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A Prospective Randomized Controlled Pilot Study to Assess the Response and Tolerability of Cold Atmospheric Plasma for Rosacea

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Keywords
Rosacea · Cold atmospheric plasma · Plasma medicine · Pilot study · Split-face study

Abstract
Introduction: Rosacea is a common, facial, chronic inflammatory skin disease. Due to its complex pathogenesis, adequate therapy of rosacea can be challenging. An innovative recent therapeutic tool is cold atmospheric plasma (CAP), which is already established in the treatment of chronic wounds and promising in different other skin diseases. ***Methods:*** In a split-face pilot study we investigated dielectric-barrier-discharged CAP in erythemato-telangiectatic (ETR) and/or papulopustular rosacea (PPR). CAP treatment was applied on lesional skin of a randomized side once daily (90 s/area) for 6 weeks. The other untreated side served as control. Co-primary endpoints were ≥1 improvement of the Investigator Global Assessment (IGA) score on the treated side compared to control and a decline of the Dermatology Life Quality Index (DLQI) after 6 weeks. Secondary endpoints included inflammatory lesion count (papules and pustules), skin redness intensity and erythema size. Adverse events (AEs) were recorded constantly. Additionally, participants were weekly assessed for symptoms, skin condition, trigger factors, skin care, treatment success, and local tolerance parameters. All p values were calculated using the Wilcoxon signed-rank test. ***Results:*** Twelve subjects (ETR, $n = 3$; ETR and PPR, $n = 9$) completed the study. DLQI was significantly improved after 6 weeks ($p = 0.007$). On the CAP-treated side, lesions ($p = 0.007$) and erythema size ($p = 0.041$) were significantly reduced compared to the control. IGA ($p = 0.2$) and skin redness intensity ($p = 0.5$) did not differ significantly between control and CAP-treated side. No serious AEs occurred and treatment was well tolerated. ***Conclusion:*** CAP is a promising new treatment of rosacea, especially for PPR.

Introduction

Rosacea is a highly prevalent and chronic inflammatory skin disease that mainly involves central areas of the face, such as the forehead, nose, cheeks, and chin [1]. It is estimated to affect up to 5.5% of the world's population, with the highest prevalence in the age group of 45–60 years [2, 3]. A multifactorial etiology is assumed, involving genetics as well as environmental factors. Furthermore, exacerbation of rosacea can be caused by a variety of endogenous and exogenous triggers, e.g., stress, heat, spicy food, menstruation, and UV radiation [4–6]. Symbiotic microbiota, in particular *Demodex folliculorum*, have been identified as another

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component of the rosacea pathogenesis with an increased density of Demodex mites in lesional skin [7–9].

By activating toll-like receptors (e.g., TLR2) and the inflammasome, Demodex induces inflammation [10]. Furthermore, the acquired immune system plays a central role in the rosacea pathogenesis [11]. Mechanistically, granulocytes, macrophages, and dendritic cells are stimulated, releasing antimicrobial peptides and pro-inflammatory cytokines [12]. Additionally, other immune cells, such as T cells, mast cells, and plasma cells, are enriched in all cutaneous rosacea subtypes [13].

The clinical presentation of rosacea is fluctuating. It is traditionally classified into four subtypes, each with its own constellation of signs and symptoms: erythemato-telangiectatic (ETR), papulopustular (PPR), phymatous, and ocular rosacea [14]. These subtypes can appear isolated, in chronological order, or coexist. The ETR subtype is characterized by vascular features of flushing, erythema, and telangiectasia (rosacea with only vascular symptoms). PPR, on the other hand, consists of inflammatory lesions (rosacea with papulopustules) [9, 14, 15].

Rosacea treatment strategies primarily include the identification and avoidance of trigger factors. Besides, different topical agents are currently listed in European recommendations for the treatment of rosacea [16, 17]. Brimonidine is approved for symptomatic treatment of erythema. In contrast, metronidazole, azelaic acid, and ivermectin are treatment options for PPR. Due to rosacea's high global prevalence with a partially serious burden on patients and the unmet need for treatments targeting more than one subtype, there is a great demand for optimization of treatment [18].

