PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Electrosclerotherapy for capillary malformations: study protocol for a randomized within-patient controlled pilot trial.
AUTHORS	Horbach, Sophie; Wolkerstorfer, Albert; de Bruin, Daniel; van der Horst, Chantal

VERSION 1 – REVIEW

REVIEWER	Philip S Bekhor Director Laser Unit Department of Dermatology Royal Children's Hospital Melbourne Vic Australia
REVIEW RETURNED	25-Feb-2017

GENERAL COMMENTS	1. Pulsed Dye Laser is NOT the standard of care for Hypertrophic CM. These lesions respond well to Alexandrite Laser, some IPLs e.g Ellipse Flex, and for isolated hypertrophic nodules Long Pulsed Nd:YAG laser. This is published in a number of papers. 2. In Aetiology mention should be made of there recently discovered GNAQ mutation. 3. More explanation should be given regarding the confusion with Bleomycin of IU versus USP versus mg. dosing. 4. The POSAS scale is only validated for scars. I do not see the logic of using it for hypertrophic CM where it is not validated and includes irrelevant symptoms such as itching or pliability.

REVIEWER	prof. Gregor Sersa Institute of Oncology Ljubljana, Slovenia Department of Experimental Oncology
REVIEW RETURNED	27-Feb-2017

GENERAL COMMENTS	The article is interesting proposal for the use of electrochemotherapy in treatment of capillary malformations. The trial is prepared according to all requirements. In order to improve it I have some suggestions/comments that might be taken into account. The general comment is why the authors introduce new term; electrosclerotherapy, while they in general stick to the SOP for the electrochemotherapy. This may be confusing, since this is not a new approach but new application of electrochemotherapy. Furthermore,

	electrochemotherapy is an established key word, which provides bigger visibility, especially in dermatology where electrochemotherapy is already well established. Page 6. Paragraph 2 and 3 need quotation of relevant literature. The vascular disrupting effect is only mentioned, but not explained. An additional sentence would be helpful. In the trial design is explained that 3 ROI will be selected and treated, the question is what will happen with the rest of the region? If it is not going to be treated it has to be explained, what will be the next step. Otherways the study is ethically questionable. Page 10. Last paragraph. Electroporation cannot be simulated by placement of the electrodes, since the patients would feel the application of the pulses. This point needs to be considered and probably also the protocol adequately modified. The authors predicted the use of two types of electrodes, plate and hexagonal. I would suggest the use of only one type. In the literature is ample evidence of the use of specific electrodes for the specific situations. In the case of the superficial lesions, Plate or Needle row electrodes would be more suitable, than the hexagonal, which are aimed for the treatment of deeper lesions, induce more pain and also cover bigger surface of the tumor. The picture of the Cliniporator is probably not necessary, and is also very commercial.

VERSION 1 – AUTHOR RESPONSE

Reviewer: #1
Reviewer Name: Philip S Bekhor
Institution and Country: Director Laser Unit, Department of Dermatology, Royal Children's Hospital, Melbourne Vic, Australia Please state any competing interests: None declared

1. Pulsed Dye Laser is NOT the standard of care for Hypertrophic CM. These lesions respond well to Alexandrite Laser, some IPLs e.g Ellipse Flex, and for isolated hypertrophic nodules Long Pulsed Nd:YAG laser. This is published in a number of papers.

Reply of the author:
We concur that we did not clearly describe this in our introduction section, we focused too much on non-hypertrophic capillary malformations. We have now adjusted the introduction section (highlighted in red) and we have mentioned the lasers that are regularly used in hypertrophic capillary malformations.

2. In Aetiology mention should be made of there recently discovered GNAQ mutation.

Reply of the author:
Thank you for this suggestion. We have now adjusted the introduction section and we added a short paragraph about the RASA1 and GNAQ mutations, highlighted in red.

3. More explanation should be given regarding the confusion with Bleomycin of IU versus USP versus mg. dosing.

Reply of the author:

The correct measure is USP in North-America and IU in the rest of the world(1), however, in the standard operating procedures for electrochemotherapy the dosages are still reported in mg and units. In order to prevent confusion about this topic we have now addressed the difference between these measures in the methods section, under 'interventions'.

4. The POSAS scale is only validated for scars. I do not see the logic of using it for hypertrophic CM where it is not validated and includes irrelevant symptoms such as itching or pliability.

