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ARTICLE DETAILS

<table>
<thead>
<tr>
<th>TITLE (PROVISIONAL)</th>
<th>Exploratory clinical trial for combination wound therapy with a gelatin sheet and platelet-rich plasma in patients with chronic skin ulcers: Study protocol</th>
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<tbody>
<tr>
<td>AUTHORS</td>
<td>Morimoto, Naoki; Kakudo, Natsuko; Matsui, Makoto; Ogura, Tsunetaka; Hara, Tomoya; Suzuki, Kenji; Yamamoto, Masaya; Tabata, Yasuhiko; Kusumoto, Kenji</td>
</tr>
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VERSION 1 - REVIEW

<table>
<thead>
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<td>REVIEW RETURNED</td>
<td>28-Jan-2015</td>
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GENERAL COMMENTS

An exploratory clinical trial for combination wound therapy with a gelatin sheet and platelet-rich plasma in patients with chronic skin ulcers: a study protocol

I suggest the following improvements to the study protocol:

1) Abstract:
   a. Page 3, line 25: 1 month is a very short period for a study on chronic skin ulcers. I suggest the inclusion of ulcers with a duration of at least 6 weeks (which is mostly used in other trials, especially on chronic venous leg ulcers).
   b. Page 3, line 32-34: The design should not include skin grafting or suturing to achieve complete healing. The given definitions when this might be performed are not clear. If the authors want to keep this option to achieve complete healing I suggest to name a defined wound size when this might be performed.
   c. Page 3, line 44: Chronic skin ulcers – in our country – are ulcers with a duration of at least 6 weeks. However, I acknowledge that this definition might not be used in other countries (but in most studies as mentioned above). Moreover, I strongly suggest the inclusion of ulcers of only one cause, e.g. only venous leg ulcers or diabetic foot ulcers. This is necessary to ensure minimal bias by concomitant therapies (e.g. compression yes/no)
   d. Page 4, line 6: „evidence evaluate“: I don’t quite understand this sentence. It’s probably just a typing mistake
2) Introduction:
   a. Page 5, line 23-25: „However, there are still issues that remain to be resolved in the treatment of those ulcers.“ It’s too generalized. The authors probably want to express that new treatment options for chronic skin ulcers are needed.
   b. Page 6, line 4: There is also a published study on EGF in a
bovine collagen matrix with sustained release.

3) Materials and methods:
   a. Page 6, line 29-30: “not expected to heal” is a strong and hard to define expression von ulcers with a duration of only 4 weeks. As mentioned above, I suggest to increase the minimal ulcer duration to at least 6 weeks and to limit the ulcers to one cause, e.g. venous leg ulcers or diabetic foot ulcers. It is also necessary to limit concomitant therapy, e.g. compression treatment yes/no. This also applies to the inclusion criteria, which are listed on page 7, lines 20-32.
   b. Page 6, line 52: “those” instead of dose
   c. Page 7, line 20-32: The inclusion criteria must include a certain wound size range.
   d. Page 8, line 5-11: Exclusion criteria must include that the use of bFGF is not allowed prior to the initiation of this study like listed on page 9, line 48-50 (subsequent therapy). Exclusion criteria must also include planned NPWT during the study period (like listed under subsequent therapy).
   e. Page 9, line 40-42: Ointment “such as” Azunol ointment is too generalized. It remains unclear what kind of ulcers (venous leg ulcers, diabetic foot ulcers) should be included. Since best practice treatment differs among different kinds of ulcers, I suggest the included ulcers to be limited to one cause (as mentioned above). Concomitant treatments should also be described more precisely. Most chronic ulcers need a wound dressing and not just an ointment.
   f. Page 9, line 48: “2 or more days prior to the administration of PRP“ means that bFGF could be applied 1 day prior to PRP administration. I think the authors wanted to say something else.
   g. Page 10, line 40-55: Evaluation criteria for the possibility of wound closure during the investigation must also include relative wound surface reduction. How exactly will the wound depth be measured?
   h. Page 11, line 9: How will the wound area be measured (grid method, length x width?). What’s the rationale for the ABI measurement? Do the authors expect improved perfusion due to PRP? The ABI range should also be included into the inclusion criteria.

VERSION 1 – AUTHOR RESPONSE

We are grateful to Dr. Martin Doerler for providing the critical comments and useful suggestions, which have helped us to improve our paper. As indicated in the responses that follow, we have taken all of these comments and suggestions into account in the revised version of our paper.

I suggest the following improvements to the study protocol:

1) Abstract:
   a. Page 3, line 25: 1 month is a very short period for a study on chronic skin ulcers. I suggest the inclusion of ulcers with a duration of at least 6 weeks (which is mostly used in other trials, especially on chronic venous leg ulcers).

