proliferation in AVM.

Major comments:

1. Inclusion criteria of the reviewed articles are "patients with congenital arteriovenous malformations" although the authors state that "the primary objective is to evaluate whether angiogenesis occurs in (all) congenital vascular malformations" . Due to the inclusion criteria the article should be clarified/modified throughout and focus in AVMs only.

Answer:

We agree with the reviewer, your comment is the same as comment 1 by reviewer 1#, so we refer to the answer to this reviewer, and the changes that we have applied accordingly throughout the text, and in the title of the new manuscript.

2. Angiogenic potential of AVMs is an interesting topic and have been suggested previously. Change accordingly the aims as it now states: "the review aims to determine whether angiogenesis is involved in AVM expansion in skin and soft tissue, and secondly, to identify which factors are involved" (p4).

This review collects the data together and is thus of value to draw conclusions.

Answer:

We revised the sentence as follow:

Therefore, this review aims to evaluate whether angiogenesis, resulting in MVP, is involved in growth of congenital vascular malformations, and specifically which histological types of lesions involved, for which purpose we focused on skin and soft tissue lesions. Secondly, we tried to identify which factors could be involved in the process of angiogenesis.

3. Mention in introduction the two inherited disorders HHT and CM-AVM.

Answer:

We added the following lines to the introduction: ***According to the ISSVA classification AVM can manifest as sporadic lesions, or occur in patients with hereditary hemorrhagic teleangiectasia (HHT) or with capillary malformation-arteriovenous malformations (CM-AVM) associated with RASA-1 mutation. Moreover, AVM can occur also in combination with other types of vascular malformations.***

Here we also added a new reference to the list: Wassef M et al.Vascular Anomalies classification: recommendations from the International Society for the Study of Vascular Anomalies. Paediatrics2015;136:e203-214

Moreover, we provided a link to the classification of ISSVA https://www.issva.org/classification

4. Add number of patients to table 3 from all studies, and if possible also to the text. If number of patient is small, state this clearly in text in order to enable the reader to assess the meaning of the finding. State in the text and tables what is the control tissue.

Answer:

The number of patients for all studies and explanation for table 3 are now provided in the supplementary data under 'table included articles' that has been used for NOS scoring.

5. Table of the angiogenic factors found in the studies, number of patients and control tissue used would highly improve the review (section 4.1). Discuss also the data in context of the patient number used in the studies e.g. which findings require additional data, which is supported by many studies and have been detected in a high number of patients.

Answer:

The table for all the included articles with explanation and patient number are now presented in supplementary data.

Table of all angiogenic factors mentioned in 4.1 is now also included

Table 5. Angiogenic Factors reported in AVM skin and soft-tissue

Angiogenic Factors	References
AGGF-1	Zhan M *et al* [28]
Ang-1	Meijer-Jorna LB *et al* [16]
Ang-2	Meijer-Jorna LB *et al* [16] Redondo P *et al* [11]
HIF-1□	Lu L *et al* [17]
Neuropilin	Lu L *et al* [17]
MMP-9	Wei T *et al* [45] Redondo P [11]
Tie-2	Redondo P *et al* [11]
TNF-□	Sainson RCA et al [40]
VEGF	Meijer-Jorna LB *et al* [16] Lu L *et al* [17]

6. TGF-beta signaling is involved in vessel maturation (recruitment of mural cells) and crosstalks with VEGFR2 signaling. VEGF also increases upregulation of multiple other growth factors e.g. BMPs,

TGFβs and PDGFs (e.g. PMID: 33021694). Add discussion to section 4.1.

