Randomized controlled trial of the effect of larvae and larval secretions on wound healing in diabetic ulcers

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In this study, the following questions will be answered:1. Does larval therapy accelerate wound healing?2. Do single larval secretions accelerate wound healing?3. Is the bacterial load in the wound reduced during larval therapy or is the microbiome...

Ethical review	Not approved
Status	Will not start
Health condition type	Other condition
Study type	Interventional

Summary

ID

NL-OMON42280

Source
ToetsingOnline

Brief title
In vivo effects of larvae and their secretions on wound healing

Condition

- Other condition
- Diabetic complications
- Skin and subcutaneous tissue disorders

Synonym
chronic wound, Diabetic ulcer

Health condition

Wondgenezing

Research involving

Human

Sponsors and support

Primary sponsor: Chirurgie
Source(s) of monetary or material Support: Biomonde, madenproductie bedrijf, Cardiff, Wales en Barsbuttel, Duitsland,Biomonde;madenproductie bedrijf;Cardiff;Wales en Barsbuttel;Duitsland. Mogelijk wordt nog extra subsidie gevraagd van het Bronovo Research Fonds;die in het verleden de laboratorium studie met maden ook heeft ondersteund.

Intervention

Keyword: Larval secretions, Larval therapy, Lucilia sericata, Wound healing

Outcome measures

Primary outcome

4. Outcomes

Primary outcome:

1. Does larval therapy accelerate wound healing?

2. Do single larval secretions accelerate wound healing?

Photographs are taken with a ruler alongside the wound, and the wound surface in maximum length and maximum width will be measured at the start of the therapy, after week 1 and then every 2 weeks. If the wound has almost healed, photographs are taken every control, which is twice a week, to have more accurate final measurements and prevent bias in the primary outcome between the groups. To check the wound size, special software will be used for measurement (Canvas X). Measurement of wound sizes with a ruler tends to overestimate the sizes in larger and more irregularly shaped wounds, so digital measurement

using software is adviced in literature. Closure of the wound surface is defined as a complete cover of the wound bed with an epithelial layer without a crusta (cicatricial tissue). One outcome assessor, who is blinded, will assess the results of the photographs without knowing which therapy was performed.

Secondary outcome

Secondary outcomes:

3. Is the bacterial load in the wound reduced during larval therapy or is the microbiome changed?

Wound cultures will be taken at the start of the therapy, after week 1 and then every 2 weeks to investigate the bacterial species present in the wound, the bacterial load and their ability to form biofilms (microscopy).

4. Are there systemic immunological and/or anti-inflammatory effects of local larval application or larval secretion application?

- Cytokines/complement will be measured in the wound fluid (e.g. C3d, C5b-C9, TNF*, IL-1, IL-6, IL-8, IL-10, PDGF, VEGF etc.). Wound fluid is collected by application of two sterile 5x5 cm gauzes (one packet) on the wound during 5 minutes in presence of 5 mL physiological saline, before start of the therapy, after the first and the third application and one week after the final application.

-CRP, BSE, leukocytes and complement activity will be measured in serum before start of the therapy, after the first and the third application and one week after the final application. The HbA1c value will be measured once before start of the therapy.

5. Does moisture balance affect the time to wound healing?

Moisture balance will be measured at the start of the therapy, after week 1 and then every 2 weeks (e.g. using WoundSense from www.ohmedics.com and/or observation by the caregiver) In chronic wounds with persistent inflammation, exudate production is out of balance. It is known that for wound bed preparation and physiological wound healing, moisture balance is important. Measurement of moisture balance can provide information about the effect of exudate production on the speed of wound healing. Furthermore, literature reports less pain in wounds with a moist environment, one of the other secondary outcomes, we will measure and correlate.

6. Is there a difference in reappearance of debris between the three groups?

Reappearance of slough or necrotic tissue after initial surgical debridement will be scored. At first, the percentage of debris is estimated by the caregiver, later this percentage will be controlled using special software (Canvas X).

7. Do patients have adverse effects of the therapy, including pain, crawling sensations, bleeding, allergic reactions etc.?

In literature, no severe side-effects have been reported. Sometimes, a tickling feeling of the larvae is noted, however after using the captured method, there are less complaints about this sensation. Pain is sometimes reported, and normally this pain can be treated by paracetamol. The origin of the pain is unknown. No allergic reactions were ever noted. After three weeks of therapy, all patients will fill in a question form in which they can score their experiences during the therapy on a scale from 1-5. Pain scores will be measured using a Visual Analog Scale (patients will be asked to compare their pain before start of the therapy and during the treatment). . Side-effects, such as bleeding or allergy, will be reported by the caregiver.