Response

As you suggested, the inclusion of ulcers with a duration of at least six weeks is commonly used. However, in our country, a duration of at least four weeks is also commonly used and we used this criterion in our previous clinical trials of a novel collagen/gelatin scaffold with the sustained release of basic fibroblast growth factor (Morimoto N, et al. Novel collagen/gelatin scaffold with sustained

b. Page 3, Line 32-34: The design should not include skin grafting or suturing to achieve complete healing. The given definitions when this might be performed are not clear. If the authors want to keep this option to achieve complete healing I suggest to name a defined wound size when this might be performed.

Response
As you mentioned, we also think that these definitions are not clear. This study is a historical control study, and our control study used these inclusion criteria; therefore, it is difficult to change the criteria. Therefore, as you suggested, we added the wound size in our criteria, as described later.

c. Page 3, line 44: Chronic skin ulcers – in our country – are ulcers with a duration of at least 6 weeks. However, I acknowledge that this definition might not be used in other countries (but in most studies as mentioned above). Moreover, I strongly suggest the inclusion of ulcers of only one cause, e.g. only venous leg ulcers or diabetic foot ulcers. This is necessary to ensure minimal bias by concomitant therapies (e.g. compression yes/no)

Response
As you mentioned, we think it is better to select ulcers with only one cause in order to evaluate the efficacy of PRP. However, it is difficult to collect an adequate number of such patients at our hospital. This study is the first clinical trial to investigate the safety and efficacy of PRP application covered with our gelatin sheet, and we focused on the safety of this combination therapy in the present study. In the next step, we intend to conduct a trial choosing specific ulcers, such as diabetic ulcers or venous leg ulcers.

d. Page 4, line 6: „evidence evaluate“: I don’t quite understand this sentence. It’s probably just a typing mistake

Response
As you suggested, we changed the description, as follows: This protocol will provide evidence to compare the efficacy of PRP covered with a hydrocolloid dressing and that of PRP covered with a gelatin sheet in the treatment of chronic skin ulcers.

2) Introduction:

a. Page 5, line 23-25: „However, there are still issues that remain to be resolved in the treatment of those ulcers.” It’s too generalized. The authors probably want to express that new treatment options for chronic skin ulcers are needed.

Response
As you suggested, we changed the description as follows: However, new treatment options for chronic skin ulcers are needed.

b. Page 6, line 4: There is also a published study on EGF in a bovine collagen matrix with sustained release.

Response
As you suggested, we cited the above paper.

3) Materials and methods:

a. Page 6, line 29-30: „not expected to heal“ is a strong and hard to define expression von ulcers with a duration of only 4 weeks. As mentioned above, I suggest to increase the minimal ulcer duration to at least 6 weeks and to limit the ulcers to one cause, e.g. venous leg ulcers or diabetic foot ulcers. It is also necessary to limit concomitant therapy , e.g. compression treatment yes/no. This also applies
to the inclusion criteria, which are listed on page 7, lines 20-32.

Response
As you mentioned, we also think that “not expected to heal” is a strong and difficult to define expression. This study is a historical control study, and our control study used these inclusion criteria; therefore, it is difficult to change the criteria. However, as you suggested, we added the wound size in our criteria, as described later.

Our control study is unpublished and was used for the application for approval of the NPWT devise in our country. As we mentioned above, we intend to conduct a trial choosing specific ulcers, such as diabetic ulcers or venous leg ulcers, in the next step after confirming the safety of our combination therapy in this study.

As for concomitant therapy, we added the following description: Concomitant therapies, such as compression treatment, in cases of venous leg ulcers will be continued in addition to before entry, because it is important to evaluate the efficacy of PRP under the same conditions.

b. Page 6, line 52: “those” instead of dose

Response
As you suggested, we changed “dose” to “those.”

c. Page 7, line 20-32: The inclusion criteria must include a certain wound size range.

Response
As you suggested, we added the wound size between 3 and 30 cm², because the ulcer should be covered with PRP derived from 60 ml of whole blood taken from the patient.

d. Page 8, line 9-11: Exclusion criteria must include that the use of bFGF is not allowed prior to the initiation of this study like listed on page 9, line 48-50 (subsequent therapy). Exclusion criteria must also include planned NPWT during the study period (like listed under subsequent therapy)

Response
We believe that the therapies of bFGF and NPWT are prohibited treatments and not suitable to be cited in the exclusion criteria.

e. Page 9, line 40-42: ointment “such as” Azunol ointment is too generalized. It remains unclear what kind of ulcers (venous leg ulcers, diabetic foot ulcers) should be included. Since best practice treatment differs among different kind of ulcers, I suggest the included ulcers to be limited to one cause (as mentioned above). Concomitant treatments should also be described more precisely. Most chronic ulcers need a wound dressing and not just an ointment.