Answer:

Sentence added to Discussion 4.1 as follow:

TGF- β signalling is involved in vessel maturation (recruitment of mural cells) and crosstalks with VEGFR2 signalling. VEGF also increases upregulation of multiple other growth factors e.g. BMPs, TGFβs and PDGFs.*(Pulkkinen et al., 2020)*

7. Add data of studies of D. Rothbart, I.A. Awad, J. Lee, J. Kim, R. Harbaugh, G.R. Criscuolo Expression of angiogenic factors and structural proteins in central nervous system vascular malformations. Neurosurgery, 38 (1996), pp. 915-924. T. Kiliç, M.N. Pamir, S. Küllü, F. Eren, M.M. Ozek, P.M. Black Expression of structural proteins and angiogenic factors in cerebrovascular anomalies. Neurosurgery, 46 (2000), pp. 1179-1191 ***Answer:***

We already added data from the articles mentioned into the results and discussion in the manuscript.

8. Add studies about the role of genetic mutations in sporadic AVMs and CM-AVM e.g MAPK21, KRAS, EPHB4 and discuss in context of angiogenesis in AVM (section 4.1) . These all have effects to endothelium function. Add what is known about the role of these mutations in human endothelial cell proliferation/migration. A table of genetic mutations in AVMs can be found e.g. PMID: 30342234. Also Nguyen, Boon Vikkula et al. 2020. The Genetic Basis of Vascular Anomalies.

Answer:

We added data regarding the genetic mutation from the articles mentioned above, under heading: Genetic Involvement.

9. AGGF1 has been shown to be an anti-inflammatory factor and also to regulate PIK3CA function in angiogenesis (e.g. pmid:27522498). Modify the text accordingly. (p.10) **Answer:**

There are not many data regarding the role of AGGF1 in the occurrence of AVM, although we try to show the role of AGGF1 expression in the occurrence of angiogenesis in this text. Sentences was added and revised:

Recently, a new angiogenic and anti-inflammmatory agent, Angiogenic Factor With GPatch and FHA Domains 1 (AGGF1), was described in Klippel-Trenaunay syndrome. This congenital vascular disorder is associated with both VM and CM. AGGF1 is involved in both physiological and pathological angiogenesis. A study found AGGF1 to be highly expressed in activated EC and MC of AVM. The PI3K pathway is a regulator of cell growth and dysregulation of this pathway could support the proliferation of endothelial cells and disruption of vasculogenesis. Mutation of PIK3CA, the gene encoding the subunit of PI3K, is associated with the presence of AGGF1 expression and will result in the dysregulation.[28,43,44] AGGF1 is unfortunately not specific for vascular malformations, since expression was also detected in vascular tumors[28]. The mechanisms of AGGF1 in angiogenesis will have to be further evaluated in order to understand its role in AVM.

Minor comments:

1. The authors use abbreviation VM to both vascular malformation (e.g. abstract) and venous malformation (Figure 1). Please, modify.

Answer:

We revised in the abstract. We use now consistently word 'vascular malformations' only without abbreviation and we used 'VM' for venous malformation.

2.For clarity, use abbreviations that are used by ISSVA e.g. CM-AVM instead of CAVM.

Answer:

We consulted the latest classification of ISSVA 2018 which still uses the abbreviation CAVM; please note the availability of the classification: ISSVA Classification of Vascular Anomalies ©2018
International Society for the Study of Vascular Anomalies
Available at *https://www.issva.org/classification*

We agree that in some cases in the classification CM-AVM is used (see also reply to your comment 3).

3. Differentiate clearly in text intracranial and extracranial AVMs.

Answer:

The text in section 4.4 had been revised in order to clarify intracranial and extracranial AVM.

4. Current ISSVA classification is from y. 2018, add reference to the website.

Answer:

We agree with the reviewer, and have changed the text (see reviewer #4, comment no.2). It was our omission that we mixed up 2016 and 2018. We also provided a link to the website:

https://www.issva.org/UserFiles/file/ISSVA-Classification-2018.pdf

5. Add figure text to graphical abstract **Answer:**
We extensively revised the graphical abstract. (see the answer in reviewer #1, minor comment no.2)

6. Row 38-41 add reference. **Answer:**
We added the reference as follow:
A case study by Redondo et al [10] further questions the current definition of vascular malformations. The authors reported extensive growth of AVM located in the trunk of a 51years old man, showing the destructive consequences of histologically proven vascular proliferation. Moreover, serum levels of angiogenic factors were increased compared to control tissue. Eventually, the patient died due to multi-organ and renal failure.