Cold atmospheric plasma (CAP) has emerged as a promising new tool for the treatment of diverse medical indications. CAP is well established for wound therapy and has shown promising results in the treatment of cancer, acne, psoriasis, and other immune-mediated skin diseases [19, 20]. It contains various active agents, yet reactive oxygen and nitrogen species are considered to be the most relevant [21, 22]. CAP acts as an immunomodulatory effector by its impact on inflammatory cytokines and immune cells in different skin diseases [23–25]. In addition, CAP has well-characterized antimicrobial properties, including activity against Demodex mites [26, 27].

Mechanistically, CAP devices can be classified into two different types: *direct plasma* (e.g., generated by dielectric barrier discharge [DBD]) or *indirect plasma* (jet plasma). DBD plasma devices consist of a single electrode covered with a dielectric using the skin as a counter-electrode. Hence, plasma is generated directly on the skin surface [28]. In the light of its promising properties, we utilized DBD-generated CAP to investigate in a pilot study its response and tolerability in the treatment of rosacea.

Materials and Methods

Study Design

Our study was conducted as a prospective, investigator-initiated, randomized controlled study at the Clinic for Dermatology and Venereology of the University Medical Center Rostock, Germany. Recruitment time was from February to May 2022. The study included participants with ETR and/or PPR. Exclusion criteria involved any topical or systemic treatment of rosacea, e.g., with corticosteroids, antibiotics, immunosuppressive agents, or laser therapy within the 4 weeks prior to participation; pregnancy; age below 18 years; implants (especially if electroconductive); arrhythmia; heart failure during the previous 6 months; or suffering from epileptic seizures [29]. Daily skin care routine could be continued, all other rosacea-directed treatments were prohibited during the duration of the study.

Plasma Treatment and Photo Documentation

All study participants performed a randomized half-sided treatment for 6 weeks. The device used for this study was the DBD device PlasmaDerm® Flex (CINOGY Technologies GmbH, Duderstadt, Germany). The PlasmaDerm® device consists of a control unit connected to a handset. A disposable plasma applicator (spacer) is connected to the handset. The spacer is positioned directly on the skin area to be treated and ensures a gap with environmental air between spacer and skin. For safety reasons, the device can only be started by pressing the on button, thus no changes of technical parameters are possible. Via a display timer, an exact treatment duration of 90 s per area is adjusted.

Computer-based randomization was applied to allocate treatment to a CAP-treated and an untreated control half. Treatment was performed on affected skin of the chin, cheek, and forehead. The nose was omitted from the treatment to ensure an exact separation of both face sides. All participants were thoroughly instructed, how to use their CAP devices, and the first treatment was conducted on site. At home, they were asked to perform treatment in front of a mirror and correct application on curved face areas was ensured by constant swinging of the spacer.

Study visits took place at baseline, after three, and after 6 weeks of CAP treatment. At baseline and after 6 weeks (shown in Fig. 1), a standardized photo documentation was performed with frontal and lateral photos, which were evaluated by two blinded investigators. In case of disagreement, a third investigator was consulted. Outside of the study, participants were offered to perform CAP treatment on both face halves for an additional 6 weeks.

Primary Endpoints: IGA Score and DLQI

As primary endpoints, the Investigator's Global Assessment (IGA) of Rosacea severity score and the Dermatology Life Quality Index (DLQI, 0–30) were gathered at baseline and week six (shown in Fig. 1). The IGA combined erythema assessment with the amount and size of inflammatory lesions resulting in five grades (grades: 0–4) (Table 1) [30]. IGA improvement of ≥1 on the treated side compared to control side was considered as treatment success.