8. What is the cost-effectiveness comparing the three therapies?

The costs of each therapy will be analyzed and related to the effectiveness of the therapy (the primary outcome).

Study description

Background summary

Larval therapy is widely used today for the treatment of acute and chronic wounds in patients. Annually, more than 15.000 patients receive larval therapy in Europe. The exact mechanisms of action of larvae in wound treatment are only recently becoming better understood. The US Food and Drug Administration

registered maggot debridement therapy (510(k) #33391 as a wound treatment method in 2004. The Inspectie voor de Gezondheidszorg (IGZ) in the Netherlands approved the larva Lucilia sericata as a medicine in 2014 (September 1st). Three randomized clinical trials (RCTs) have shown the debridement potential of larvae, while other beneficial effects of larvae on wounds, including anti-infection, immunomodulation, angiogenesis, and tissue remodeling and regeneration, have been widely reported clinically and are supported by numerous in vitro studies. One (small) RCT showed a lower incidence of wound infections in vivo during larval therapy, compared to hydrogel application. The RCT from Opletalova et al., that focused on debridement, showed a faster wound bed preparation during the first week with larval therapy compared to surgical debridement, but there was no difference in percentage of slough after 15 days. The other two clinical studies compared larval therapy with hydrogel application and both significantly reduced the time to debridement with larval therapy. The VenUS II trial from Dumville et al. demonstrated that wounds were debrided within 14 days using larvae versus 72 days using hydrogel application. This trial was the only clinical study that investigated the time to healing and bacterial load during larval therapy, and could not show improved wound healing rate or bacterial reduction in the wound during larval application. While debridement, according to the current knowledge of the wound healing process, is essential to progress from the inflammatory phase to the proliferative phase, an increased wound healing rate was expected. Maybe, the discrepant result can be explained by the unclear inclusion criteria of the trial; e.g. different sizes of wound areas and percentages of slough were included, which finally resulted in a median larger ulcer area in the larval group comparing to the hydrogel group, and a quantity of debris that varied from 26 up to 100%. Patients with immune-related diseases and malignancies were not excluded from the trial, however these underlying diseases could significantly interfere with the process of wound healing. To conclude, further research, especially a (randomized) clinical trial, is needed.

Study objective

In this study, the following questions will be answered:

1. Does larval therapy accelerate wound healing?
2. Do single larval secretions accelerate wound healing?
3. Is the bacterial load in the wound reduced during larval therapy or is the microbiome changed?
4. Are there systemic immunological and/or anti-inflammatory effects of local larval application or larval secretion application?
5. Does moisture balance affect the time to wound healing?
6. Is there a difference in reappearance of debris between the three groups?
7. Do patients have adverse effects of the therapy, including pain, crawling sensations, bleeding, allergic reactions etc.?
8. What is the cost-effectiveness comparing the three therapies?

We hypothesize that larvae and/or their excretions/secretions (ES) accelerate wound healing (this study does not focus on debridement as primary outcome). In several trials no difference in (debriding) effect was shown between free larvae and larvae, captured in small, permeable bags, so-called Biobags, that allow free exchange of larval ES. Therefore we choose to test only the bagged larvae.

We would like to compare three groups: larvae in Biobags (1), larval secretions gel (under GMP controlled conditions produced) (2) and a control group (3).

This protocol is written, following the guidelines for design and conduct of clinical trials in wound care, as described by Eskes et al. in the journal of Wound Repair and Regeneration, 2012.

For references, see above in the paragraph 'Background of the study'.

Study design

1. Trial setting:
The trial takes place in two general hospitals in the Hague, the Netherlands, that have recently merged: The Medical Center Haaglanden (MCH) and the Bronovo Hospital. In the future, probably the Leiden University Medical Center in Leiden, the Rijnland hospital in Leiderdorp and the Isala Klinieken in Zwolle will participate.

2. Patients: see paragraphs below.

3. Interventions: see paragraphs below.

4. Outcomes: see paragraphs below.

5. Sample size and statistics
It was determined that 76 participants per group are needed to detect a reduction in median healing time from 16 weeks to 10 weeks (i.e. a constant hazard ratio of 1,6 between two groups) with 80% power and a two sided significance level of 5%. In literature, the median healing varies between 12 and 34 weeks (13 weeks, Warriner et al, 2011; 21-34 weeks, Pickwell et al, 2013; 12-16 weeks, Gul et al, 2006; 14 weeks, Wilarusmee et al, 2014). We think that in our trial a reduction from 16 weeks to 10 weeks will be a realistic and clinical relevant difference to measure.