7. For readability explain e.g. what are p21 and p27 (p7, rows 41 and 42) as these may not be familiar to all the readers of the journal.
Answer:
We adjusted the sentence as follows:
Interestingly, the same study found p21 (a cell cycle dependent protein) to be expressed in immature vessels and p27, a cycle dependent kinase inhibitor which reduces the cell cycle, to be expressed in mature vessels only.

8. Change The VEGF family is one of the most important factors (p10, row 10) **Answer:**
The sentence is changed into:
The VEGF family is one of the most important factors in endothelial cell migration and sprouting.

9. Add pg/ml to p8 row 29.
Answer:
We added the pg/mg, the sentence is as follows:
Average VEGF and VEGFR concentrations in primary AVM were 4.80±1.34 pg/mg protein and 61.80±20.85 pg/mg (p<0.05) compared to recurrent AVM 21.50±0.27 pg/mg and 545±243 pg/mg (p>0.05)(Pavlov et al., 2011)***.***

10. Change to stage III and and to stage II (p11, rows 29-30) **Answer:**
The sentences in the manuscript were changed as follows:
Expression of VEGF in stage II and stage III were similar (p=0.7). However, expression of VEGFR2, Neuropilin 1 and Neuropilin 2 was found increased in stage II compared to stage III (p=0.03)

11. "1.8 to 6.4" is this a fold change? (p13 row 4) **Answer:**
Thank you for the correction: It was a mistake, and it's not supposed to be there. It was removed from the text.

12. "AVMs occur mostly intracranial", add a reference **Answer:**
After we expanded the review search to cerebral , the data on prevalence are unclear. Thus, we deleted this information.

13. "In contrast, pyogenic granuloma, diffuse dermal angiomatosis and acro-angiodermatitis are all reactive capillary lesions and have similar morphologic and immunophenotypic features AVM? which could indicate AVM to be a reactive lesion as well[4]". Please clarify the sentence and what is considered to be a reactive lesion. (p13) **Answer:**

Reactive lesions occur in response to acquired stimuli such as for example inflammation, trauma or hypoxia.
We revised the sentence:
In contrast, pyogenic granuloma, diffuse dermal angiomatosis and acro-angiodermatitis are all reactive capillary lesions, which occur in response to acquired stimuli such as inflammation trauma or hypoxia/ ischemia. Since they also show, at least episodically, the histomorphology and immunophenotypic features of microvascular proliferation, it could well be that microvascular proliferative activity in AVM also represents a reactive process as well.[4].

14. Another study of colon related to intussusception and mucosal prolapse believed that AVM in the intestinal walls were also of a reactive process as a result from the mechanic forces during those conditions trigger the angiogenesis. Modify or omit the sentence. (p13) **Answer:**

The sentenced was omitted.

2nd Editorial decision
12-May-2021

Ref.: Ms. No. JCTRes-D-20-00134R1
A LITERATURE REVIEW OF MICROVASCULAR PROLIFERATION IN ARTERIOVENOUS MALFORMATIONS OF SKIN AND SOFT-TISSUE
Journal of Clinical and Translational Research

Dear Mrs Utami,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Jun 11, 2021.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: Despite a range of clear improvements by the authors, many previous remarks have not yet been sufficiently addressed.

Major comments:
I applaud the authors for focusing the text towards AVM. However, this change has not been consistently applied throughout the text, particularly in the abstract (E.g. "In all studies, angiogenesis was noted only in the arteriovenous types of vascular malformations")

I agree with reviewer #2 that a clear rational is often missing in the paragraphs, especially in the Results and the Discussion sections. These often feel as tedious and lengthy summaries of facts that will scare readers away. Please look into "topic sentences" to solve this.

