

# Comparative, prospective, and double-blind study of transdermal products that claim to produce CO<sub>2</sub>

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## Abstract

Numerous cosmeceutical products claim to produce CO<sub>2</sub> or transcutaneous carboxytherapy.

Transcutaneous carboxytherapy produces a positive action by increasing microcirculation and stimulating the extracellular matrix.

Many products are sold over the counter and on social networks without scientific studies to support their claims.

We have conducted a prospective, comparative, double-blind study comparing four products that claim to produce transdermal CO<sub>2</sub>. Only one of them had a scientific research study to support the action claimed.

Volunteers used all four products, and two independent dermatologists checked the action through videodermoscopies.

The results clearly show that the products that had scientific support from previous research produce transdermal CO<sub>2</sub>, the therapeutic action claimed.

**Carboxytherapy** consists of the therapeutic use of carbon dioxide (CO<sub>2</sub>) in its gaseous state by subcutaneous injection. (1)

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CO<sub>2</sub> immediately diffuses at the cutaneous and muscular microcirculatory level when administered subcutaneously.

CO<sub>2</sub> increases vascular tone and produces active microcirculatory vasodilatation at the vascular level.

CO<sub>2</sub>-induced vasodilatation results from its direct activity on arteriole smooth muscle cells. (2)

CO<sub>2</sub> promotes the Bohr effect, where the affinity of the hemoglobin for oxygen decreases due to an increase in carbon dioxide, which means that extra oxygen is realized in tissues that need it the most. (3)

The Bohr effect refers to the shift in the oxygen dissociation curve caused by changes in the concentration of carbon dioxide.

The Bohr effect facilitates its release in the tissues, resulting in higher tissue oxygenation and angiogenesis. (4)

Hemoglobin has 20 times more affinity for CO<sub>2</sub> than O<sub>2</sub>. When we inject CO<sub>2</sub>, the hemoglobin catches the CO<sub>2</sub>, releasing oxygen and increasing its partial O<sub>2</sub> pressure in the area.

The extra oxygen produces stimulation of the fibroblast and an increase in ECM (extracellular matrix) quality.

CO<sub>2</sub> can be used alone as a base treatment to help regeneration or in combination with other procedures to synergize the outcomes. (5)

**Transdermal Carboxytherapy** is a new treatment option that provides carbon dioxide through the skin's superficial layers. (6)

*Sakai et al. "Transcutaneous Application of Carbon Dioxide causing Artificial Bohr effect" reported that transcutaneous CO<sub>2</sub> was beneficial for therapeutic purposes via increased blood flow and microcirculation as evaluated by laser Doppler and intensification of tcpO<sub>2</sub> in ischemic tissues, providing evidence of the Bohr effect in vivo.*

*Leibaschoff et al. "A Prospective Clinical and Instrumental Study on the Effects of a Transcutaneous Cosmeceutical Gel that Claimed to Produce CO<sub>2</sub>." (7) The video capillaroscopy evaluated the effect of transdermal CO<sub>2</sub> gel and observed the microcirculation improvement, equivalent to the increase in microcirculation observed after subcutaneous CO<sub>2</sub> injection.*

Now we want to compare the effects of four gels that claim to produce transdermal carboxytherapy.

Study Protocol

The study is a comparative, prospective, and double-blind study of different transdermal products that claim to produce CO<sub>2</sub> and therefore increase microcirculation.

We compared the effects of the four gels, A, B, C, and D, that claim to produce transdermal carboxytherapy.

Gel A confirmed that outcome by research studies; the other three, Gels B, C, and D, were bought on the internet, and they do not show any scientific support for their claims.

Twelve volunteers used all four products, and two independent dermatologists checked the action through videodermatoscopes.

Dr. Gustavo Leibaschoff, director of the study, was the only one that knew the source of the four gels, ingredients, and references.

The 12 volunteers knew the letters of the gels but not the ingredients or the source.

Independent physicians performing videodermatoscopes before and after the study did not know the gel's letters or ingredients.

Twelve volunteer patients were selected.

48 hours before the study started, patients received a videodermatoscopes of both earlobes with photographic records for later comparison.

Five average pictures per earlobe were taken at the beginning and end of the study, and 200 photos were taken during the entire study.

After the first round of videodermatoscopes, volunteers gathered in a different room and were randomly divided into groups 1, 2, and 3, with 4 patients in each.

All patients received the indications written and orally to use the gels at home and in the earlobes.

All patients received Gel A.

Gel B was given to group 1, Gel C to group 2, and Gel D to group 3.

### **Indications**

The patients should use Gel A in their right or left earlobe. They must apply and leave it for 45 minutes and continue using it in the chosen lobe for five days. For control purposes, they must send a picture each day with the gel in place.

For the remaining gel (B, C, or D, according to the group), they must use and leave the gel for 25 to 45 minutes in the other earlobe for five days, according to the instructions of each product. For control purposes, they must send a picture each day with the gel in place on the right and left earlobe.

After five days of applications ended, we waited for 24 hrs.; then, patients returned to the office and received another videodermatoscopes, in the same way as at the beginning of the study, taking pictures of both earlobes. The average ambient temperature of the image captured at the beginning and completion was  $23.04 \pm 0.7$  °c.

## Instruments

**Videodermatoscopes** is a non-invasive technique that allows in vivo, magnified ( $\times 10$ ) observation of skin details and structures not visible to the naked eye. It may be performed using handheld devices, computer-assisted digital systems, or videodermatoscopes. (8) The handheld dermoscopies is the most used device because it is user-friendly and relatively inexpensive.

It is not a mere magnifying glass but a more complex instrument that allows the visualization of the cutaneous microstructures of the different skin layers.

Videodermatoscopes has been demonstrated to be helpful in a wide variety of cutaneous disorders, including ectoparasitic infestations, cutaneous/mucosal infections, hair and nail abnormalities, psoriasis, and other dermatologic, as well as cosmetic, conditions.

Depending on the skin disorder, videodermatoscopes may be helpful in differential diagnosis, prognostic evaluation, and monitoring response to treatment. Nowadays, it represents an essential and relatively simple aid in daily clinical practice. (9)



The mission of a dermoscopes is to bring the skin into the picture brilliantly and make medical photography high-performing, consistent, and timesaving.

It is the combination of state-of-the-art technology with intuitive software, reliable hardware, and the best available camera.

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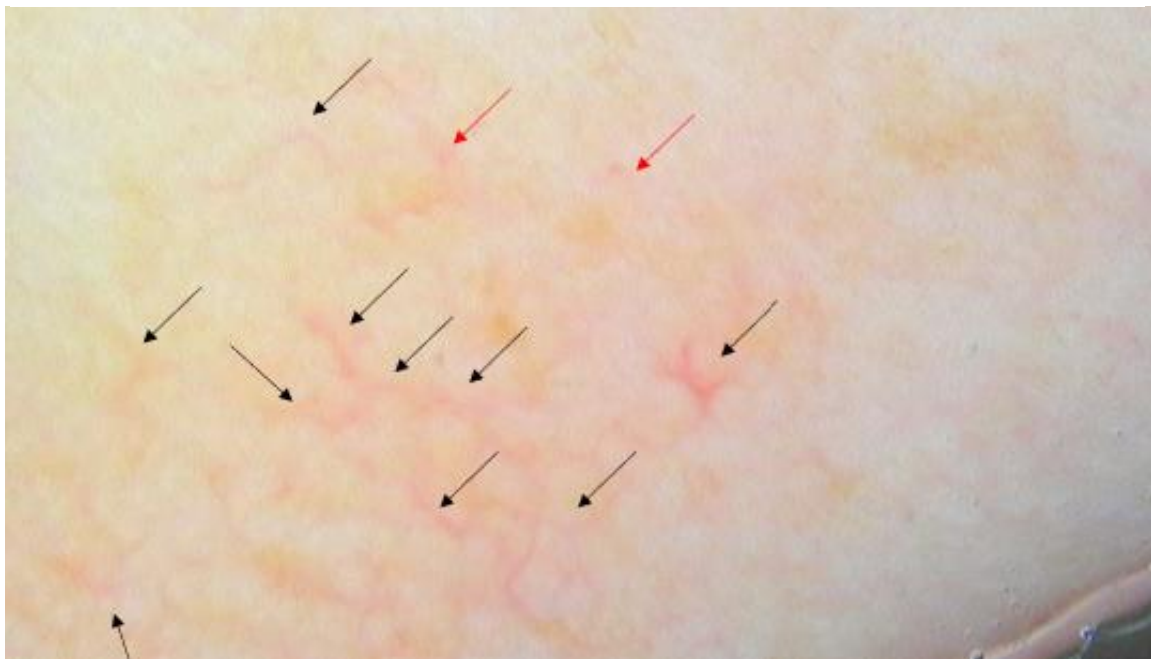


Equipment used videodermatoscopes with a 20-immersion lens (Fotofinder Leviacame, Germany). With up to 13 Mega Pixels resolution, a large field of view, and an ergonomic form, Leviacam combines compact size with outstanding performance. The result: 13 megapixels, 220 grams light.

The integrated Twin Light LEDs allow for immersion and polarized dermoscopies, and the option to switch between modes enables the visualization of different skin lesion structures.

