INFORMED CONSENT

STUDY ON THE EFFECTIVENESS OF HYPOTHERMAL SULPHUROUS WATER IN WOUND HYGIENE

INFORMED CONSENT

- **PARTICIPATION**

You have been selected to participate in a research project in which you can freely choose whether to participate. To enable you to make your choice, the potential benefits and possible risks of participating in the study will be explained below. This form describes the objective of the project and the procedures that will be carried out, as well as how your health information will be used and who will have access to it.

Your doctor will answer any questions you may have about the study or about this form. Therefore, please ask your doctor for clarification regarding any term or information that you do not understand. After reading this informed consent, in case you agree to participate in the study, you will be asked to sign this form. You will be given a copy of the informed consent form to which you can always refer and which we therefore advise you to keep.

GENERAL INFORMATION

- **CHRONIC WOUNDS**

Skin ulcers that are difficult to heal are skin lesions that have arisen for at least four weeks, which, after an initial positive response to treatment with traditional or advanced dressings, suddenly become non-responsive [PMID: 16799373]. In Italy, about 2 million people are affected by chronic ulcers. In the United States, there are approximately 6.5 million chronic pressure ulcers, venous stasis ulcers and diabetes [PMIDs: 22117872] each year. The healthcare cost for an unhealed ulcer is on average 135% higher than that of a healed wound. The treatment of "difficult" ulcers is an articulated and complex process, in which a global and multi-specialist view of the clinical picture is required. The choice of the most appropriate medical interventions to facilitate the healing of the wound is represented first and foremost by the correct preparation of the wound, the concept of "wound hygiene" which represents the heart of the activity of the expert in difficult wounds or vulnologist [PMID: 32160083]. Wound hygiene (https://www.nurse24.it/images/allegati/Wound_Hygiene_JWC.pdf) consists of 4 steps: the first is wound washing, [PMID: 22336796]. Along with washing, there is manual or mechanical removal of debris and non-vital tissues, reactivation of the wound fundus, and dressing. Wound chronicity is, in about 80% of cases, due to a form of bacterial colonization known as wound biofilm, an extremely complex form of polymicrobial symbiosis, which provides protection against antimicrobial agents and host defenses, a phenomenon underlying multiple antibiotic resistance [PMID: 35113669]. Chronic wounds also have pH changes, with a tendency to increase the pH of the wound bed. The decrease in pH towards acidity is one of the signals of progression towards healing. [PMID: 17091276]. The timely, multidisciplinary approach to the patient makes it possible to identify the needs and limitations of any previously attempted approaches. The use of thermal waters, in particular sulphurous thermal waters, for the treatment of wounds has been known for

millennia, as reported by ancient written testimonies. The healing value on the lesions of the Albule Waters, specifically, is cited by Pliny the Elder who defines them as "holy waters" for their therapeutic properties and describes how soldiers wounded during the war were specially sent to these springs. To date, the possible use of thermal waters on wounds has been the subject of at least 95 publications and scientific studies. In addition, recent scientific studies have confirmed the favorable effect on wound healing of some thermal waters through the stimulating action of fibroblasts and keratinocytes [PMID: 36982430] , [PMID: 37761993] the cells that allow re-epithelialization. The presence of an ulcer or skin lesion, in addition to the painful symptoms, the impact on the patient's daily life and activities and family and economic commitment can also be a factor favoring systemic infections, osteomyelitis (bone infection), reduced mobility leading to irreversible immobility, decline in health status with an increase in morbidity and mortality. In addition, chronic lesions have an increased risk of tumor transformation [PMID: 24075006], called Marjolin ulcers.