Minor comments:
- Considerable English editing and spelling checks are still required. (E.g. abstract, line 1) "ArterioveRnous malformations (AVM) are defined as being quiescent vascular masses composed of mature vessels. However, recent studies reported areas of microvascular proliferation (mvO)"
- The use of abbreviations is still deplorable (double definitions, unnecessary abbreviations, misspellings, previously abbreviated words used in full, etc.)
- If the search was also developed in collaboration with a librarian/information specialist please mention this in the text.
- Date of last search is still missing.
- The text states that Newcastle-Ottawa scales were assessed "together", not "independently" (p.6).
- It still don't understand what you, in relation to AVM MVP, mean by leukocyte 'defense mechanisms'(p.11)
- Please be precise: although most people will know what a PCR implies you should specify that it is GENE expression (p.12). Because of this imprecision it is completely unclear when you are referring to protein/factor expression or gene expression throughout the text. Also, no need to abbreviate to qRT-PCR as you only use it once. Or be complete and write "quantitative real-time reverse-transcriptase polymerase chain reaction".
- Theoretically' and 'potential' is redundant (p. 15)
- Flow chart Fig.: The number of excluded studies should ideally also be listed per exclusion reason.

Reviewer #3: Authors responded carefully to the reviewers' comments and submitted an improved version of their review manuscript.

In the abstract please revise microvascular proliferation abbreviation (MVO)- to (MPV).

Reviewer #5: The authors have answered my questions and modified the manuscript. Language editing is required particularly in the genetics section (4.6), which has some repetition and unclear sentences.

As examples:

page 13
"Increased levels of MMP-9 are often found in the structurally unstable vasculature.[35] It is also found in extracranial AVMs
[55]. Extracranial AVM shows an increase in MMP-9."

page 14
"Furthermore genetic studies have unravelled a number of inherited mutations in which AVM play a role."
Authors state previously that most of the mutations are sporadic. Change e.g. to: Furthermore studies have unravelled a number of genetic mutations in AVM.

"Several genetic mutations are associated with malignant neoplasm,but some of these including the KRAS gene mutations relates to angiogenic factors. Notably, activated KRAS gene mutations were found in a proportion of patients with AVM of brains."
First sentence can be omitted. Use brain AVM not AVM of brains.

"Nikolaev et al identified a KRAS mutation in cerebral AVM lesions that coincided with dysregulation of the MAPK-ERK pathway; pathways also associated with the development of a number of types of cancer.[66] "
modify to "pathway, which is also.."

Row 33 p14 change section

p10
"Brain FGF was found"
Omit brain

Authors' response

Rebuttal Letter:

Thank you for all the reviewers' critical comments and feedbacks on our manuscript.
We have taken into account all the feedbacks and improved our manuscript.

Reviewer #1:
Despite a range of clear improvements by the authors, many previous remarks have not yet been sufficiently addressed.

Major comments:

1. I applaud the authors for focusing the text towards AVM. However, this change has not been consistently applied throughout the text, particularly in the abstract (E.g. "In all studies, angiogenesis was noted only in the arteriovenous type of vascular malformations")

Answer:

In the 16 articles that were included in the study based on the search criteria angiogenesis was reported only in the AVM type of malformations. We adapted the text accordingly:

In these studies angiogenesis was reported only in the AVM-type of vascular malformations.

2. I agree with reviewer #2 that a clear rational is often missing in the paragraphs, especially in the Results and the Discussion sections. These often feel as tedious and lengthy summaries of facts that will scare readers away. Please look into "topic sentences" to solve this.

Answer:

Following the comments of reviewer 2 we extensively revised the text of the article, but new data including explanatory text had to be added following the requests of reviewers in the first round of revision. We revised the text again and we feel that now all sentences in each subchapter clearly relate to the heading (topic) of the respective subchapters.