Reply of the author:
For lack of a better outcome measurement instrument, we decided to use the global assessment score (assessed by both patient and physician) which is frequently used in studies on capillary malformations. However, since we also wanted a more detailed assessment of color, vascularity, nodularity, et cetera, we were in need of a more detailed instrument. Since there was no validated questionnaire available for capillary malformations, we had the choice to either use a self-developed questionnaire which was not validated at all, or a modified version of a validated score for scars, which addressed similar constructs. Some items do not seem relevant, like itching, but are relevant since we are inducing a sclerosing effect with the bleomycin treatment which can cause symptoms similar to those in scars. We therefore decided to try using the POSAS score as an outcome measurement instrument in this study, alongside the global assessment score. As this is a pilot study, in which we aim to determine the feasibility of the study protocol, one major focus will be to assess if the POSAS score is suitable as an outcome measurement instrument or not. We will certainly discuss this issue in the final manuscript of this study.

Reviewer: #2
Reviewer Name: Prof. Gregor Sersa
Institution and Country: Institute of Oncology Ljubljana, Slovenia, Department of Experimental Oncology Please state any competing interests: None declared

Please leave your comments for the authors below

1. The article is interesting proposal for the use of electrochemotherapy in treatment of capillary malformations.
The trial is prepared according to all requirements.
In order to improve it I have some suggestions/comments that might be taken into account.

Reply of the author:
We thank the reviewer for his valuable comments regarding our manuscript.

2. The general comment is why the authors introduce new term; electrosclerotherapy, while they in general stick to the SOP for the electrochemotherapy. This may be confusing, since this is not a new approach but new application of electrochemotherapy. Furthermore, electrochemotherapy is an established key word, which provides bigger visibility, especially in dermatology where electrochemotherapy is already well established.

Reply of the author:
We fully understand this remark of the reviewer and we agree that using the term electrochemotherapy would enhance the visibility of our manuscript as it is a well-established definition. The reason that we chose for 'electrosclerotherapy' in this study was that we use bleomycin for its sclerosing mechanism of action and not as a chemotherapeutic agent. Bleomycin sclerotherapy is well-known as the 'state of the art' treatment option for larger vessel malformations (e.g. venous and lymphatic malformations), but is not feasible for capillary malformations due to the small diameter

of capillary vessels in which localized intravascular injections are difficult to perform. In this study, we hypothesized that the combination of bleomycin and electroporation would enhance the local sclerosing effect of bleomycin rather than the cytotoxic effect. However, we admit that we do not yet have any pre-clinical evidence in this patient category to support this hypothesis.
Nevertheless, we think that using the definition 'chemotherapy' may cause unnecessary confusion about this therapy among both physicians and patients, as chemotherapy is not usually applied in patients with these kinds of benign vascular malformations and can therefore be mistakenly viewed as 'too aggressive' or 'high-risk'. We encountered this while we were submitting our protocol to our IRB: when we used the term 'electrochemotherapy' they were hesitant to approve this study, but when we explained that we wanted to use it as a form of sclerotherapy (which is a regular therapy in vascular malformations) they were quickly convinced.
We are therefore inclined to use the definition 'electrosclerotherapy' in this patient group, however, we do agree with your remark. We think that we may be able to solve this issue by a more elaborate explanation in the abstract and the introduction in which we clarify that electrosclerotherapy is identical to electrochemotherapy. We will also add electrochemotherapy as a key word for this manuscript, so that researchers will be able to easily find this article in biomedical search engines. We have made these adjustments to the manuscript, highlighted in red.

3. Page 6. Paragraph 2 and 3 need quotation of relevant literature. The vascular disrupting effect is only mentioned, but not explained. An additional sentence would be helpful.

Reply of the author:
We concur that this topic deserves a more elaborate explanation. We have now added references of relevant literature to the introduction section and we have added an extra paragraph to the discussion section about the immediate and delayed vascular disrupting effect of ECT.

4. In the trial design is explained that 3 ROI will be selected and treated, the question is what will happen with the rest of the region? If it is not going to be treated it has to be explained, what will be the next step. Otherways the study is ethically questionable.

Reply of the author:
We thank the reviewer for bringing this matter to our attention. In this pilot study we follow-up 3 pre-selected regions within the capillary malformation: only 1 region is treated with electrosclerotherapy, 1 region is treated with bleomycin alone and 1 region is not treated at all. These 3 ROIs will be where we perform the baseline and follow-up measurements. The rest of the capillary malformation is not treated, and will also not be subjected to measurements. As the goal of this pilot study was (among others) to investigate the safety of this treatment option, we wanted to start by treating just a small area within the capillary malformation. If the results of our pilot study confirm the safety of this treatment option in capillary malformations (and no severe local skin complications occur, such as skin necrosis), we would propose EST for treating the entire CM (perhaps in more than one treatment session). We have now further clarified our approach in the methods section under 'interventions'.