The normal dermoscopies shows the characteristics of visible capillaries on the skin, absence of edema, vascular plexus visible in 10-30% of healthy patients, absent dilated capillaries, absent ramification, absent neoangiogenesis, absent avascular areas, absent hemorrhages, and dynamic capillary flow. (10)

Black arrows horizontal capillaries, red arrow dot capillaries



### Image processing (before and after)

An average of 5 images per earlobe were taken at the beginning and end of the study (200 total pictures during the entire study).

The capture was made in jpeg format with dimensions of 1920\*1032.

And the images analyzed in dimensions of 1920\*1032

Purpose

**Test points-type of study:** Double-blind, observational, prospective, and comparative study

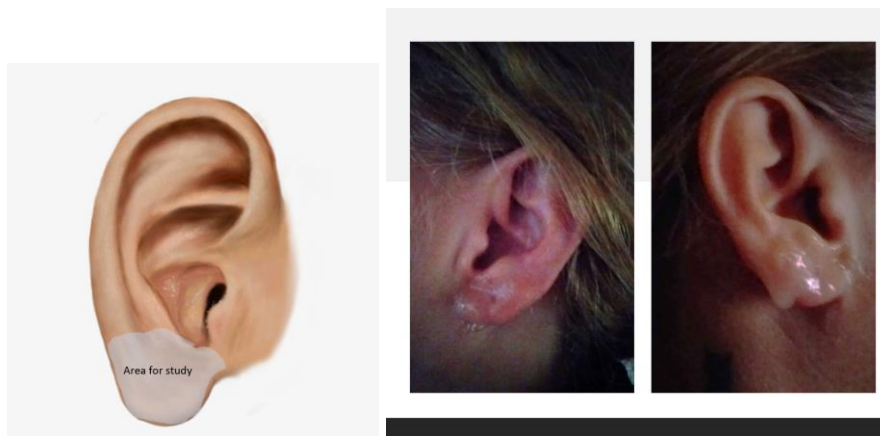
**Epidemiology:** 10 Volunteers females (10 F) complete the whole treatment as specified. According to Fitzpatrick classification, skin type II and III, and the average age was  $38 \pm 11$  years

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### Ear lobe placement areas

Each patient received oral and written instructions to avoid errors.

The gel applications were used by the patient at home. The place of application was the earlobes.



Patients recruited for this study were volunteers from private practice. According to the Helsinki declaration, they completed the informed consent, and written authorization was placed in their medical records.

The skin of the ear lobe is equal to any part of the body. The benefit it presents is that the area is smaller, and the capillaries are detected more efficiently, allowing repeat shots in the same places.

### - Inclusion Criteria

- Adult females and males between the ages of  $38 \pm 11$  years
- Skin type II and III
- Women who are regular users of skin treatment products on the face and hands with no history of allergic reaction or sensitivity to such products
- Individuals in general, good health, with no skin diseases or conditions, such as psoriasis or eczema
- Free of any clinically significant disease

#### Exclusion Criteria

- Women who are currently pregnant or nursing or may potentially be pregnant
- Those taking medications that may affect the study results, including anti-inflammatory agents such as Ibuprofen, Naproxen, Aspirin, etc.
- Those were having a skin treatment procedure on the test areas, such as a chemical peel, tattoo removal, etc., within 30 days of study initiation
- Those exhibiting abnormal skin conditions, such as inflammation, sunburn, or scars in the test sites, which may interfere with the study
- Those who report allergies or sensitivity to skincare products

- Those who have performed wax depilation of the face within 14 days before the baseline
- Those who have excessive sun exposure (greater than 1 hour per day) during the study period

## Methodology:

Transdermal Carboxytherapy is the use of CO<sub>2</sub> through the skin's superficial layer. (11)

## Products:

### Gel A



The non-injectable treatment "CO<sub>2</sub> Lift" manufactured in Japan (Gel A). The product contains two gels: Gel 1, a mixture of Magnesium Carbonate, and Gel 2, a mixture of Gluconolactone.

After mixed and applied to the skin, it provides the same powerful effects on the microcirculation observed when CO<sub>2</sub> is injected into the dermis.

CO<sub>2</sub> Lift is a Japanese patented technology under #[2016-20309](#) issued on Feb. 4, 2016.

This product, which has published scientific studies that support its therapeutic action, was named Gel A.(12)

## Gel B



The package states:

"CO2 Gel Pack by Dr. Select is a beauty salon-grade face mask that you can do in your home. Revolutionary needle-free carboxy therapy helps boost the skin's metabolism and natural production of Oxygen providing deep hydration. This breakthrough therapy generates CO2 bubbles that contain a formula to stimulate skin to restore beauty, energy, and health."

**INGREDIENTS (1):** Glycerin, water, citric acid, xanthan gum, placenta extract, water-soluble proteoglycan,  $\alpha$ -arbutin, Na citrate, pentylene glycol, polyglyceryl laurate-10, BG

**INGREDIENTS (2):** Water, propanediol, tamarind gum, sodium hydrogen carbonate, pentylene glycol, sodium hyaluronate, acetyl tyrosine, auren extract, kudzu root extract, button extract, BG

We have not found any scientific studies on Gel B.

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## Gel C



The prospect states:

Mini Carboxy System CO2 Gel is an innovative skin care system which allows you to enjoy the benefits of Carboxytherapy without needles. It produces CO2 through combining gel and a sheet mask and transfers it to the skin cells through the gel layer. CO2 promotes the rise of Oxygen (O2) supply, which is critical for cell metabolism.

We have not found any scientific studies that support the claims of Gel C.

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## Gel D



Esthetic House Secret 19 CO2 Esthetic Formula Carbonic Mask: the procedure takes place without injections and the use of special equipment, painlessly. Noninvasive carboxytherapy can be used either alone or included as an additional step in professional skin care cosmetic procedures,

which can significantly enhance their effect.

We have not found any scientific studies to support the claims of this product, Gel D.

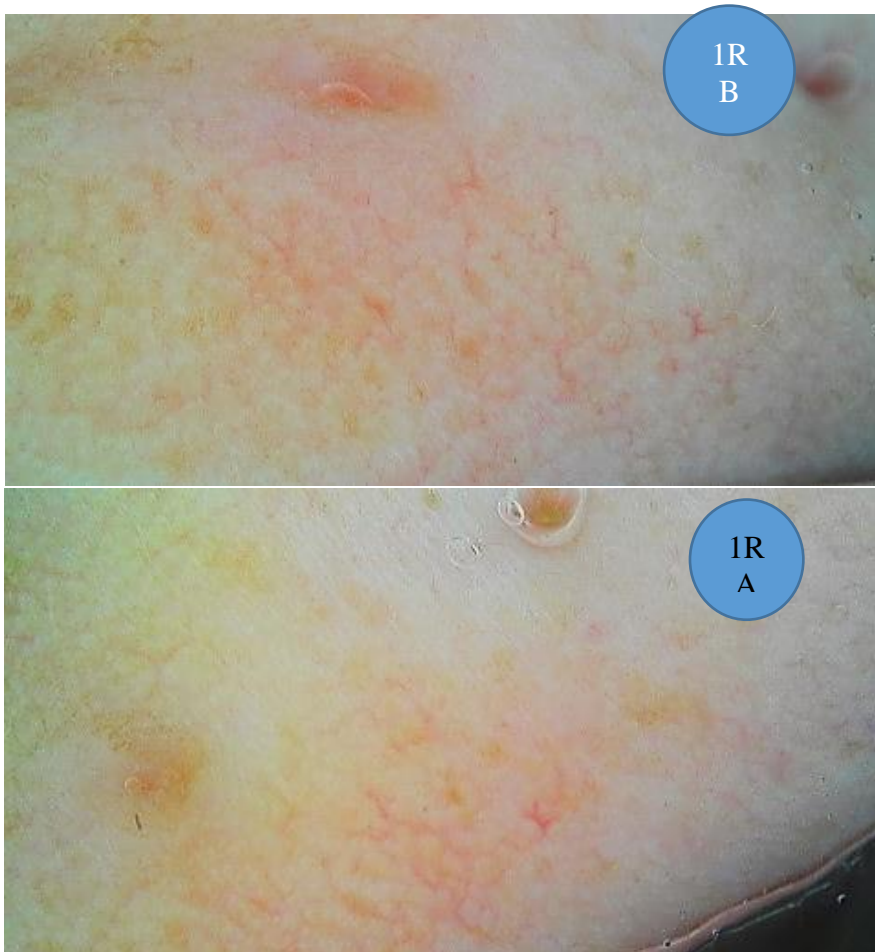
## Results

### **R -Right; L -Left; B -Before; A -After**

#### Patient 1

RB: Basal photo: branched and tortuous clustered capillaries are observed. Absence of angiogenesis.