Minor comments:

1. Considerable English editing and spelling checks are still required. (E.g. abstract, line 1) "ArterioveRnous malformations (AVM) are defined as being quiescent vascular masses composed of mature vessels. However, recent studies reported areas of microvascular proliferation (mvO)"

Answer:

We apologize for the misspellings which were introduced during revision of, and not noticed; we checked this throughout the text.

Specifically the line in the abstract reads now as follows: ***Arteriovenous malformations (AVM) are defined as being quiescent vascular masses composed of mature vessels. However, recent studies reported areas of microvascular proliferation (MVP) in AVM, indicating a process of angiogenesis.***

2. The use of abbreviations is still deplorable (double definitions, unnecessary abbreviations, misspellings, previously abbreviated words used in full, etc.)

Answer:

Thank you for this feedback. We carefully corrected all misspelling, unnecessary abbreviations that we could find throughout the manuscript, and additional editing took place to further improve the readability.

3. If the search was also developed in collaboration with a librarian/information specialist please mention this in the text.

Answer:

The hermeneutic approach of reviewing literature was chosen and worked out in collaboration with a librarian. We have added to the method section of the article:

After consulting a librarian, a hermeneutic systematic approach was applied, since this method suits well the multifaceted subject of the study. The process consisted of: 1. searching and citation tracking in PubMed and Web of Science to gather articles on

angiogenesis in vascular malformations, and 2. analysis and interpretation of the articles on potential factors inducing MVP in the lesions (Figure 2).
In addition,we have acknowledged him in the acknowledgement section of the manuscript:
Rene Spijker, MSc. is gratefully acknowledged for advices concerning the hermeneutic approach to review the literature.

3. Date of last search is still missing.
Answer:
The last search was applied on the first manuscript revision process on 28th March 2021.
We added a sentence in the method section:
Latest update of the search took place during the second revision of this manuscript (May 2021).
During the second revision process (this revision), we found a new article that had been published on 31st March 2021: **Ryu JY, Kim YH, Lee JS, *et al.* Oscillatory shear stress promotes angiogenic effects in arteriovenous malformations endothelial cells. *Mol Med* 2021; 27**
We have placed it in the table of included articles (Table 3) and added the findings in the result and discussion section of this manuscript.

4. The text states that Newcastle-Ottawa scales were assessed "together", not "independently" (p.6).
Answer:
We tried to explain that two authors (AMU and SA) screened the articles independently, but at the same time (same period), and consensus was reached since it's a straight forward and objective scoring system. We have changed the word 'together' into 'independently', and added a sentence:
The quality assessment for all the included articles was performed by 2 investigators (AMU and SA) independently using Newcastle-Ottawa Scale (NOS). NOS is a straight forward and objective scoring system, and resulted in consensus between both investigators.

5. It still don't understand what you, in relation to AVM MVP, mean by leukocyte 'defense mechanisms'(p.11)
Answer:
The paragraph was not clear and we have revised it as follow:
TNF-α is a cytokine also thought to be involved in angiogenesis. TNF-α is pro-angiogenic in vivo, but promotes apoptosis in vitro[40]. In cultured AVMs exposed to IL-1β, IFN-γ, TGF-β and TNF-α. Intercellular adhesion molecule (ICAM) expression was highly increased, but E-selectin and vascular cell adhesion molecule (VCAM) were not expressed. The apparent dysregulation of leukocyte adhesion molecules expression may pose a barrier to leukocyte infiltration, thus inhibiting local inflammation (as a potential contributor to angiogenesis).

6. Please be precise: although most people will know what a PCR implies you should specify that it is GENE expression (p.12). Because of this imprecision it is completely unclear when you are referring to protein/factor expression or gene expression throughout the text. Also, no need to abbreviate to qRT-PCR as you only use it once. Or be complete and write "quantitative real-time reverse-transcriptase polymerase chain reaction".