- **H2S**

H2S is a colorless gas that is produced in small quantities by our body by means of enzymatic and non-enzymatic processes. H2S has recently been included among the so-called "gasotransmitters", together with nitric oxide (NO) and carbon monoxide (CO), because like these it takes part in intra- and extra-cellular signaling processes (PMID: 34944543/ PMID: 32783196).
The efficacy of sulphurous waters containing H2S has been proven on almost all systems [https://doi.org/10.1016/B978-0-12-814253-0.00010-3]. In the field of vulnology, this gas can be administered to patients exogenously, in the form of H2S donor molecules, using the gases that are released from sulphurous waters through localized skin application or through whole-body balneotherapy or balneotherapy limited to the area affected by the lesion [https://doi.org/10.3390/app11188402]. In recent years, due to the selection of multi-drug resistant germs, the approach to chronic lesions with complex biofilms has turned to molecules that are not strictly pharmacological and to natural compounds that have multiple activities (anti-inflammatory, immunoregulatory, etc.) including antibacterial activity based on metabolic pathways so that the bacteria that make up the biofilm cannot yet develop resistance. In addition, the use of these compounds is economically and ecologically more sustainable than synthetic derivatives. As also observed in other fields of application, H_{2S} counteracts the "cytokine storm" characteristic of acute or chronic inflammatory states regardless of their etiology (through the reduction of inflammatory molecules such as the interleukins IL-1β, IL-6, TNFα, [PMID: 30927279] and is also able to act as a stimulus for the formation of new tissue, induces the orderly deposition of Collagen, increase the formation of new vessels, especially in conditions of oxygen deficiency or poor circulation such as in the case of vascular disease [PMID: 19955410] and protects the myocrocirculation from damage secondary to diabetes [PMID: 32186933]. H2S, transforming into Pentathionic Acid H2S5O6, represents a powerful antibacterial and antifungal [PMID: 17778264]. For these effects, balneotherapy with sulphurous waters have been included in the WHO Global Action Plan for Chronic Diseases (NCDs) of 2013-2023 (World Health Organization. Global Action Plan for the Prevention and Control of Noncommunicable Diseases 2013–2020. 2013. Available online).

- **AIMS**

She was asked to participate in this research project because she had a chronic, hard to heal lesion requiring specialist treatment.
If you accept, you will be asked to undergo a course of medications that uses sulphurous thermal water to induce wound healing. The study will take place at the Terme di Roma spa, Acque Albule, Bagni di Tivoli, Rome.

The aims of this study are:

- Evaluation of the wound healing process

-Evaluation of the change in skin integrity (by measurement of TEWL-trans epidermal water loss)

- Evaluation of pH changes at the wound bed level

-Evaluation of microbial colonization in terms of both quantitative and qualitative changes:

- Execution of qualitative and quantitative microbiological swab of the wound
- Direct visualization of the biofilm in vivo using a microscope
- Immediate visualization Critical colonization with fluorescence imaging devices
- Evaluation of changes in the wound microbiota
- Evaluation of scar stability at 3mm from healing

-Evaluation of the possible onset of recurrences 3 months after recovery

The objectives will be evaluated at each session based on the changes in the parameters at T0 (pretreatment) and T1 (posttreatment), and every 48 hours until re-epithelialization.

The final check on the stability of healing (follow-up) will be performed at 90 days after the complete wound healing and end of the intervention (Tf).

- **STUDY DESIGN**

This is a single-blind, non-pharmacological double-arm randomized interventional trial.

Interventional means that he will be subjected to a treatment (dressings) performed using the sulphurous thermal water, microbiologically pure, authorized for hydrotherapy (drinking) of the Tivoli thermal center for wound hygiene, but no pharmacological treatment. Single-blind means that you and the doctors who perform the treatment will know the type of treatment performed but not the researchers who will analyze the data deriving from the study, therefore not being able to intervene on them, to further guarantee the objectivity of the results. Double-arm means that subjects who will join the study will be randomly divided into two groups that will undergo two different treatments: group A, for which thermal water for wound hygiene will be applied by compress of gauze for 20 minutes; and group B for which thermal water for wound hygiene will be applied by immersion of the limb in thermal water for 20 minutes.

You will not be given the opportunity to choose which group to join, nor will you be given the opportunity to change groups before or during the study.