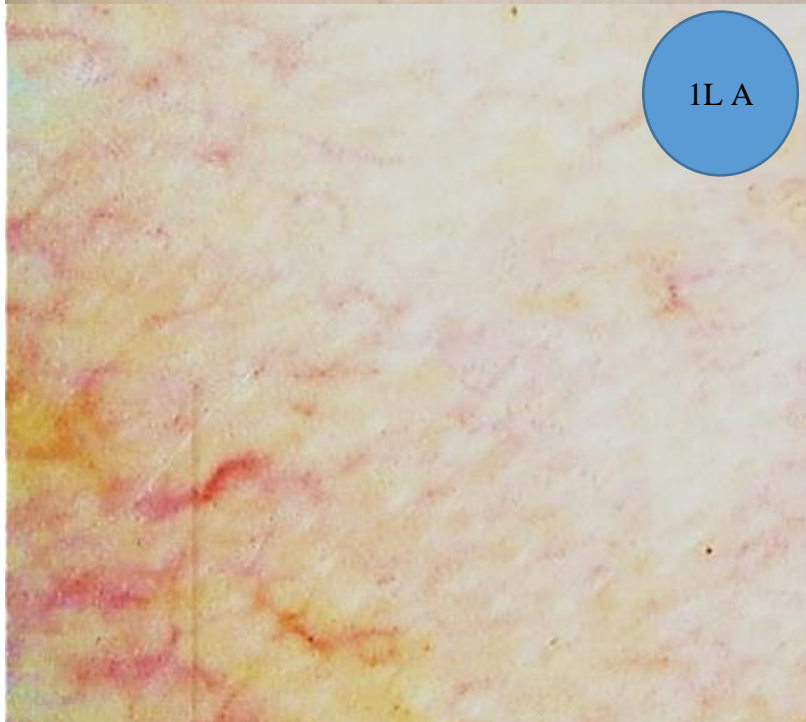
RA: Post-treatment photo: capillary increase and density are observed. Branched, linear, and pointed scattered capillaries. Presence of angiogenesis.



## Patient 1

LB: Basal photo: few capillaries are observed, absence of branched capillaries, absence of angiogenesis.

LA: Post-treatment photo: an increase in the number and density of capillaries is observed. Some branched, tortuous capillaries, others pointed. Increased angiogenesis.

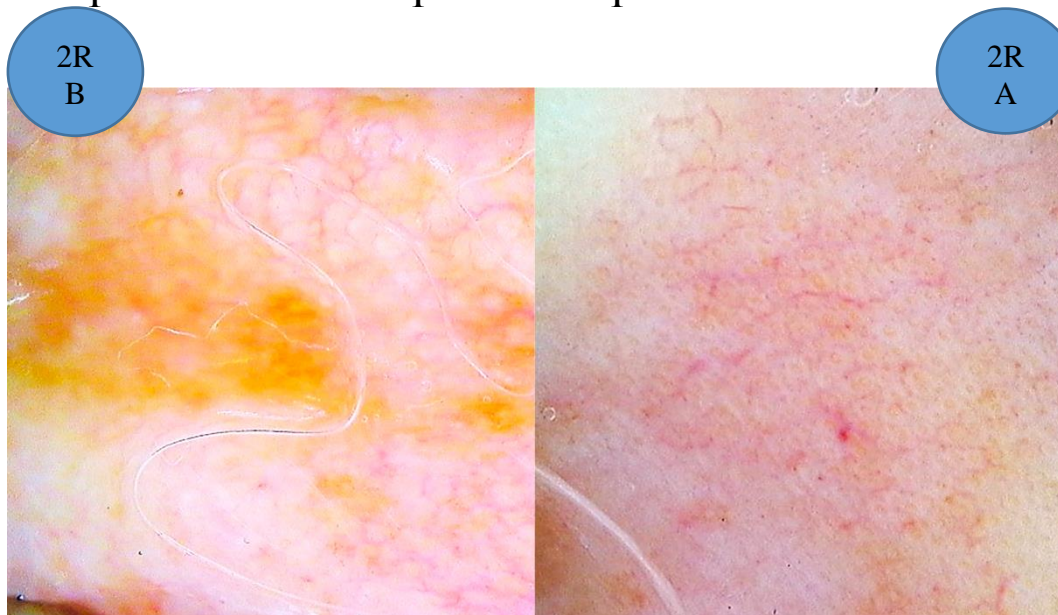


## Patient 2

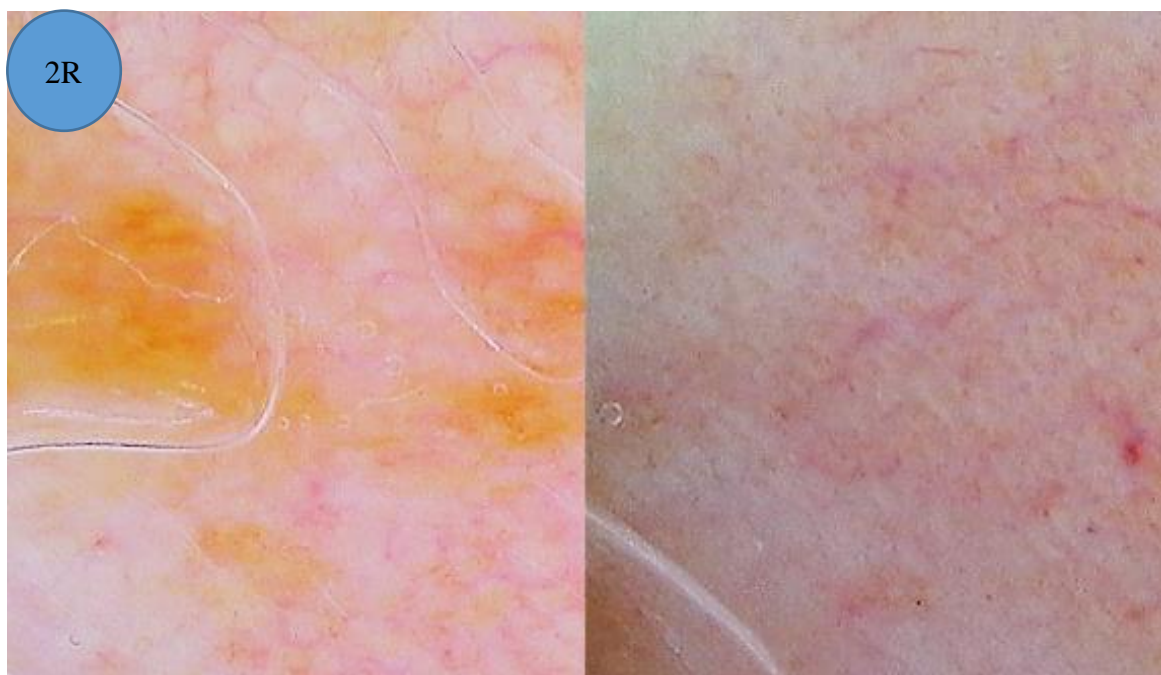
RB: Basal photo: the presence of branched capillaries is observed.

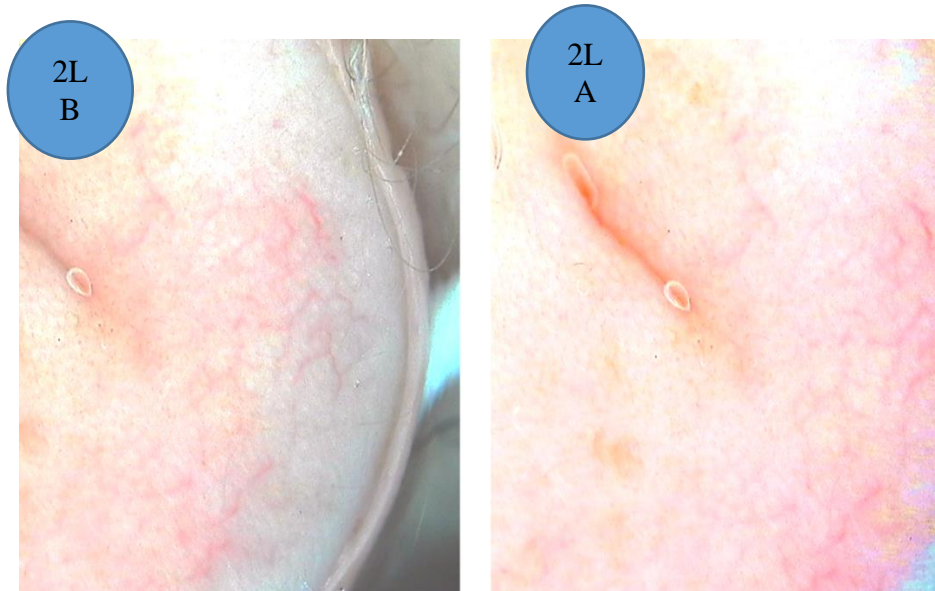
RA: Post-treatment photo: increase in branched capillaries and presence of new pointed capillaries are observed.

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2 R before and after shows greater increase in microcirculation.