Answer:
Thank you for your comment, we have corrected the sentence:
The expression of the vasculogenic factors stromal derived factor (SDF-1α) and hypoxia inducible factor (HIF-α) gene were determined by quantitative real-time reverse-transcriptase polymerase chain reaction.

a. Theoretically' and 'potential' is redundant (p. 15)
Answer:
We have corrected the sentence.

b. Flow chart Fig.: The number of excluded studies should ideally also be listed per exclusion reason.
Answer:
We have revised the Flow Chart (Figure 2). The number of excluded studies were not presented in the flow chart figure as we are presenting the hermeneutic framework by snowballing method and citation tracking on relevant articles based on exclusion criteria on table 2, thus number of exclusion study were not recorded.

Reviewer #3:
Authors responded carefully to the reviewers comments and submitted an improved version of their review manuscript.
In the abstract please revise microvascular proliferation abbreviation (MVO)- to (MPV).
Answer:
Thank you for your feedback. We have revised this misspelling and also others throughout the manuscript.

Reviewer #5:
The authors have answered my questions and modified the manuscript. Language editing is required particularly in the genetics section (4.6), which has some repetition and unclear sentences.
As examples:
1. page 13
"Increased levels of MMP-9 are often found in the structurally unstable vasculature.[35] It is also found in extracranial AVMs
[55]. Extracranial AVM shows an increase in MMP-9."
Answer:
Thank you for your feedback.
We have deleted the repetitive sentences and corrected as follow:
MMP-9 has the ability to degrade vascular extracellular components including collagen types IV and V, fibronectin, and elastin, and. increased levels of MMP-9 are often found in structurally unstable vessels.[37] MMPs have also been reported in extracranial AVMs [55], which could explain the absence of a number of extracellular components in the AVM lesion[21].

2. page 14
"Furthermore genetic studies have unravelled a number of inherited mutations in which AVM play a role."
Authors state previously that most of the mutations are sporadic. Change e.g. to: Furthermore

studies have unravelled a number of genetic mutations in AVM.
Answer:
Thank you for your suggestion, I have corrected the sentence and put it in the first sentence of the paragraph:
'Recently, several studies have unravelled a number of genetic mutations in AVM.

3. "Several genetic mutations are associated with malignant neoplasm,but some of these including the KRAS gene mutations relates to angiogenic factors. Notably, activated KRAS gene mutations were found in a proportion of patients with AVM of brains."
First sentence can be omitted. Use brain AVM not AVM of brains.
Answer:
We have omitted the first sentence and revised to:
Activated Kirsten-Rat sarcoma 2 viral oncogene homolog (KRAS) gene mutations were found in a proportion of patients with brain AVM.

4. "Nikolaev et al identified a KRAS mutation in cerebral AVM lesions that coincided with dysregulation of the MAPK-ERK pathway; pathways also associated with the development of a number of types of cancer.[66] "
modify to "pathway, which is also.."
Answer:
Thank you for your suggestion, we have revised the sentence:
Nikolaev et al identified a KRAS mutation in cerebral AVM lesions that coincided with dysregulation of the MAPK-extracellular-signal-regulated kinase (MAPK-ERK) pathway, which is also associated with the development of a number of types of cancer.[60]

5. Row 33 p14 change section
Answer:
We have changed the section.

6. p10
"Brain FGF was found"
Omit "brain"
Answer: The word was omitted.
FGF was found to stimulate VEGF expression in vascular smooth muscle cells and has a role in modifying fibroblasts in smooth muscle cells that will form cerebral AVM lesions. In AVM lesions, this FGF is reported to be expressed in perivascular tissue and tunica media.

3rd Editorial decision
16-Jun-2021

Ref.: Ms. No. JCTRes-D-20-00134R2
A LITERATURE REVIEW OF MICROVASCULAR PROLIFERATION IN ARTERIOVENOUS MALFORMATIONS OF SKIN AND SOFT-TISSUE
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers: