PNKCELULITIS STUDY

Pilot study for ultrasound evaluation of structural changes in subcutaneous tissue in patients with cellulite after a multidisciplinary anti-cellulite treatment

(PnKcelulitis program®)

STUDY PROTOCOL

Protocol Code: PNK-CEL-2016-06

(Version 1.0 dated 05 May, 2016)

Principal investigator:

Dr. Esther Roe

Department of Dermatology.

Hospital de la Santa Creu i Sant Pau

1 ABSTRACT

1.1 Type of study

A pilot, prospective, single blind, no controlled study with ultrasound evaluation of skin in patients undergoing treatment with a multidisciplinary program that includes diet, physical exercise and cosmetic cream.

1.2 Sponsor's identification

PNKDIET, S.L.U.

C/ Roger de Llúria, 58.

08009 Barcelona

Tel: +34 934 877 589.

1.3 Study title

Pilot study for ultrasound evaluation of structural changes in subcutaneous tissue in patients with cellulite after a multidisciplinary anti-cellulite treatment (PnKcelulitis program). PNKCELULITIS Study

1.4 Protocol Code:

PNK-CEL-2016-06

1.5 Principal investigator:

Dr. Esther Roe

Department of Dermatology.

Hospital de la Santa Creu i Sant Pau

1.6 Research collaborator

Dr. Esther Serra Baldrich

Department of Dermatology.

Hospital de la Santa Creu i Sant Pau

1.7 Study location

Department of Dermatology.

Hospital de la Santa Creu i Sant Pau

Barcelona (Spain)

1.8 Study design

Prospective, no controlled pilot study on the structural changes in subcutaneous tissue observed by skin ultrasound, in patients with edematous fibrosclerotic panniculopathy (cellulite) after a multidisciplinary treatment program including diet, physical exercise and cosmetic cream.

1.9 Primary objective

To evaluate the structural changes (trabecular texture) in subcutaneous tissue detected by skin ultrasound in patients with cellulite after a multidisciplinary treatment that includes diet, physical exercise and cosmetic cream.

1.10 Secondary objectives

- To evaluate changes in skin thickness assessed by skin ultrasound after multidisciplinary treatment.
- To evaluate the changes in cellulite severity in the involved areas after the multidisciplinary treatment from photographic images.

1.11 Disease in study

Non-infectious skin cellulite or edematous fibrosclerotic panniculopathy

1.12 Therapy in study

Multidisciplinary program including diet, physical exercise and cosmetic cream (PnKCelulitis® program)

1.13 Primary Outcome Measure

Differences in trabecular structure of subcutaneous tissue between prior to starting multidisciplinary treatment and after the end of this treatment, assessed by skin ultrasound.

1.14 Study population and number of subjects

Women with non-infectious skin cellulite (edematous fibrosclerotic panniculopathy), submitted to the PnKCelulitis® multidisciplinary treatment program including diet, physical exercise and cosmetic cream.

The number of patients expected to be included is 30.

1.15 Follow-up and duration of the study

Patient follow-up will be 10 ± 1 weeks to 18 ± 1 weeks, depending of treatment duration because of the degree of cellulite.

2 INDEX

3 GENERAL INFORMATION

Study title	Pilot study for ultrasound evaluation of structural changes in subcutaneous tissue in patients with cellulite after a multidisciplinary anti-cellulite treatment (PnKcelulitis® program). PROCESS study
Protocol Code:	PNK-CEL-2016-06
Type of study	A pilot, prospective, single blind, no controlled study with ultrasound evaluation of skin in patients undergoing treatment with a multidisciplinary program that includes diet, physical exercise and cosmetic cream.
Therapy in study	Multidisciplinary program including diet, protein supplements, physical exercise and cosmetic cream (PnKCelulitis® program)
Sponsor's details	PNKDIET, S.L.U. C/ Roger de Llúria, 58. 08009 Barcelona Tel: +34 934 877 589.
Principal investigator:	Dr. Esther Roe Department of Dermatology. Hospital de la Santa Creu i Sant Pau
Research collaboration	Dr. Esther Serra Baldrich Department of Dermatology. Hospital de la Santa Creu i Sant Pau
Primary objective	To evaluate the structural changes (trabecular texture) of subcutaneous tissue detected by skin ultrasound in patients with cellulite after a multidisciplinary treatment that includes diet, physical exercise and cosmetic cream.
Study population	Women with non-infectious skin cellulite (edematous fibrosclerotic panniculopathy), submitted to the PnKCelulitis®multidisciplinary program including diet, physical exercise and cosmetic cream.
No. of subjects	A pilot study is expected to include 30 patients
Follow-up	Patient follow-up will be 10 ± 1 weeks to 18 ± 1 weeks, depending on the degree of cellulite. With two controls: initial and final

4 INTRODUCTION AND RATIONALE OF THE STUDY

Cellulite is a skin disorder that modifies the subcutaneous adipose tissue causing changes in the body shape and skin. It is accompanied by changes in the microcirculation of the connective tissue, causing morphological, histochemical and biochemical modifications to the tissue. It is characterized by the padded and nodular appearance of the skin in sloping areas.

Also known as 'deforming skin panniculitis', 'nodular lipodermatosclerosis', 'gynecological lipodystrophy' or 'edematous fibrosclerosis', among others, it is a disorder that affects 85-98% of women after puberty and is considered a women's secondary sexual feature[1,2].

With a multifactorial etiology, its physiopathology is complex and includes: expansion of the subcutaneous fat, formation of fibrotic septa in the dermis, dermal laxity and atrophy[3].

In addition to the direct effect of hormonal changes; genetic factors ,a family tendency to abnormal fat deposition and poorly developed muscle mass are some of the factors influencing the development of cellulite. Bad eating habits are other non-hormonal risk factors for the development of cellulite. Increased intake of carbohydrates and fats increases hyperinsulinemia which, among other effects, intensifies the lipogenesis process. Also, a poorly balanced diet with a lot of preservatives and salt in food increases fluid retention and edema.[4].

Some additional factors are poor physical exercise and a sedentary lifestyle that disrupt the proper functioning of microcirculation and support the accumulation of adipose tissue[4].

A wide range of therapies have been suggested for the treatment of cellulite, from weight loss (the most frequent), to treatments of skin massage and the use of topical agents and oral supplements. Massages reduce edema and there is also some evidence of increased collagen synthesis after such treatments. Likewise, their benefits might be explained by their likely effects on fibroblast activity stimulation and decreased adipocyte activity. Generally, topical creams contain different agents but have been poorly studied. Xanthines, botanical extracts, fragrances, and retinoid and PPAR receptors ligands seem to provide some results, through the reduction of adipogenesis and an increased thermogenesis that would improve microcirculation and the collagen synthesis. In addition, many oral agents for weight control (hydroxycitrate, epigallocatechin gallate, conjugated linoleic acid, etc.) are being researched and some of these agents, such as conjugated linoleic acid (CLA), seem to improve the signs of cellulite. Regular exercise and a proper diet can help control weight and therefore the appearance of cellulite.[2]

The PnKCelulitis® Program is a new treatment aimed at improving the signs of cellulite by combining different types of therapies. Its multidisciplinary approach includes: diet, physical exercise and the topical application of a cream. The dietary regimen includes a hypocaloric diet and protein supplements. The diet, which in the first few weeks is also ketogenic with a very restricted supply of carbohydrates, is able to increase lipolysis and reduce adipose tissue also improving fluid drainage. The supply of protein

supplements along with physical exercise seeks to increase muscle mass and reverse the underlying flaccidity, while improving microcirculation and mobilizing fat deposits. The treatment is complemented by the application of a cosmetic cream whose components contribute to reduce lipogenesis and improve circulation.[5].

This study is aimed at evaluating the degree of benefit obtained through this new multidisciplinary anti-cellulite treatment, semi-objectively, from the changes observed in the dermis structure and the subcutaneous tissue by means of skin ultrasound[6], as well as the visual changes on skin appearance in the areas involved.

5 STUDY OBJECTIVES

5.1 Primary objective

To evaluate the structural changes (trabecular texture) in subcutaneous tissue detected by skin ultrasound in patients with cellulite after a multidisciplinary treatment that includes diet, physical exercise and cosmetic cream.

5.2 Secondary objectives

- To evaluate changes in skin thickness assessed by skin ultrasound after multidisciplinary treatment.
- To evaluate the changes in cellulite severity in the involved areas after the multidisciplinary treatment from photographic images.

6 STUDY DESIGN

6.1 Type of study

A pilot, prospective, single blind, no controlled study with ultrasound evaluation of skin in patients undergoing treatment with a multidisciplinary program that includes diet, physical exercise and cosmetic cream. .

6.2 Randomization process

Not applicable

6.3 Type of control

Not applicable.

6.4 Masking Techniques

Both the ultrasound images and the photographic images will be blindly assessed by two independent researchers, who will not know the identity of the patients evaluated.

7 SUBJECT SELECTION

7.1 Study population

Women with non-infectious skin cellulite (edematous fibrosclerotic panniculopathy), submitted to the PnKCelulitis® multidisciplinary treatment program including diet, physical exercise and cosmetic cream.

7.2 Inclusion criteria

- Women aged over 18
- Women who, regardless of their inclusion in this study, will undergo the anti-cellulite multidisciplinary treatment program (PnKCelulitis® program)
- Women who agree to participate and sign the Informed Consent

7.3 Exclusion criteria

- Pregnant or lactating women.
- Women in whom the ketogenic diet is contraindicated and therefore they cannot be on the multidisciplinary program's diet. These contraindications include:
    - Eating disorders, alcoholism, and/or drug addiction.
    - Severe psychological disorders (eg schizophrenia, bipolar disorder, depression ...).
    - Treatment with dicumarinic anticoagulants (Sintrom®) or cortisone.
    - Liver failure.
    - Severe kidney failure (gfr <30).
    - Type 1, and type 2 Diabetes mellitus if insulin dependent.
    - Hemopathies.
    - Cancer.
    - Cardiovascular or cerebrovascular diseases.
    - Acute attack of gout.
    - Nephrolithiasis verified by ultrasound.
    - Cholelithiasis verified by ultrasound.
    - Electrolyte imbalance, according to medical criteria.
    - Orthostatic hypotension.

7.4 Number of subjects

The number of subjects expected for this pilot study is 30 women.

7.5 Rationale of sample size

In an exploratory pilot study to test a concept, it has been estimated that 30 patients will be sufficient to analyze results.

Being a pilot study, it is understood that the estimation of the sample size is not necessary.

7.6 Recruitment period

We expect a 1-month recruitment period

7.7 Criteria for withdrawal or dropouts

Dropouts are those women who do not comply with the treatment schedule or do not complete treatment time established in the protocol of the multidisciplinary program.

8 TREATMENT DESCRIPTION

Multidisciplinary program for the treatment of cellulite

The PnKCelulitis® Program consists of a multidisciplinary program that includes diet, protein supplements, physical exercise and anti-cellulite cream. Its duration depends on the degree of cellulite and is structured in 3 parts called "processes" (restructuring process, drainage process and toning process). Each of them consists of:

- Dietary regimen. The dietary regimen during the first part of the program (restructuring process 1 and 2) consists of a ketogenic low calorie diet (800-1200 kcal/day) based on protein, and vitamin and trace element supplement intake. The dietary regimen during the drainage and toning processes is based on a non-ketogenic low calorie diet (1,200 - 1,500 kcal/day) accompanied by protein products and vitamin and trace element supplements.

- Physical exercise. Toning exercises that increase in difficulty and intensity in each process.

- Application of a cosmetic cream. It is a cosmetic cream consisting of: red coralline algae, caffeine, red vine L-carnitine, and phosphatidylcholine.

According to the PnKCelulitis® program, the duration of the multidisciplinary treatment will vary between 61 days and 110 days, depending on the degree of cellulite assessed by the doctor who prescribes said treatment, according to the following scheme:

	Restructuring		Drainage	Toning
	1	2		
GRADE 1	7 days	14 days	20 days	20 days
GRADE 2	14 days	7 days	30 days	30 days
GRADE 3	20 days	20 days	40 days	30 days

Multidisciplinary treatment will be prescribed and controlled independently of this study by the prescribing physician as well as by the physical trainer and a team of dietitians from the Pronokal Group® Center. The doctor will be in charge to refer the patients to the Dermatology Service of the Santa Creu i Sant Pau Hospital to propose their participation.

9 STUDY MEASUREMENTS AND ENDPOINTS

9.1 Study endpoints

9.1.1 Primary endpoint

Differences in trabecular structure of subcutaneous tissue between the time prior to the multidisciplinary treatment and after the end of the treatment, assessed by skin ultrasound:

- Indented subcutaneous fascicles in the dermis (length: measured from the center of the base to the point of maximum indentation in the dermis)

9.1.2 Secondary endpoints

- Changes in other skin structural parameters between the time prior to the multidisciplinary treatment and after the end of said treatment, assessed by skin ultrasound:
  - Thickness of skin: thickness of dermis, thickness of epidermis and total thickness of the skin
  - Changes in the echogenicity of the dermis
  - Presence of dermal edema
  - Presence of nodules in the subcutaneous areolar tissue

- Changes in cellulite severity in the areas involved, between the time prior to the multidisciplinary treatment and after the end of treatment, assessed from photographic images, using the validated photonumeric scale of Hexel et al. [7], which evaluates the following parameters:
  - Number of visible depressions (0 = none, 1 = 1 to 4; 2 = 5 to 9; 3 = 10 or more depressions)
  - Depth of depressions (0 = No depressions; 1 = superficial depressions, 2 = medium depth depressions, 3 = deep depressions)
  - Morphology of the epidermis (0 = flat, 1 = "orange skin" look; 2 = "cottage cheese" look; 3 = quilted look
  - Grade of laxity or sagging (0 = absence of laxity/sagging; 1 = slight; 2 = moderate; 3 = severe)
  - Severity in accordance with Nürnberger and Muller scale(0 = zero grade; 1 = first grade; 2 = second grade; 3 = third grade)

  The addition of the scores obtained will provide a final score that will allow the cellulite classification:

  From 1 to 5 = mild cellulite

  From 6 to 10 = moderate cellulite

  From 11 to 15 = severe cellulite

9.2 Study plan and procedures

9.2.1 Recruitment of patients

Patients will be referred to the Dermatology Department of Hospital de la Santa Creu i Sant Pau, from the PronoKal Group® Center by the doctor who prescribes it regularly.

During the consultation, the dermatologist will explain the study to the patient and propose their participation. The patient will be provided with the information sheet and will be asked to sign the informed consent.

9.2.2 Follow-up of patients

Patient follow-up will be 10 ± 1 weeks to18 ± 1 weeks, depending on duration of treatment depending on the degree of cellulite.

Only two controls will be performed: initial (prior to the multidisciplinary treatment) and final (after the end of the multidisciplinary treatment).

Initial visit

The inclusion and exclusion criteria will be assessed and the signed informed consent will be obtained. Demographic data (age) are recorded and the degree of cellulite is evaluated following the Nürnberger and Muller scale (0 = zero grade, 1 = first grade, 2 = second grade, 3 = third grade). The dermatologist will perform skin ultrasound and will take pictures.

Final visit

The dermatologist evaluations will be repeated: evaluation of the cellulite grade, accomplishment of skin ultrasound and photographic images.

9.2.3 Procedure for taking photographs

Photographs of the gluteal area (posterior) and hips (right and left), with the patient standing upright, 50 cm apart using a tripod, with an amplitude of focus from the iliac crest to the knee, always in the same place and the same lighting conditions.

Patients should wear disposable paper underwear, exposing the largest possible gluteal surface. Photographs will not include tattoos or other types of marks that identify the patient. Digital images will be saved and identified with the patient code.

The images will be assessed subsequently using the validated photonumeric scale, by two independent evaluators, blindly, that is, with a new coding that prevents them from identifying the patient or the visit to which it corresponds. The final result will be the average between both evaluations.

9.2.4 Procedure for the assessment of skin ultrasound

Ultrasound changes will be assessed at the end of the study. The ultrasound images will be saved with the patient and the visit code. At the end of the study, ultrasound changes will be assessed by two evaluators, independently and blindly. The final result will be the average between both evaluations.

The parameters to be controlled are:

- Dermis thickness
- Hypodermis thickness
- Total skin thickness
- Dermis echogenicity
- Assess presence of dermal edema
- Presence of nodules in areolar subcutaneous tissue

- Indented subcutaneous fascicles in the dermis (length: measured from the center of the base to the point of maximum indentation in dermis)
- Presence and quantity of perpendicular fibrous septa

10 STATISTICAL ANALYSIS

10.1 Descriptive statistics

Descriptive statistic of all the variables collected in the Data Collection Notebook, with frequency tables for nominal variables and measures of central tendency and dispersion for the continuous variables. 95% confidence intervals (95% CI) will be estimated for the latter.

10.2 Analysis of objectives

For the analysis of the primary objective, we will study the mean values of the trabecular structure and skin thickness, comparing the results before and after treatment. Similarly we will compare the scores of the photographic images (overall score and scores of each scale). Qualitative variables will be compared using Fisher's exact test, and the quantitative variables will be compared by means of the student's t test (in the case of 2 groups) or by the one-way ANOVA test (for 3 or more groups). If the conditions of the Student t test or the Anova test are not met, we will use the non-parametric test of the Mann-Withney U (2 groups) or the Kruskall-Wallis test (for 3 or more groups).

For all tests, the significance level will be set at $p < 0.05$.

10.3 Responsibility for statistical analysis

The CRO responsible for the statistical analysis of data will be:

CROSSDATA – Punta Alta
Enric Granados 145, 2º-1º
08008Barcelona
Tel.: +34 935 177 609.

11 REPORT OF ADVERSE EVENTS

The clinical evaluation of the subjects and safety of the multidisciplinary treatment is not the purpose of this study. Patients will only be visited before and after treatment. Therefore, no intermediate control will be performed to assess the safety and tolerability of the multidisciplinary anti-cellulite treatment during its application.

However, if patients report any adverse effects to the anti-cellulite treatment, the doctor will record it in the Data Collection Notebook, specifying:

- Type of reaction

- Intensity: mild (no limitation of daily living activities, patient may have mild discomfort), moderate (cause some limitation of daily living activities, patient may experience increased discomfort), severe (inability to perform daily living activities; patient may report intolerable pain or discomfort).
- Duration
- Causality with the DHA dietary supplement treatment under study: definitive, likely, possible, unlikely, unrelated, unknown.
- Therapy administered
- Resolution

If a subject reports a serious adverse event (see sidebar) during the time of this study, the physician should gather all available data and report them immediately (if possible within 24h.) by fax, telephone or e-mail to the Sponsor, who will implement the established Pharmacovigilance procedures in accordance with current legislation.

SERIOUS ADVERSE REACTION: is any adverse reaction that can be classified in one or more of the following categories:
- Lethal
- Life threatening
- It causes significant or persistent discapacity/ disability
- It causes hospitalization or prolongs hospitalization

Congenital anomalies/birth defects and serious adverse clinical consequences associated with use under conditions other than those set out in the Summary of Product Characteristics (SPC), overdose or abuse are also included.
Medical judgment should be applied when deciding whether an event or reaction is serious in other situations. Major adverse reactions or events that do not pose immediate threat to life, or do not cause death or hospitalization but may endanger the patient, should be deemed serious.

Sponsor:

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12 ETHICAL ISSUES

12.1 General considerations

This study will be conducted in accordance with current regulations, international accepted ethical standards of Good Clinical Practice (CPMP/ICH/135/95) and the principles established in the latest version of the Declaration of Helsinki.

12.2 Evaluation by an Authorized Clinical Research Ethics Committee

Prior to its initiation, this study will be submitted to the Clinical Research Ethics Committee (CREC) of the Foundation of Health Management of the Santa Creu i Sant Pau Hospital to obtain its approval.

12.3 Patient information and informed consent

The patient will be informed about the study issues for their knowledge and written informed consent will be requested for participation in the study (Attachments 2 and 3).

12.4 Data confidentiality

Patient's identity in the study forms and in the digital files of the ultrasound and photographic images will be coded. The investigating physician will maintain a separate confidential record linking the identification codes with the patients' names.

13 PRACTICAL CONSIDERATIONS

13.1 Workplan

The principal investigator will be in charge of including the patients referred to his office. Once the patient has read the Patient Information Sheet (Attachment 2) and confirmed their willingness to participate, the investigator will obtain the signature of the Informed Consent Form (Attachment 3) and must keep them with the rest of the documentation of every subject.

The doctor will record the data requested in the CRD and will perform the skin ultrasound and the taking of photographs. The ultrasound images will be saved digitally, identified by the patient and the visit code; and will be saved to be evaluated at the end of the study, blindly, by the principal investigator and by another evaluator separately. Similarly, the photographs will be saved in digital files identified with the patient and visit code. The latter will be recoded before evaluation by two independent evaluators to ensure the blindness of the patient and the visit in which the photographs were taken.

The investigator will not perform any clinical follow-up of the patients during the multidisciplinary medical treatment and will only visit the patients prior to and after the anti-cellulite program.

13.2 Monitoring

CROSSDATA (CRO) will carry out remote monitoring of all patients, with follow-up reports of all of the study visits. If necessary, there will be on-site monitoring visits at the center.

13.3 Financing the study

The Sponsor will be in charge of the study expenses.

13.4 Disseminating the study results

The overall data will be used by the whole project, not individually.

With the results obtained, we will issue the final study report, which will be deemed confidential.

With the overall data we will try to maximize the exploitation of the study results in the form of publications or reports in conferences, always mentioning the study.

14 REFERENCES

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