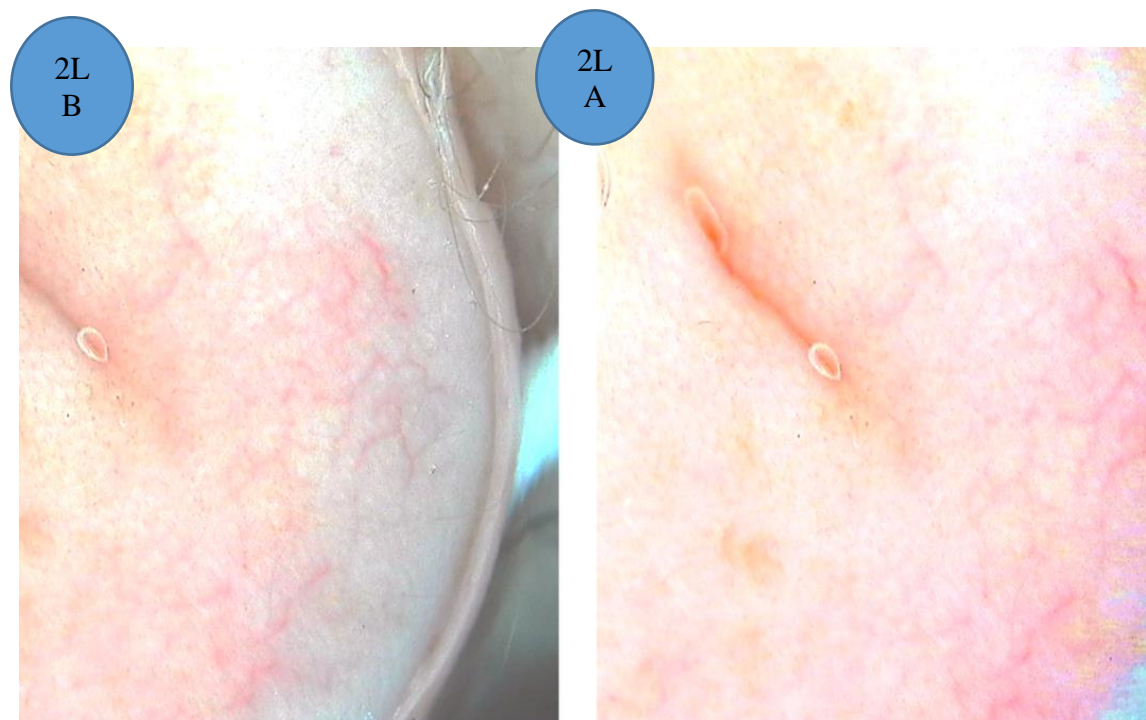


## Patient 2

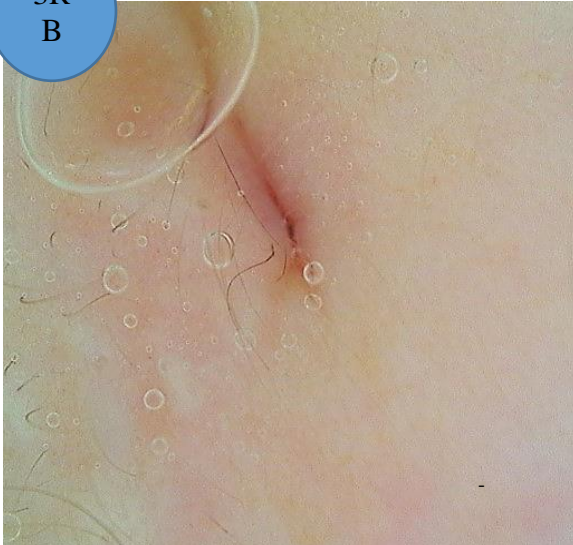
LB: Basal photo: multiple branched and scattered capillaries are observed forming a network.

LA: Post-treatment photo: there is an overall decrease in the number of capillaries, although in sectors there is an increase in the density of the same and dilated capillaries.

2L better increase



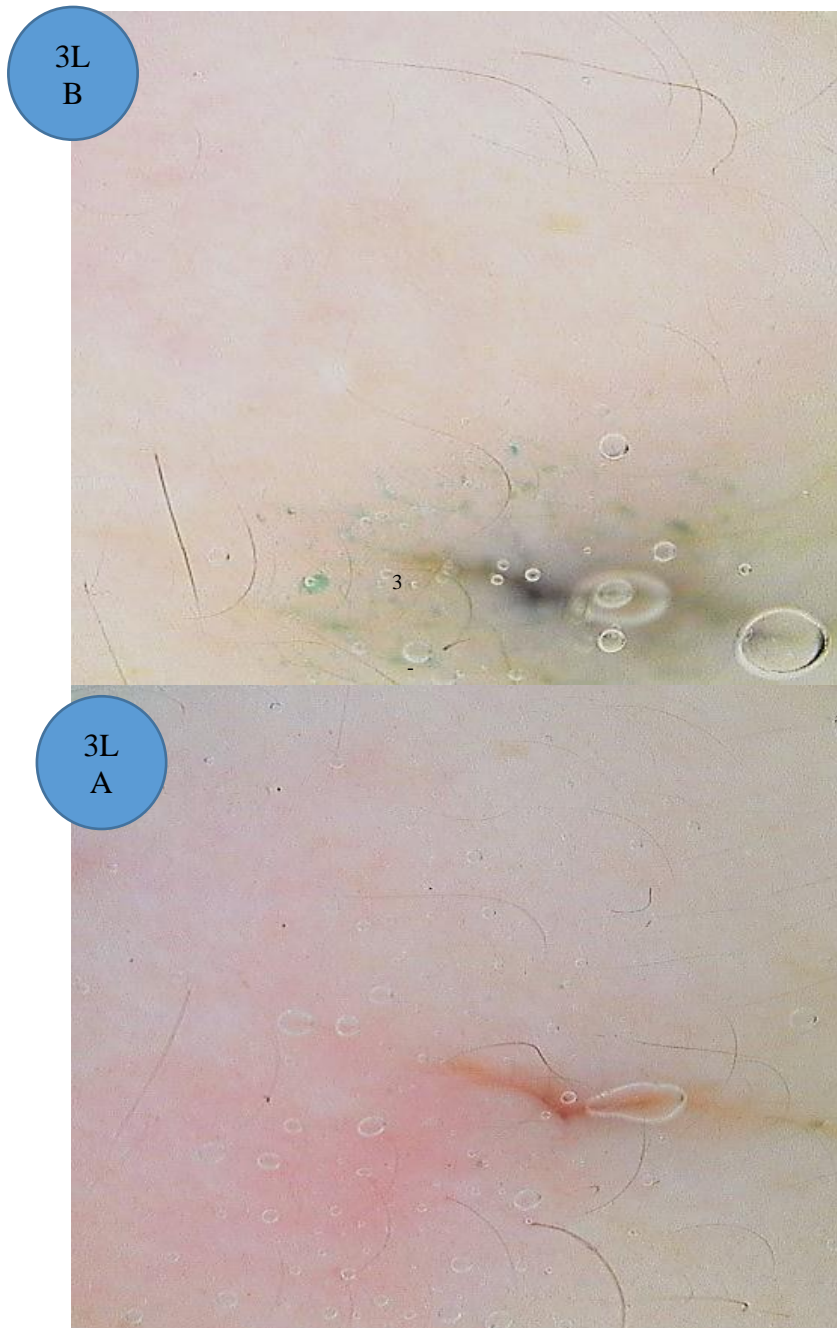
3R  
B



3R  
A



RB: Basal Photo: absence of capillaries observed  
RA: Post-treatment photo: no change from baseline



### Patient 3

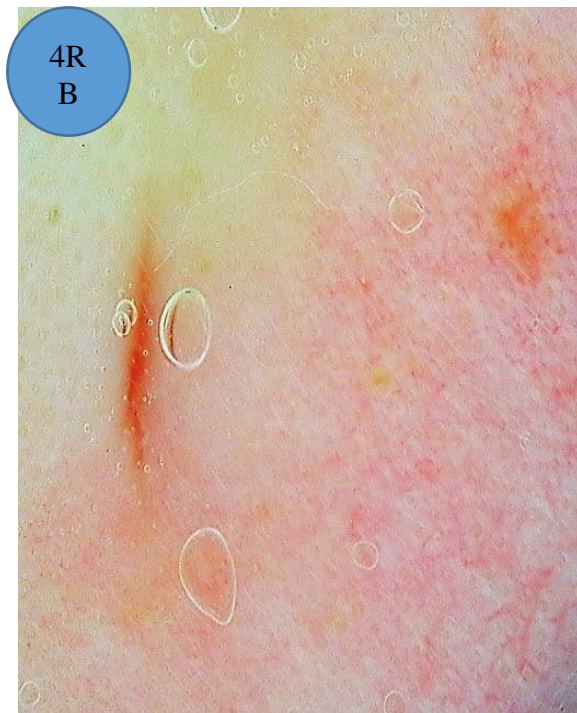
LB: Basal photo: absence of capillaries observed (only one branched capillary is seen).

LA: Post-treatment photo: no change from baseline photo.

## Patient 4

RB: Basal photo: multiple branched capillaries observed.