5. Page 10. Last paragraph. Electroporation cannot be simulated by placement of the electrodes, since the patients would feel the application of the pulses.

This point needs to be considered and probably also the protocol adequately modified.

Reply of the author:
We agree that placement of the electrodes alone will not suffice to simulate electroporation. Even more so because all ROIs will be anesthetized with local anesthesia.
The patient is not aware of the treatment allocation, and is not allowed to look during the procedure. However, we concur that there isn't a good way to fully blind the patient, as the patient may feel where on the body the pulses are applied. This is a limitation of the study, however, we do not see

any other way to simulate the EST pulses without interfering with our study design. We have now addressed this issue in the methods section, highlighted in red.

6. The authors predicted the use of two types of electrodes, plate and hexagonal. I would suggest the use of only one type. In the literature is ample evidence of the use of specific electrodes for the specific situations. In the case of the superficial lesions, Plate or Needle row electrodes would be more suitable, than the hexagonal, which are aimed for the treatment of deeper lesions, induce more pain and also cover bigger surface of the tumor.

Reply of the author:
We thank the reviewer for this valuable comment. We discussed with IGEA which electrodes would be most suitable for our patient population, and they recommended to try these two electrodes. As it is a pilot study, which focuses on determining the feasibility of our study protocol, one of our goals was also to find out which electrodes work better for capillary malformations. We started with the plate electrodes, as these do not penetrate the skin and are therefore less painful/harmful. We discussed that the hexagonal needles might be necessary for deeper lesions. We have now also added this explanation to the methods section.
Our study has already started and we have only used the plate electrodes so far and we already see very promising results in the ROIs that were treated with electrochemotherapy. So, we think that the reviewer has a good point and that there may be no need to use the hexagonal needle electrodes in this study!

7. The picture of the Cliniporator is probably not necessary, and is also very commercial.

Reply of the author:
We agree with the author that the picture of the Cliniporator is probably not necessary. We have now deleted this figure from the manuscript.

1. Stefanou A. Not again! BMJ (Clinical research ed). 2001;322(7285):548.

VERSION 2 – REVIEW

REVIEWER	Philip S Bekhor Royal Children's Hospital Melbourne Australia
REVIEW RETURNED	09-Apr-2017

GENERAL COMMENTS	It will be interesting to see if bleomycin has any role in the management of hypertrophic PWS

REVIEWER	prof. Gregor Sersa Institute of Oncology Ljubljana Slovenia
REVIEW RETURNED	05-Apr-2017

GENERAL COMMENTS	I have no further comments. The comments raised were answered adequately.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Philip S Bekhor
Institution and Country: Royal Children's Hospital, Melbourne, Australia
Please state any competing interests: None declared

Please leave your comments for the authors below

1. It will be interesting to see if bleomycin has any role in the management of hypertrophic PWS

Reply:
We fully agree with the reviewer. We therefore included a 'control' group of regions of interest within the PWS which will only be treated with bleomycine. We are curious to see if this would also have an effect on the PWS.

Reviewer: 2
Reviewer Name: Prof. Gregor Sersa
Institution and Country: Institute of Oncology Ljubljana, Slovenia
Please state any competing interests: None Declared

Please leave your comments for the authors below

I have no further comments.

The comments raised were answered adequately.

VERSION 3 – REVIEW

REVIEWER	Philip S Bekhor Royal Children's Hospital Melbourne Australia
REVIEW RETURNED	21-May-2017

GENERAL COMMENTS	I have commented on previous versions of the paper have no more comments to make other than page 4 line 20, it is better English to say bleeding rather than Bleedings.

REVIEWER	prof. Gregor Sersa Institute of Oncology Ljubljana Slovenia
REVIEW RETURNED	26-Apr-2017

GENERAL COMMENTS	I have no further comments

Correction: *Electrosclerotherapy for capillary malformations: study protocol for a randomised within-patient controlled pilot trial*

Horbach SER, Wolkerstorfer A, de Bruin DM, *et al*. Electrosclerotherapy for capillary malformations: study protocol for a randomised within-patient controlled pilot trial. *BMJ Open* 2017;7:e016401. doi: 10.1136/bmjopen-2017-016401

'Sanne M Jansen' was missed off the original author list. The correct author list and affiliations should read:

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