- **PRACTICE ACTIVITIES AND COMMITMENT BY THE PATIENT IN TERMS OF TIME**

The maximum period of time for your participation in the study will be the time necessary for the complete healing of your injury(s). During this period he will have to go to the Tivoli spa every 2 days, where he will perform a wound care and medical check-up and carry out the medication with the application of pure sulphurous water.
The times of evaluations and therapies will be cadenced as follows:

- **T0**: will undergo a medical examination, his clinical, demographic, anthropometric, and diagnostic data will be recorded. After general classification, microbiological swab of the wound with antibiogram, wound bottom swab will be carried out for determination of the microbiota, measurement of the lesion pH, measurement of the skin integrity index (TEWL), instantaneous determination of the bacterial load in vivo by fluorescence photography (Moleculight®), photographic acquisition of the lesion and microscopic photography (1000x bluetooth microscope). Finally, your lesion will be staged according to specific scales for the type of lesion you present (vascular ulcer, autoimmune ulcer, post-traumatic wound, decubitus, burn, etc.). On the same day he will be assigned to one of the two intervention groups (A-compress, B-immersion) and undergo the first session of medication with sulphurous waters. He will continue the sessions every 48 hours until the injury(s) heal (with a break on Sundays).
- **T1**: within 10 minutes since the end of wound hygiene (both group A and B) a wound swab for microbiome will be once more performed, all determinations of pH, TEWL, photographic surveys for bacterial load, microscopic, and staging according to a specific scale. Finally, the lesion(s) will be medicated with non-interactive dressings, for the sole management of the exudate. If necessary, a compressive bandage or pressure off load will be provided.
- **Tf**: The final check will be performed 90 days after the end of the treatments, in order to get a photographic surveys and assess scar stability or detect any new lesions onset.
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All treatments will be carried out by medical wound care specialists with sterile/disposable devices in accordance with current hygiene and safety regulations. Samples will be identified and labeled at origin and transported by medical personnel to the selected analysis laboratory.

- **LIABILITY**

As a patient enrolled in this study, you have certain responsibilities.

You will therefore have to:

- Complete all scheduled visits and treatments.
- Refer to the study physician your complete medical history.
- Provide the physician with all relevant diagnostic investigations performed. Ask your study referring doctor if you have any questions or concerns about the medical documentation to be submitted when asked.

- During your the study time, you are kindly request to strictly follow all instructions that will be given to you. Otherwise, you will not be able to participate in the study. If you are not sure what you are being asked to do, talk to your study referring doctor.

POTENTIAL ADVERSE EVENTS

The selection process and the medical examinations you will undergo before the medication are designed to identify patients who are eligible to the study, which will always take place under strict medical supervision.

In general, there are no adverse effects associated with the application of microbiologically pure thermal water on selected skin lesions.

Any possible side effects deriving from the local application of thermal water such as: skin dryness, perception of skin tension, are all to be understood as transitory. Rare self-limiting skin irritative phenomena (sulfur dermatitis) are described in the literature in patients with pre-existing skin reactivity after bathing in sulphurous thermal water; all transient phenomena, which will be treated by washing with tap water, application of soothing cream and temporary prohibition of sun exposure of the area involved. Patients will be monitored during each session for the onset of any bacterial superinfections. In the event that a bacterial superinfection occurs, it will be treated with immediate exclusion from the protocol, microbiological sampling and antibiogram (Swab Copan®, Copan Italia), double antisepsis with PHMB (prontosan solution for irrigation ® BBraun, Germany) and sodium hypochlorite 0.05% (Amukine Med®, Angelini, Italy), dressing for bacterial uptake in DACC (Cutimed Sorbact gel ®, Essity, Sweden) with change at 24hh until microbiological confirmation. Subsequently, systemic antibiotic therapy will eventually be started to be agreed with an infectious disease specialist.

POTENTIAL BENEFITS

Treatment with sulphurous water can lead to an improvement in skin inflammation, edema, infection until complete healing of the lesion(s) can be induced.

ALLOWANCE

Your participation in this study is free of charge:

-You will NOT receive any compensation from your participation in this research study

- **No financial contribution will be required** on your part for the medical examination or treatment.

In some cases, it may be suggested to you to purchase generic aids (therefore not specific brands) necessary for you (bandages or containment stockings, diabetic foot shoes, walking aids, etc.) or for the wound healing. Such economic participation is not sponsored, it is optional and is not always necessary nor request.

Treatments and medical examinations will be provided **free of charge**.

You will NOT have to incur any expenses for medical procedures (Medical examination, microbiome swab, pH measurement, TEWL, photographs and microscope determinations.)

- **VOLUNTARY PARTICIPATION/WITHDRAWAL**

You are free to choose whether to take part in the study and can also change your mind at any time. If you decide not to participate or stop participating in the study after starting it, it will not affect your future treatments and care; there will be no penalties or loss of benefits to which you are entitled. If you wish to withdraw from the study, you should contact the study doctor or the staff of the reference centre and inform them of your decision. (see contact section *).
The Sponsor, the Ethics Committee or the regulatory authorities may also decide to terminate the study, at any time and for any reason. The study physicians may also decide that you should no longer participate in the study if it is in your best interests or if you do not follow the instructions provided to ensure proper participation.

- **APPROVAL**

The following study and relative consent have been approved by the Ethical Board of UNIVERSITY COMMITTEE FOR RESEARCH (CAR-IRB) of University of Rome Foro Italico

Number: CAR 214/2024

- **CONTACTS***

If, at any time, you have any questions about the study or your rights or if you need to contact the doctors in charge of the study, who will always be present during your use at the spa, you can send an email to the following address:

vulnologiatermale@gmail.com

INFORMED CONSENT FORM

On the / / , the undersigned .....

was informed by Dr. Dr. .....

About the medical research study in question "THERMAL VULNOLOGY". I believe that I have had the opportunity to read the information and ask questions to which comprehensive answers have been given, which I have understood.

I voluntarily agree:

- To participate in the study
- Follow the instructions given by the study doctor

I agree to undergo a microbiological swab sample wound

Either I agree or I don't agree

I agree to undergo the swab for the determination of the wound microbiome

Either I agree or I don't agree

If for some reason I wish or need to stop continuing the study, before the final visit, I agree to be contacted by letter or telephone or other means of communication to provide important information about my state of health

Either I agree or I don't agree

I would like to be informed of any results from the tests performed during the research study that could provide me with a concrete and direct benefit

By signing this form I agree:

a) To the processing of my personal data deriving from the study for scientific purposes *by the School of Specialization in Thermal Medicine, Faculty of Medicine and Surgery of the Sapienza University of Rome, Policlinico Umberto I.
(*mandatory consent for study enrollment)

b) To the transfer of my personal data, for scientific-statistical purposes to an Excel database, associating a numerical or alpha-numeric code, with my name and surname*
(*mandatory consent for study enrollment)

c) To consent to photographic taking during the execution of the medical treatment that will be carried out on my person, which will not include identifying elements of my person (face, facial features, etc.) and therefore will completely guarantee the anonymity of my identity.
(*mandatory consent for study enrollment)

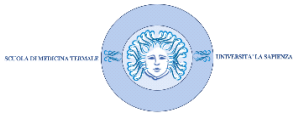
d) To give my specific consent to the processing of my personal data relating to the use of photographs depicting parts of my body during medical treatment for reasons of scientific dissemination (scientific publication or national and international congresses)
(**consent not mandatory for study enrollment)

e) Not to raise exceptions against the publication of any photographs or audio-video footage for the purposes described above (scientific dissemination). The photographs indicated above and/or the publication of articles, photos or audiovisual footage will not entitle you to any compensation.
(*mandatory consent for study enrollment if consent is provided d))

I understand that at any time and without giving any reason I can withdraw my consent and stop participating in the study and that this will not affect the treatment and care I will receive in the future.

Patient's name and surname in block letters

Place and Date of Signature



Informed consent 20/10/2024

Signature of the **doctor** collecting consent

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