Response
We fully understand your suggestion; however, our control study used these criteria and it is difficult to change the criteria only for the current study, as mentioned above. We intend to conduct a trial choosing specific ulcers, as you suggested. Our criteria contain various ulcers, so we can set prohibited therapies, although it is difficult to set recommended therapies. In this study, we did not prohibit the use of wound dressings and therefore added a description of the wound dressings with respect to the application of activated PRP and dressing changes.

f. Page 9, line 48: „2 or more days prior to the administration of PRP“ means that bFGF could be applied 1 day prior to PRP administration. I think the authors wanted to say something else.

Response
As you suggested, “three or more days” is correct.

g. Page 10, line 40-55: Evaluation criteria for the possibility of wound closure during the investigation must also include relative wound surface reduction. How exactly will the wound depth be measured?

Response
As you suggested, we added a description of the wound area of less 3 cm².
As for the evaluation of wound depth, we used a depth gauge for evaluating the wound depth.

h. Page 11, line 9: How will the wound area be measured (grid method, length x width?). What’s the rationale for the ABI measurement? Do the authors expect improved perfusion due to PRP? The ABI range should also be included into the inclusion criteria.

Response
As we described in the section on the use of digital photography for the healing assessments, we took gross photos of the ulcers with a calibrator and evaluated the wound area using an image editing software program.

As for the ABI measurements, surgical procedures are contraindicated in cases of ABI values below 25 or 30 mmHg. We intend to evaluate the efficacy of PRP in these patients and therefore did not set the ABI in our criteria.

**VERSION 2 – REVIEW**

<table>
<thead>
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<td>REVIEW RETURNED</td>
<td>13-Mar-2015</td>
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**GENERAL COMMENTS**

The authors have addressed all comments of my first review. Given the fact that this is a study protocol and that new research in this field is highly important, I recommend the publication of this manuscript after some minor revisions:

1) Please include a limitations section in the discussion mentioning that this study included ulcers of various causes and the possible bias by different concomitant therapies (various wound dressings...). You may also state your plans for the future in the discussion (study limited to one ulcer cause, eventually standardized concomitant therapy). This also limits the conclusions with regard to efficacy of the PRP/gelatin hydrogel sheet. It still allows an evaluation of the safety. This should shortly be discussed.

2) I still don’t understand why bFGF must not be applied „3 or more days” before inclusion or during the study. Why should bFGF be applied 2 or 1 day before the study? Do the authors mean „3 or less days” prior to the beginning of the study?

3) Please include that wound depth is measured by using a depth gauge in your manuscript (not only in your response)

**VERSION 2 – AUTHOR RESPONSE**

The authors have addressed all comments of my first review. Given the fact that this is a study protocol and that new research in this field is highly important, I recommend the publication of this manuscript after some minor revisions:

1) Please include a limitations section in the discussion mentioning that this study included ulcers of various causes and the possible bias by different concomitant therapies (various wound dressings...). You may also state your plans for the future in the discussion (study limited to one ulcer cause, eventually standardized concomitant therapy). This also limits the conclusions with regard to efficacy of the PRP/gelatin hydrogel sheet. It still allows an evaluation of the safety. This should shortly be
discussed.

Response
As you suggested, we added a description in the discussion as follows: Regarding the limitation of this study, this study included ulcers of various causes that could be potential for bias by different concomitant therapies. Therefore, we will plan the clinical study limited to one ulcer cause with standardized concomitant therapy in the next step. This is our first trial using the PRP/gelatin hydrogel sheet and we focused mainly on safety in this study and evaluate the efficacy in the next step limited to one ulcer cause (Page 14, line22).

2) I still don’t understand why bFGF must not be applied „3 or more days“ before inclusion or during the study. Why should bFGF be applied 2 or 1 day before the study? Do the authors mean „3 or less days“ prior to the beginning of the study?

Response
We mean that the washout period of bFGF will be necessary for three or more days before the administration of PRP. The use of bFGF will be prohibited from three or more days prior to the administration of PRP until the end of this study. We added a description as follows: The use of basic fibroblast growth factor (bFGF) will be prohibited from three or more days prior to the administration of PRP (wash-out period of bFGF) and during the study period (Page 10,line 3).

3) Please include that wound depth is measured by using a depth gauge in your manuscript (not only in your response)

Response
As you suggested, we added a description as follows: The wound depth measured by using a depth gauge is reduced by an average of 50% or more (Page 11,line10)
Exploratory clinical trial of combination wound therapy with a gelatin sheet and platelet-rich plasma in patients with chronic skin ulcers: study protocol

Naoki Morimoto, Natsuko Kakudo, Makoto Matsui, Tsunetaka Ogura, Tomoya Hara, Kenji Suzuki, Masaya Yamamoto, Yasuhiko Tabata and Kenji Kusumoto

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