The calculation above assumed an exponential distribution of time to healing and an assessment of equality of survival curves using the exponential maximum likelihood test, assuming an accrual period of 156 weeks (3 years) and a maximum follow-up time of 168 weeks (so follow up ranging from 12 up to 168 weeks for the last resp. first patient included), and no dropouts.

The estimated percentage of patients with completely healed wound after 1 year is 72-90%; Jeffcoate et al, 2006, Treece et al, 2004. The assumption above corresponds to an expected percentage of 89% healed wounds after one year in one group and 97% healed wounds in another group with a positive effect of the treatment.

The time to healing between the three groups is investigated using survival analysis (kaplan meier curves and log rank test). Cox analysis is performed to correct for wound size, duration of ulcer and ulcer type (stratifying factors during randomization). For the patients with wounds that won*t heal and have an amputation, the time to wound healing will be censored at the moment of amputation. The estimated percentage of patients in these groups requiring amputation is less than 9% (Orneholm et al. 2015; in this publication 9% minor and major amputations had to be performed in a group of 770 diabetic patients, however 25% had major peripheral vascular disease which will be excluded in our trial).
The patients whose therapy has been changed after 4 weeks will be analyzed in the group in which they are allocated, in conformity with the intention-to-treat principle. The follow up of these patients will continue until the wound has been closed.

A secondary analysis will be performed per protocol. Details of the per protocol analysis will be specified in the statistical analysis plan. In another secondary analysis, the groups will be compared corrected for the baseline HbA1c values.

Baseline characteristics of patients and wounds in the three different groups will be reported using descriptive statistics. These characteristics include: mean age, sex, mean size of ulcer, median duration of ulcer, toe-brachial index, smoking, BMI, antibiotic therapy, bacterial colonization. The outcomes of wound cultures will also be described.

The repeated measurements of inflammatory markers (e.g. cytokines, CRP, complement factors etc.) will be analyzed with linear mixed models including a time x treatment interaction effect, using transformations where appropriate.

6. Randomization
We will randomize all patients in a stratified way to prevent large differences in wound characterics between the groups, such as wound surface, duration of ulcer and ulcer type (Wagner I-III, Texas AI-III and BI-III). Each patient will be assigned to a group using randomization software, especially developed for clinical trials (e.g. on the website: https://www.tenalea.com/nkiavl/ALEA). The caregiver will randomize the patient and start the therapy. Which therapy will be started is registered in the patient record.

7. Blinding
The outcome assessor is blinded and will only assess the results of the

photographs and the measurements without knowing which therapy was performed. Caregivers cannot be blinded. Patients are not blinded. Although coping style can possibly affect wound healing, psychological factors such as anxiety or depression do not influence the time to complete healing of chronic wounds (Vedhara et al. 2010, Diabetologia).

8. Intention-to-treat
All randomized patients are analyzed in the group to which they are allocated.

9. Funding
This study is financially supported by Research Foundation Bronovo, The Hague, The Netherlands. Laboratory facilities are provided by the Leiden University Medical Center, Leiden, The Netherlands.

Sterile larvae (Lucilia sericata) and larval secretions gel are a generous gift from Biomonde, GmBH, Barsbuttel, Germany.

10. Follow-up
All patients will be followed until the wound has been closed, with a maximal follow-up of 168 weeks. When there is no effect of the treatment after 4 weeks, the therapy may be changed by the caregiver.

11. Ethics
The ethic committee has to evaluate this protocol.

12. Final remarks
For future trials, we would like to investigate dose-reponse relations of larvae and larval secretions, and different ways of application and their effect on wound healing. Of course, we need to analyze the results of this trial before any other study can be initiated.

Intervention

3. Interventions:

All patients that are included will have surgical debridement of the wound at the start of the study, preferably under local anaesthesia (Lidocaine 1%). Surgical debridement is performed again during policlinical visits if slough reappears after initial debridement. After initial debridement, patients are randomized and the fully debrided wound will receive one of the three therapies described below.

- Application of larvae in biobags. Larvae will be changed twice a week at the wound care unit (outpatient department).
o 5-8 larvae/cm2 wound surface, which is the adviced dose in literature and by the manufacturer*s guide. This is the dose which was also used in the other RCT*s and it was effective for debridement of the wounds. If a patient is not

present at his or her appointment, there is no risk that the larvae will puppate and become flies in the Biobags. Larvae need a dry environment and enough oxygen to puppate and develop, which they do not have in the bags. They won*t survive.