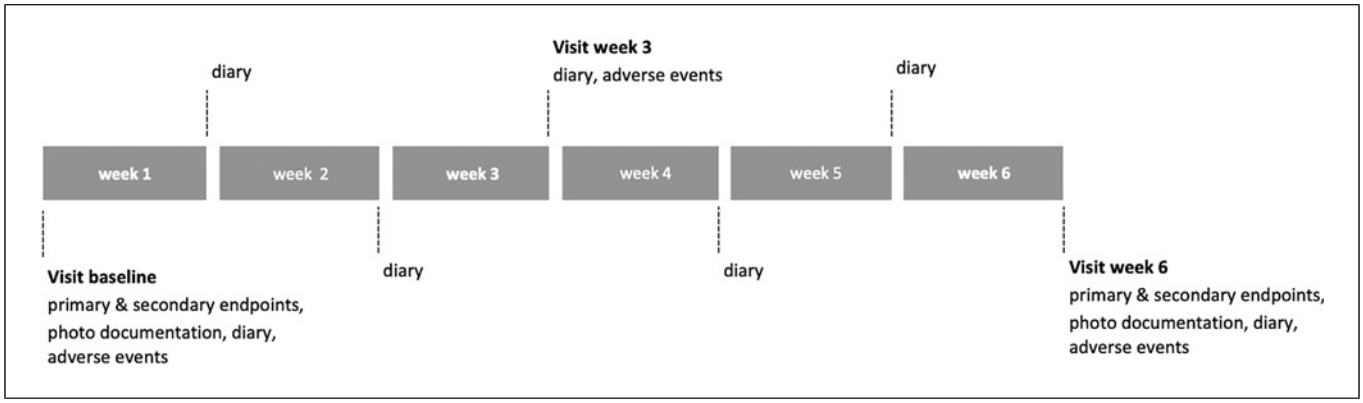

Fig. 1. Study timeline indicating visits and endpoints recorded.

The DLQI score is a clinical assessment questionnaire developed specifically for dermatologic conditions and measures the individual quality of life (QOL) restriction [31]. It consists of a 10-item scale and reaches scores from 0 to 30. In our study, an improvement from baseline to week six on the treated half in comparison to the control half was considered as treatment success.

Secondary Endpoints: Lesion Count, Skin Redness Intensity, and Erythema Size

All secondary endpoints were assessed at baseline and after 6 weeks. The absolute lesion count was assessed in PPR and included numbering of papules and pustules by the investigator. For evaluation of skin redness intensity and erythema size in ETR, the photos were assessed using the picture-analyzing program ImageJ® (available at https://imagej.net/ij/index.html, last accessed March 8, 2023).

The analysis of skin redness intensity was performed as previously described by Logger et al. [32]. In short, to quantify the mean red intensity, the color space CIE method $L^*a^*b^*$ (L, lightness; a, green-red ratio; b, blue-yellow ratio) was utilized (shown in Fig. 2a–c). First, the most intense visible facial erythema (=lesional skin) was defined as the region of interest (ROI) 1 using frontal images. Second, a non-lesional area was defined as non-lesional ROI 2. Third, the image was converted to CIE $L^*a^*b^*$ color space. The proportion of red (α) of both ROIs was calculated separately and then their ratio was formed ($\Delta\alpha$).

Facial erythema was measured in a stepwise process (shown in Fig. 2d). First, the erythema was identified as ROI 3 in the lateral photos. Second, a white sticker of determined size was used as size reference. Third, the size of ROI 3 was calculated in relation to the reference point.

Rosacea Diary

The diary was obtained from the German awareness campaign "Aktiv gegen Rosacea," which is sponsored by Galderma Laboratorium GmbH, modified for the purposes of our study and answered weekly [33] (shown in online suppl. Tables 1, 2; for all online suppl. material, see https://doi.org/10.1159/000533190). It enabled our participants to rate on a five-point scale the subjective expression of main (erythema and/or papules/pustules) and additional symptoms (burning, stinging, dry appearance) from "not affected" to "very severe," and their current skin condition from "very good" to "severe." Furthermore, it assessed the appearance of predefined triggers (weather, spicy foods, alcohol, stress), application of specific skin care products, notification of CAP treatment success, and occurrence of local discomfort (irritation or pain). Furthermore, regular CAP utilization was monitored. In addition, participants were able to comment on their subjective experience with CAP in a free-text field.

Adverse Events

Adverse events (AEs) were recorded separately for both face sides adhering to Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 and their relation to CAP treatment was assessed [34]. Accordingly, appearance only and/or with a higher intensity on the CAP-treated face side was regarded as treatment-related.

Statistics

IBM SPSS Statistics for Windows (version 25, IBM Corp., Armonk, NY, USA) was used for statistical analysis. All p values were derived from Wilcoxon signed-rank tests. A p value of $p < 0.05$ was regarded as statistically significant. The p values were not adjusted for multiple testing.

Results

Baseline Characteristics

Twelve participants were included. Their median age was 50.5 years. Each participant had used topical and/or systemic guideline medication for rosacea prior to the study. Baseline characteristics are summarized in Table 2.