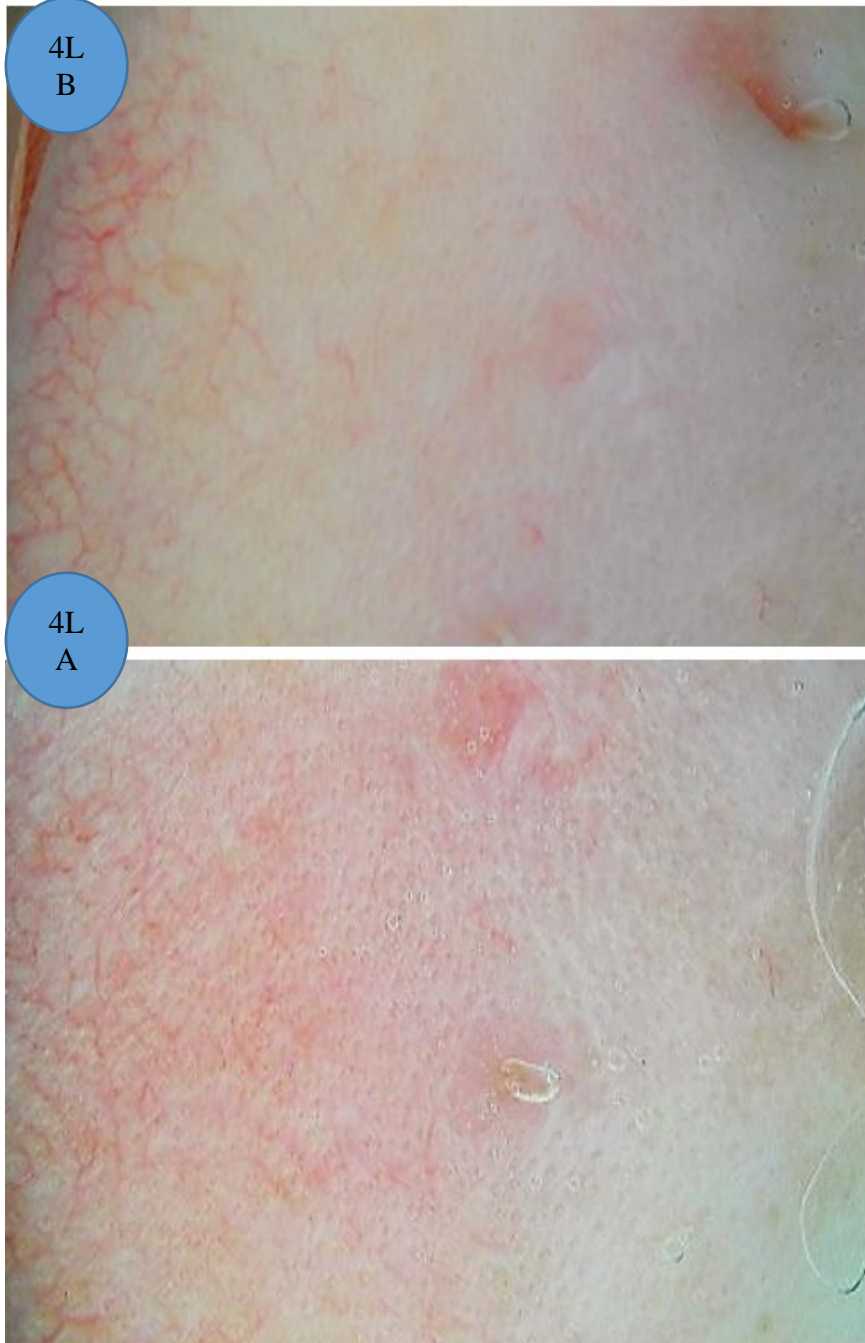
RA: Post-treatment photo: decrease in the number and density of capillaries is observed.



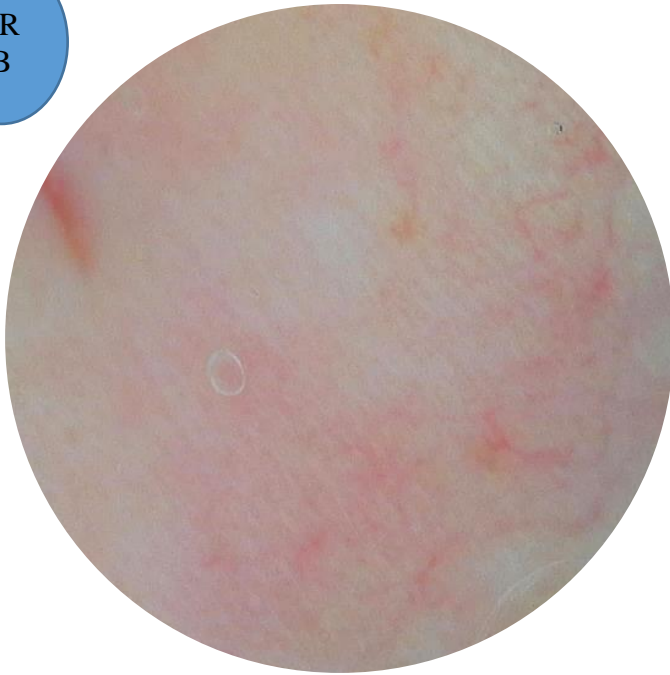
Patient 4:

LB: Basal photo: multiple branched and tortuous capillaries observed

LA: Post-treatment photo: increase in the number and density of capillaries is observed.

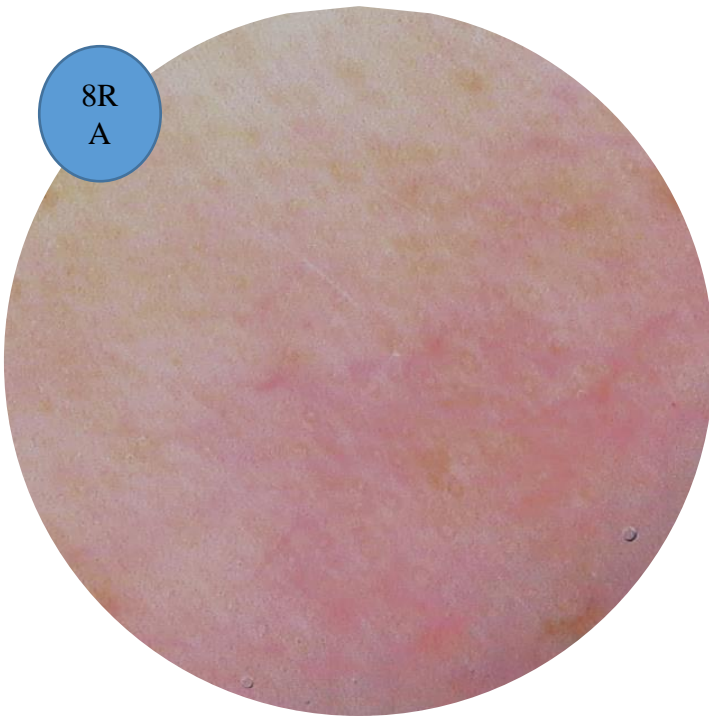


8R  
B



## Patient 8

8R  
A



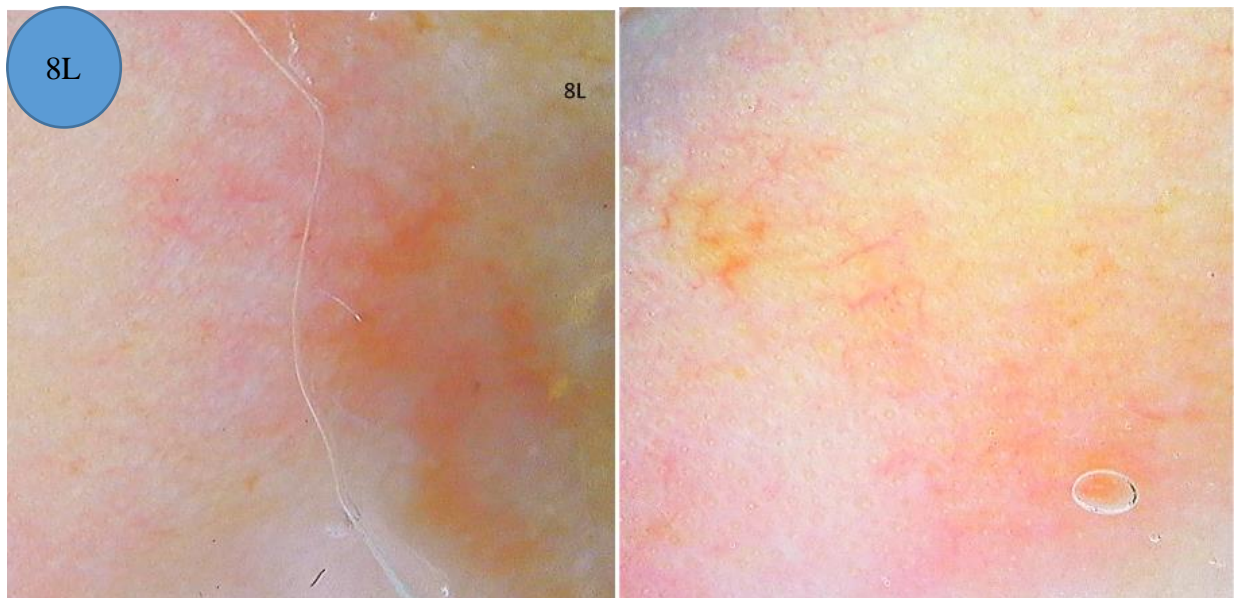
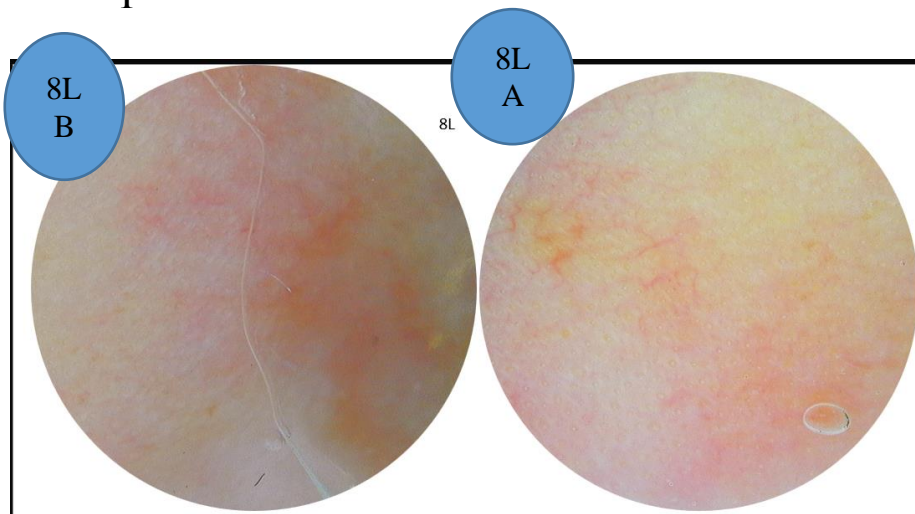
RB: Basal photo: the presence of fine branched capillaries observed.