- Application of larval secretions in (hypromellose) gel once a day. Patients receive a tube with larval secretions gel 0.05% (this is not the definitive dosage, first a pilot study will be performed to test the optimal dosage, see also C1 protocol, July 2015, version 2), which can be stored at room temperature for a month. Previous and recent studies have shown that larval secretions are temperature tolerant. Once a day, a thin layer of the gel is applied on the wound by the patient or the wound nurse at home. The wound is covered by a wound film dressing. Patients come back at the policlinical outpatient department every one or two weeks for wound inspection. If the wound of a person has almost healed, the controls will be twice a week to prevent bias in the primary outcome (time to wound healing).
o Larval secretion gel, comparable with 5-8 larvae/cm2 wound surface/24 hours), produced under GMP controlled conditions by Biomonde, Cardiff, UK. More information about the dosage can be found in the IB and IMPD of this RCT.

- Control: standard care. Dry wounds are treated by hydrogel and a hydrocolloid dressing. On more exudative wounds alginate is applied. Wounds will be inspected at the wound care unit every one or two weeks. If the wound of a person has almost healed, the controls will be twice a week to prevent bias in the primary outcome (time to wound healing).

If a patient has multiple wounds, all wounds receive the same therapy. The largest wound of this patient will be included in the study for analysis. Surgical debridement is performed if there is reappearance of slough (after initial debridement) and will be scored in each group. Other basic wound care, that will be the same for these 3 groups, includes compression therapy if there is peripheral edema (arterial blood supply is not compromised in these groups with a minimal toe-brachial index of 0.7). All wounds will be finally covered with an absorbing bandage and a hydrophilic bandage for fixation.

All patients are treated until the wound has closed, with a maximal follow-up of 168 weeks. When there is no effect of the treatment after 4 weeks, the therapy may be changed by the caregiver. In acute settings, e.g. if a patient develops a systemic infection or an acute imflammation, of course the therapy can/must be changed if necessary.

Study burden and risks

There are no severe side-effects known for any of these therapies. Patients who receive larvae on their wounds complain sometimes of pain, but pain killers, such as paracetamol, are enough to reduce the complaints. Allergies for larvae are not known, but could happen. Allergies for hydrogel or alginates are

possible as well. In the case of an allergy, the therapy will be discontinued immediately.

The persons in the study will have four extra venous punctures: one before start of the therapy, one after the first and third week of the initial treatment and the last puncture one week after ending the therapy. At the same time of the puncture a wet gauze is applied on the wound for five minutes to obtain wound fluid for laboratory research. Several times photographs of the wounds will be taken. Once, the person will be asked to fill in a questionnaire. Finally, for the group which receives live larvae, it is necessary to come to the policlinical department for a change of the biobag with larvae twice a week.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Diabetic patients with chronic wounds, defined in this study as a wound without any healing tendency within a period of 4 weeks, with sufficient arterial circulation (toe-brachial index > 0.7) ;- Mono- or bilateral lower extremity wound without healing tendency within 4 weeks after the initial injury/origin.
- Wounds with at least 50% slough (yellow wound surface) or necrosis (black wound surface)
- Wound surfaces from 10 up to 40 cm2
- Age >18 years
- Classification: Wagner 1-3 / Texas AI-III and BI-III

Exclusion criteria

- Use of immunosupressive medication
- Other auto-immune diseases than Diabetes Mellitus
- Malignancies (unless cured in the past)
- Use of more than 4 units alcohol a day
- Pregnancy/lactation
- Classification: Wagner 0, 4-5, Texas A0, B0, C0-III and D0-III
- Unable to understand dutch language
- Unable to visit the hospital multiple times during a longer period

Study design

Design

Study type:	Interventional
Intervention model:	Other
Allocation:	Randomized controlled trial
Masking:	Single blinded (masking used)
Control:	Active
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Will not start
Enrollment:	228

Type:	Anticipated

Medical products/devices used

Product type:	Medicine
Brand name:	Larvae en biobag © (unregistered medicine in the Netherlands, approved by the IGZ)
Generic name:	Larvae en biobag ©
Product type:	Medicine
Brand name:	Lucilia sericata secreta gel 0.05%
Generic name:	Lucilia sericata secreta gel 0.05%

Ethics review

Approved WMO Date:	19-05-2015
Application type:	First submission
Review commission:	METC Leiden-Den Haag-Delft (Leiden) metc-ldd@lumc.nl

Not approved Date:	15-04-2016
Application type:	First submission
Review commission:	METC Leiden-Den Haag-Delft (Leiden) metc-ldd@lumc.nl

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
EudraCT	EUCTR2015-000613-51-NL
CCMO	NL51756.098.15