Table 1. Investigator global assessment score

Grade	Description	Amount/size of inflammatory lesions	Erythema
0	Clear	None	None
1	Almost clear	Very few small papules/pustules	Very mild
2	Mild	Few small papules/pustules	Mild
3	Moderate	Several small or large papules/pustules	Moderate
4	Severe	Numerous small and/or large papules/pustules	Severe

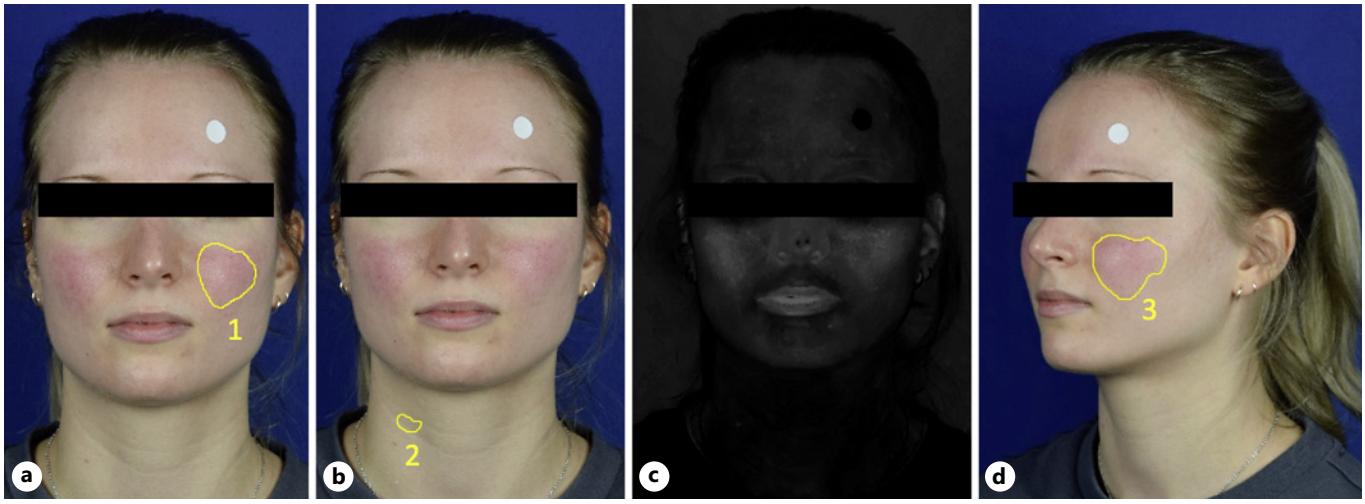

Fig. 2. Photo analysis of a female participant with ETR using ImageJ. **a** Erythema was defined as region of interest (ROI) 1 in the frontal images. A white sticker served as size reference. **b** ROI 2 was selected on a non-lesional region of the neck. **c** CIE L*a*b color space filter was applied to analyze mean intensity of skin redness α separately for ROI 1 and ROI 2, which were then set in relation to each other (resulting in $\Delta\alpha$). **d** Erythema size (ROI 3) was calculated according to the reference point in the lateral images.

Primary Endpoints

Both, in the treated (median change = −1.0) as well as the untreated (median change = −1.0) areas, the IGA scores reduced from baseline to week six ($p = 0.2$ for the difference of treatment vs. control ($median_{t\text{-}c}$) = 0.0) (shown in Fig. 3a). The median DLQI at baseline was 2.5 and decreased to 1.0 ($p = 0.007$) (shown in Fig. 3b).

Secondary Endpoints

A decrease of lesion count was observed on the treated side (median change = −3.0), however, lesions increased on the control side (median change = +1.0) ($p = 0.007$; $median_{t\text{-}c} = -4.0$) (shown in Fig. 4a). The intensity of skin redness ($\Delta\alpha$) declined on both face sides (median changes control = −1.5; CAP = −1.1; $p = 0.5$; $median_{t\text{-}c} = -0.4$) (shown in Fig. 4b).

Furthermore, a favorable change of erythema size was detected. It decreased on the CAP-treated side (median change = −1.2), while it increased on the control side (median change = +0.4) ($p = 0.041$; $median_{t\text{-}c} = -1.0$) (shown in Fig. 4c; online suppl. Fig. 1 exemplifies assessment of endpoints).