RA: Post-treatment photo shows the increase in the number and density of branched dilated capillaries.

Patient 8:

LB: Basal photo: little presence of branched capillaries is observed.

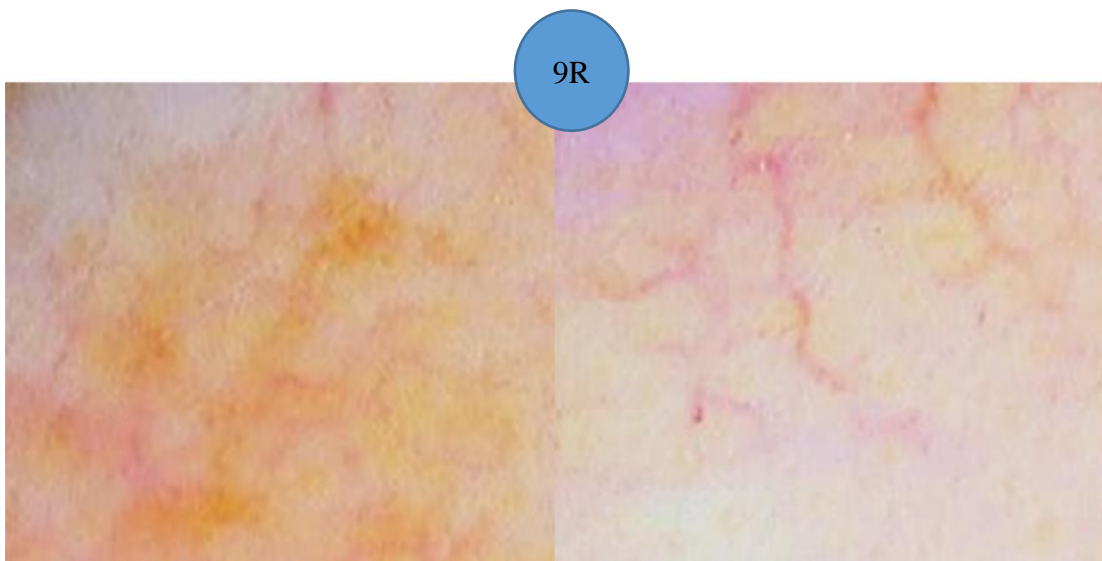
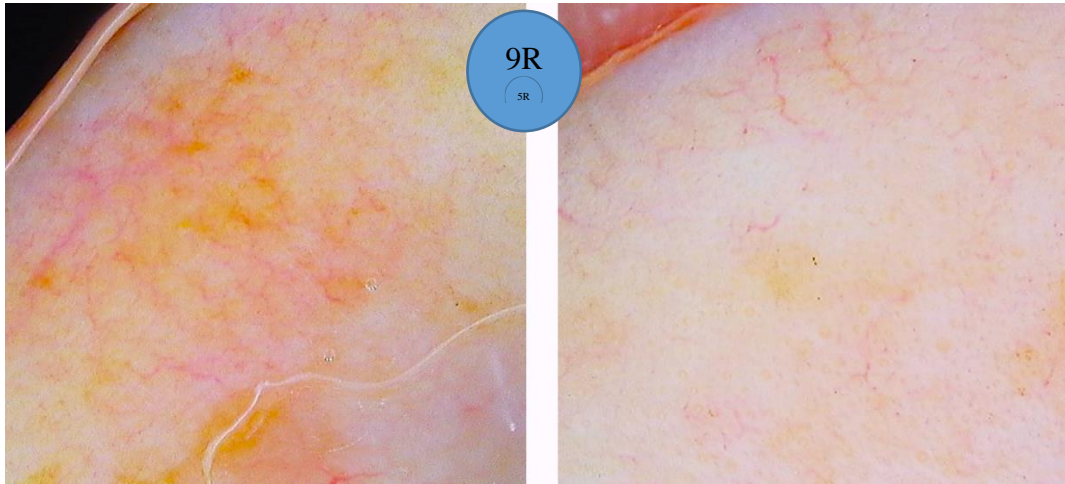
LA: Photo post treatment: no change with respect to the basal photo.



9 R before

after

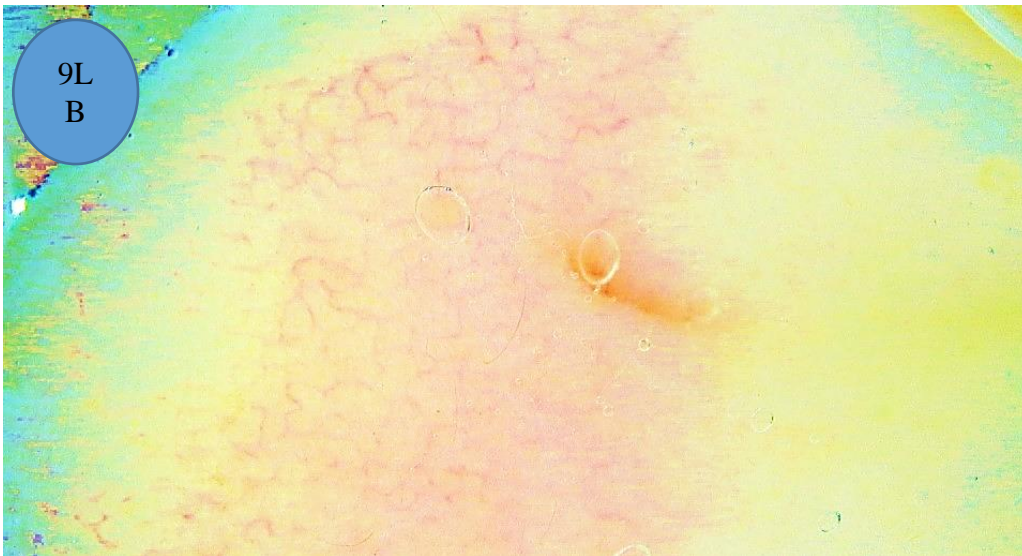
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Patient 9:

RB Basal photo: few branched capillaries observed.

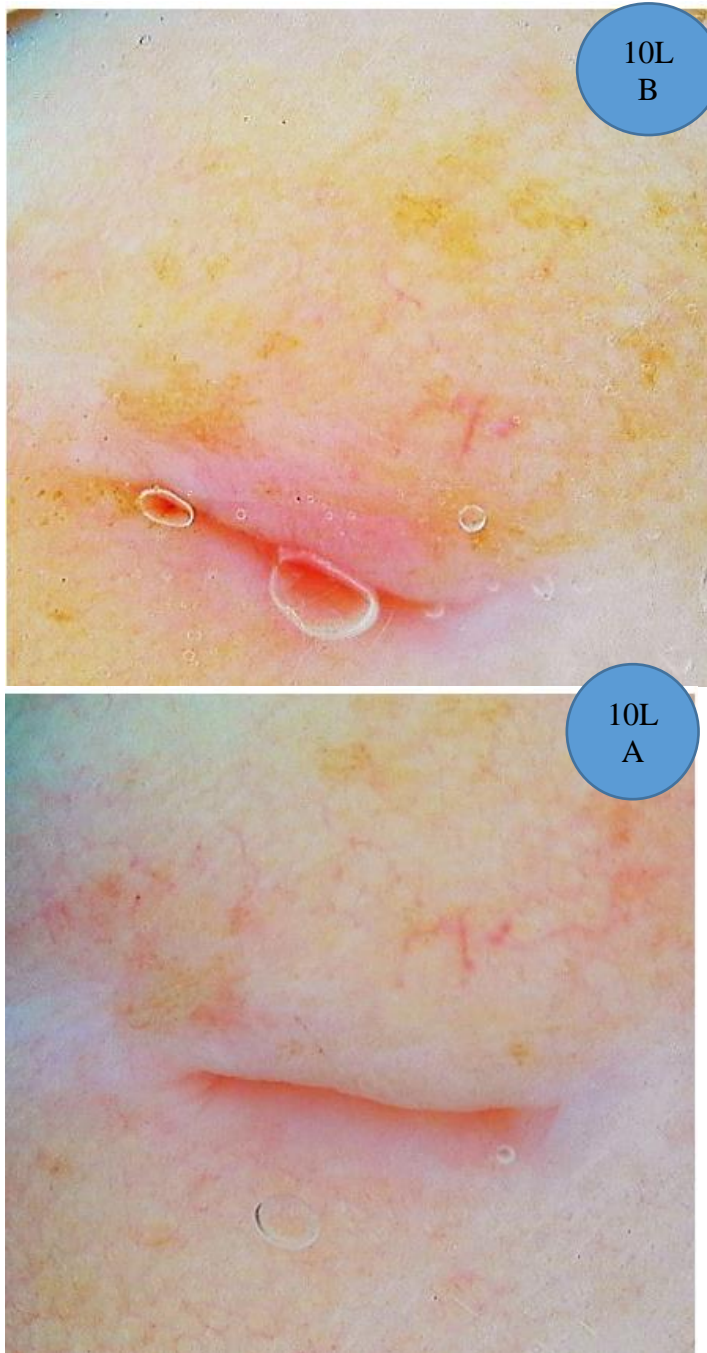
RA Photo post treatment: an increase in the number and density of capillaries is observed.



Patient 9

LB: Basal photo: multiple branched capillaries are observed.

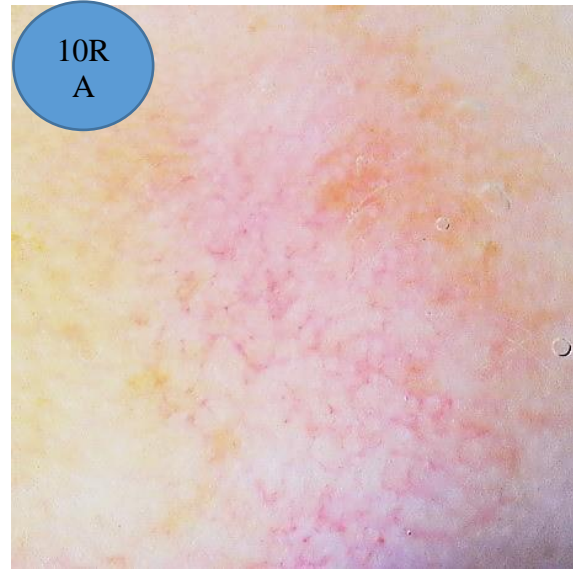
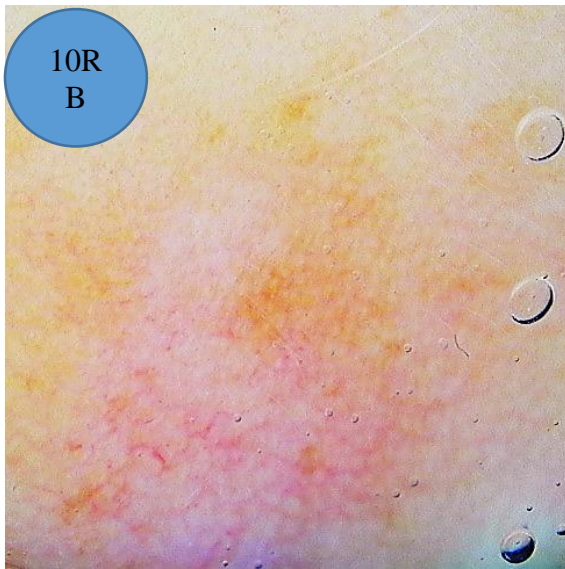
LA: Post-treatment photo: a decrease in the number of capillaries and density is observed, preserving their morphology.



Patient 10:

LB: Basal photo: presence of scarce linear capillaries is observed.

LA: Post-treatment photo: slight increase in the number and density of linear capillaries is observed.



Patient 10:

RB: Basal photo: rare, branched capillaries are observed.

RA: Post-treatment photo shows increase in the number and density of branched capillaries.



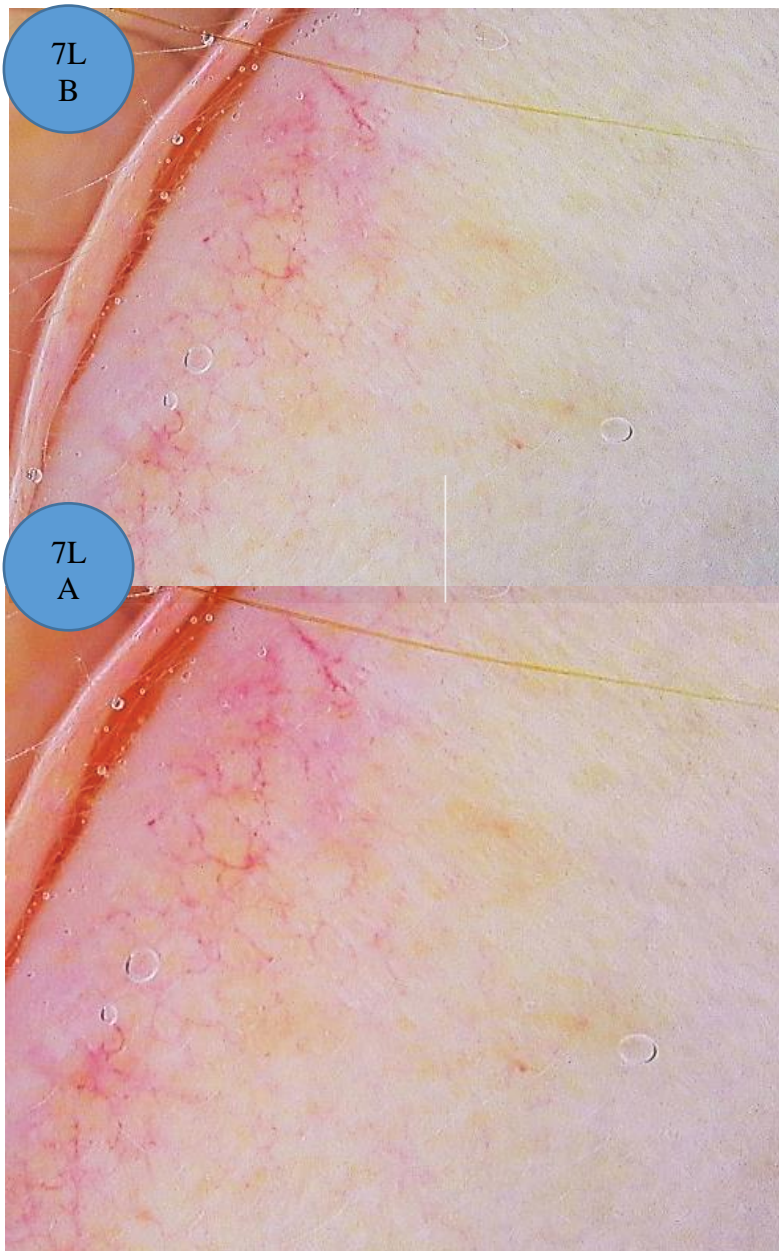
## Patient 7

RB: Basal photo: presence of branched capillaries observed.

RA: Post-treatment photo: change regarding control, increase branched capillaries.



7L



Patient 7:

LB: Basal photo: presence of fine branched capillaries observed.

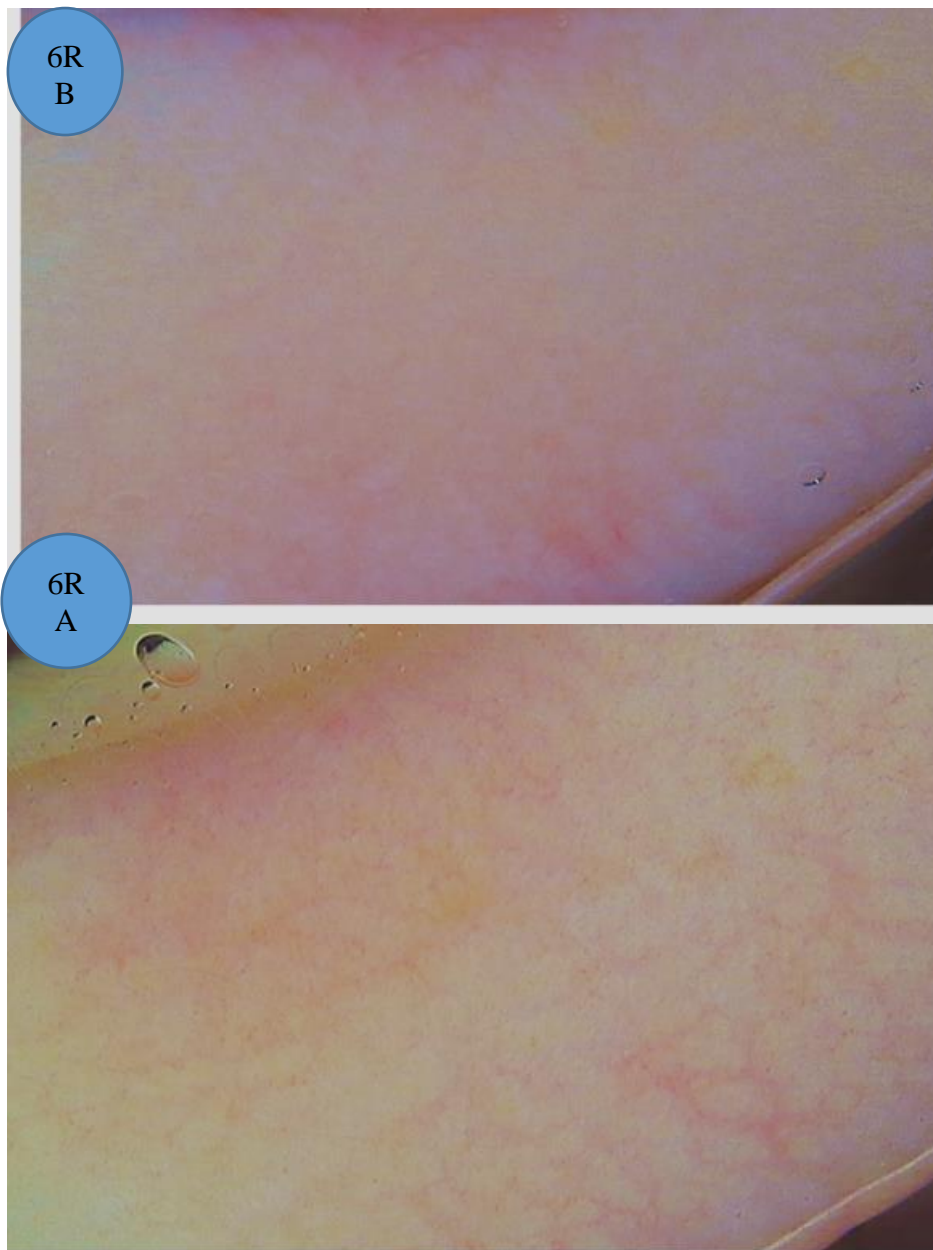
LA: Post-treatment photo: no increase of the number and density of capillaries is observed.