Rosacea Diary

CAP was administered regularly. The participants' perception of main and additional symptoms, as well as their general skin condition did not change significantly from baseline to week six. Also, the occurrence of triggering factors and application of skin care products were constant throughout the whole treatment period. Moreover, CAP did not cause irritation or pain. Most importantly, ten (83%) participants agreed that after 6 weeks, their CAP treatment has been successful. The treatment was subjectively assessed as comfortable, skin smoothing and effective in mitigating exacerbation due to triggering factors.

Table 2. Baseline characteristics

Characteristic			Value
Total patients, *n*			12
Female/male			10/2
Age, years			
Median (min-max)			50.5 (27–77)
Subtype, *n*			
ETR			3
ETR + PPR			9
DLQI score, 0–30 points			
Median (min-max)			2.5 (1–6)
Baseline medians for both face sides	Control	Treated	*p* value (control vs. treated)
IGA score, 0–4 grades	3	3	0.6
Lesion count, *n*	7	6	0.3
Intensity of skin redness Δα	13.0	11.9	0.5
Erythema size, cm^2	10.3	11.7	0.1

DLQI, Dermatology Life Quality Index; ETR, erythemato-telangiectatic rosacea; IGA, Investigator Global Assessment; PPR, papulopustular rosacea. *p* value <0.05 was considered as statistically significant.

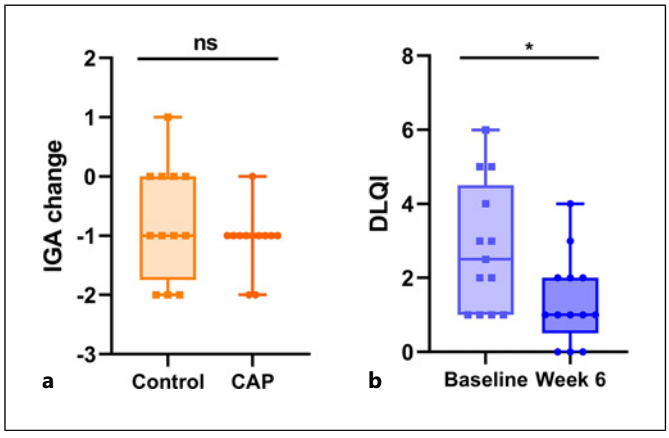

Fig. 3. Boxplots of primary endpoints. **a** IGA change from baseline to week six. **b** Proband-specific DLQI at baseline and week six. CAP, cold atmospheric plasma; ns, not significant; *$p < 0.05$.

Additional Treatment

Participants were offered to use CAP on both sides of the face for an additional 6 weeks, which was realized by eight participants (ETR, $n = 1$; ETR and PPR, $n = 7$). Notably, inflammatory lesions now also declined on the former control side (median change from week six to week twelve = −6.0, $p = 0.03$). On the contralateral side, which was CAP treated for 12 weeks, lesions could be reduced even further (median change = −2.0; $p = 0.04$). All other endpoints were not significantly changed on both sides.

Adverse Events

In total, five participants noticed AEs of five different categories (online suppl. Table 3): paresthesia ($n = 2$), tension of the skin ($n = 1$), dry skin ($n = 1$), papulopustular rash ($n = 1$), and erythema ($n = 1$). All AEs were mild and only in three cases (paresthesia, tension of the skin, dry skin) AEs were regarded as treatment-related. None of the enrolled participants terminated the study prematurely due to AEs or because of other reasons.

Discussion

CAP is used in a variety of clinical settings with great success [35]. Well-established applications include chronic wound therapy and disinfection [36]. Furthermore, different studies have suggested CAP for the treatment of cancer [37, 38]. Atopic dermatitis, psoriasis, and herpes zoster, among others, are further skin diseases in which CAP has been successfully applied [23, 39–42]. Recently, Karrer et al. [43] used a jet plasma device in a prospective, randomized, controlled, split-face trial to assess its safety and efficacy in the treatment of acne vulgaris. Reduction of particularly inflammatory lesions after ten CAP treatments within 4 to 6 weeks was significantly higher on the CAP treated than on the untreated side. Also, percentage of patients reporting improved aesthetics was higher for the treated than for the untreated side after treatment completion and at the 2- and 4-week follow-up [43].