Patient 6:

LB Basal photo: absence of capillaries.

LA: Post-treatment photo: no modifications with respect to the basal photo.



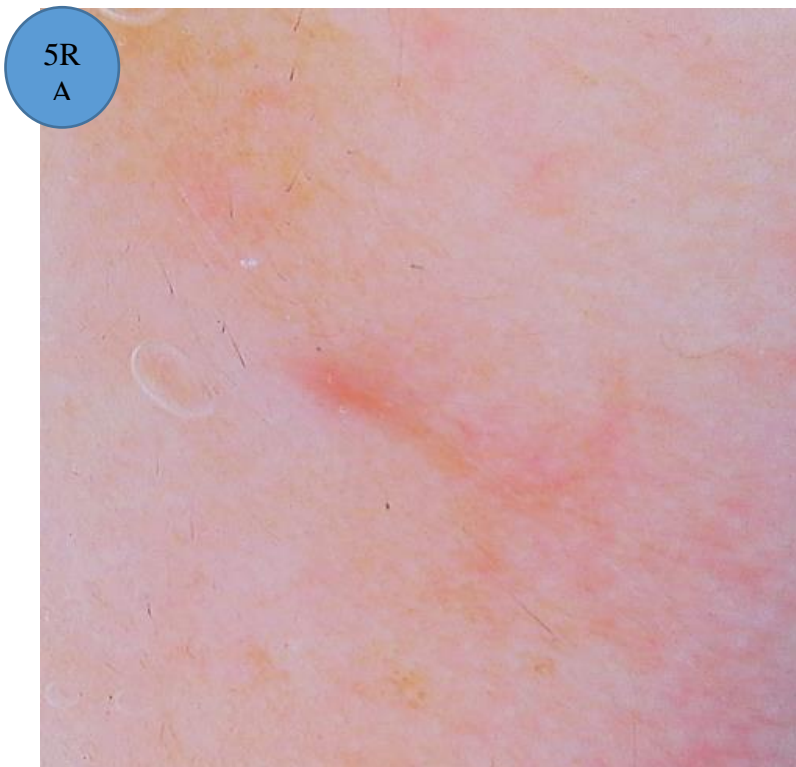
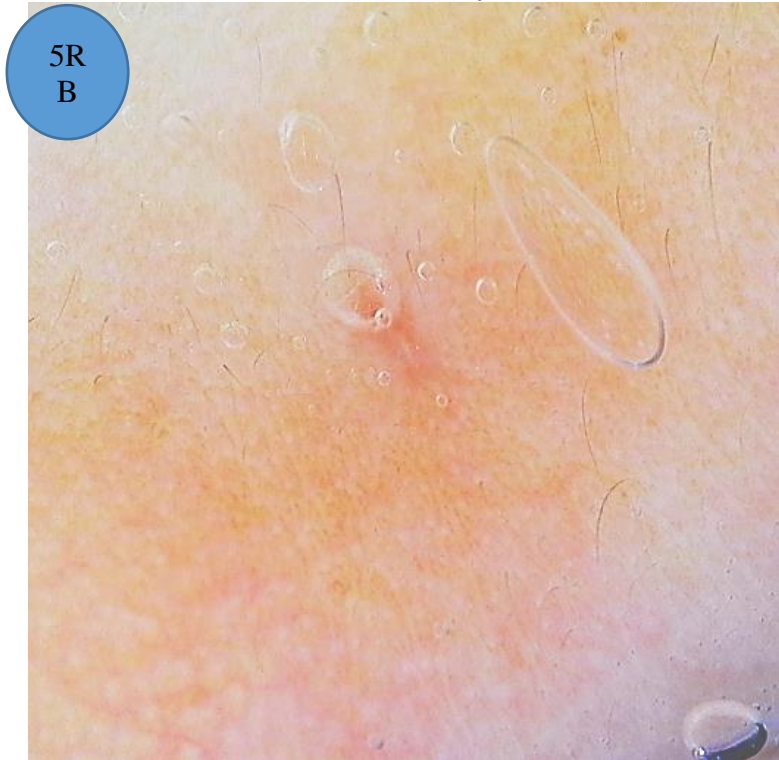
RB: Basal photo: branched capillaries observed.

RA: Post-treatment photo: increase in the number and density of capillaries.

Patient 5:

RB: Basal photo: few branched capillaries observed.

RA: Post-treatment photo: increase in the number and density of branched capillaries observed.



## Patient 5

LB: Basal photo: few branched capillaries are observed.

LR: Post-treatment photo: no increase in the number and density of branched capillaries.



In this table, you can see who received the different gels and on which side they were applied.

gel	1	2	3	4	5	6	7	8	9	10
A	Left	Right	Left	Left	Right	Right	Right	Right	Right	Right
B	Right	Left	Right	Right						
C					Left	Left		Left		Left
D							Left		Left	

## Discussion

Injectable Subcutaneous Carboxytherapy is used in various treatments for its ability to improve microcirculation in the dermis and subcutaneous tissues with an increase of the partial pressure of Oxygen (Bohr effect). (13)

It stimulates the formation of procollagen at the level of connective tissue of the skin, recovering the extracellular matrix's consistency, all functions that help tissues repair to their normal physiology (biostimulation). (14)

Injectable Carboxytherapy is used in various aesthetic pathologies (cellulite, facial aging, dark circles, stretch marks, scars, among others), (15)(16) but also in the treatment of varicose or diabetics ulcers, in limbs of diabetic patients, and in patients with alterations of arterial microcirculation.

There are new studies about the use in patients with burns. (17)

The injection of carboxytherapy produces some discomfort related to emphysema and barotrauma.

**Transdermal Carboxytherapy** is a new treatment option that provides carbon dioxide through the skin's superficial layers. (6)

Conducted studies show that transdermal carboxytherapy produces changes at the microcirculatory level equal to those with the injectable carboxytherapy.

The studies were conducted in patients without pathology, patients with diabetic foot, and patients with vaginal atrophies (GSM).

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The results showed a significant increase in microcirculation and signs of bio-regeneration after the transdermal CO<sub>2</sub>, the same changes produced with injectable CO<sub>2</sub>. (17) (18)

*Sakai et al. "Transcutaneous Application of Carbon Dioxide causing Artificial Bohr effect" reported that transcutaneous CO<sub>2</sub> was beneficial for therapeutic purposes via increased blood flow and microcirculation as evaluated by laser Doppler and intensification of tcpO<sub>2</sub> in ischemic tissues, providing evidence of the Bohr effect in vivo.*

*Leibaschoff et al. "A Prospective Clinical and Instrumental Study on the Effects of a Transcutaneous Cosmeceutical Gel that Claimed to Produce CO<sub>2</sub>." (7)*

Transdermal CO<sub>2</sub> using a transcutaneous gel allows for an easy, at-home application with no discomfort.

Therefore, we will be able to expand treatments using transdermal carboxytherapy and combine it with other treatments. (18)

The group using Gel D lost two patients because they reported that on the second day of placing the gel, the earlobe was edematous and itchy like an allergic reaction; therefore, they did not want to continue.

Only ten volunteers remained in the study.

One patient in group 1 drew attention to physicians because the videodermoscopies showed no microcirculation in both earlobes in neither the before or after.

48 hours before the study started, patients received videodermoscopies of both earlobes with photographic records for later comparison.

At the end of 5 days of application, we waited for 24 hrs and did another videodermoscopy.

The videodermoscopies of the earlobes of the nine patients that received Gel A showed that microcirculation improved. The control videodermoscopies of the earlobes of the nine patients that received gels B, C, and D, did not produce any changes in the microcirculation.

I want to comment that gels B, C, and D included a very unusual explanation of how they produce transdermal CO<sub>2</sub> in their package instructions, without any scientific support or information on the claim.

The number of patients and the duration produced a short sample.

In my opinion, being the study is double-blind, where neither the patients nor the dermatologists knew anything about the gels or earlobe chosen for the application, it offered a considerate way of checking the outcomes.

**In conclusion**, in nine out of ten patients, the videodermoscopies of earlobes that used Gel A improved the microcirculatory level. Gel A is referred to as CO<sub>2</sub>Lift®. In nine out of ten patients, the video capillaroscopy of earlobes that used gel B, C, or D showed no change in the microcirculatory level.

This prospective preliminary study on the action of four different gels, with only one validated by scientific studies, gives us encouraging results to state that some products on the market claiming to be carboxytherapy are not producing any changes in the cutaneous microcirculation.

Disclosure:

Lumisque paid for the videodermoscopies.

Independent research

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