Accordingly, in our pilot study we observed a decrease of papular and pustular lesions only on the CAP-treated side (CAP vs. control, $p = 0.007$). Just recently, the concept of PPR as a chronic demodex infection probably associated with T-cell exhaustion has been reasoned extensively [9]. CAP was shown to be capable of both, reduction of demodex count on the face comparable to topical ivermectin and modulation of immune cells [24, 44]. Hence, the positive effects of CAP on PPR detected in our study are plausible.

Transient or persistent facial erythema is the most common feature of the different cutaneous rosacea subtypes and was present in all participants of our

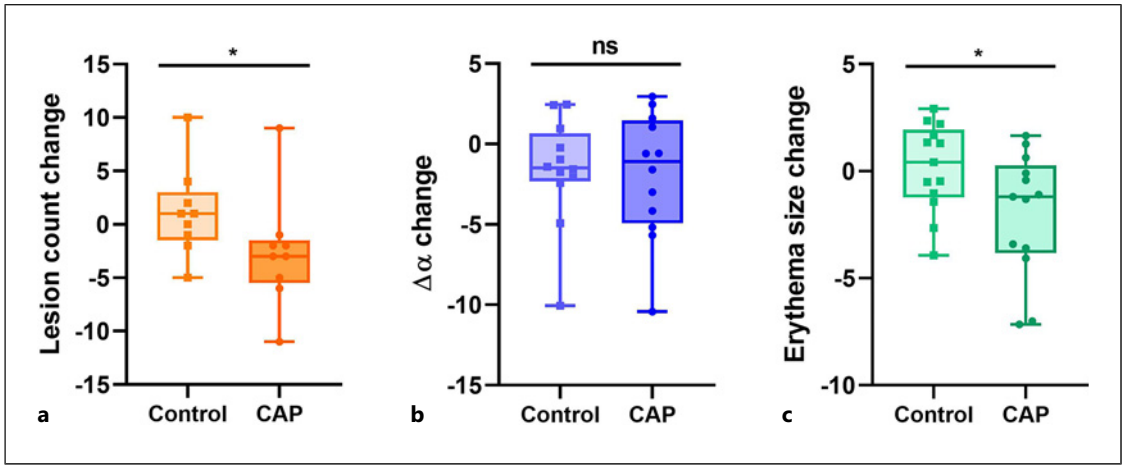

Fig. 4. Boxplots for the change of secondary endpoints from baseline to week six. **a** Lesion count, **b** intensity of skin redness $\Delta\alpha$, **c** erythema size. CAP, cold atmospheric plasma; ns, not significant; *$p < 0.05$.

study [45]. We analyzed erythema size with ImageJ, and only in the CAP-treated side a decrease was detected (CAP vs. control; $p = 0.041$). Importantly, we did not differentiate between transient or persistent erythema. Transient flushes are produced by neurogenic inflammation through various triggers [9, 46, 47]. Furthermore, in persistent erythema a perilesional redness may be distinguished, which can be based on the sustained vasodilatation and plasma extravasation induced by the inflammatory infiltrates [45, 48]. Hence, the immune-modulating properties of CAP might have been associated with the reduction of erythema size. However, the complex vicious circle of vascular and inflammatory changes in rosacea is characterized by a persistent dilation of vessels, neoangiogenesis, telangiectasias, and derma matrix degradation, which are often insufficiently cured by anti-inflammatory treatments [45]. Likewise, daily CAP treatment over 6 weeks was not able to diminish median skin redness intensity $\Delta\alpha$ more than the control (comparison of $\Delta\alpha$ for control and CAP-treated side not significant). Of note, α correlates with hemoglobin, skin blood flow, and vascularization [32]. Accordingly, in a former study of acute wound healing, we revealed that DBD-based CAP increased hemoglobin distribution in the microcirculatory system and oxygen saturation in deeper tissue layers [49]. A stimulation of microcirculation of the skin has also been shown by others [50–54]. This phenomenon might have negatively influenced the effect on skin redness intensity despite CAP's immune-modulating efficacy. Nevertheless, absolute $\Delta\alpha$-values decreased on both sides, control and treatment. Hence, further experiments are necessary to elucidate the detailed implication of CAP on the rosacea pathogenesis, in particular its immune-modulating activity.

Rosacea is often associated with a considerable burden for patients. Thus, it can negatively affect QOL, as well as social and psychological well-being [55–58]. In our study, we assessed the individual QOL using the common DLQI questionnaire. Although our study participants did not have a high strain at baseline, we observed a significantly improved DLQI after 6 weeks ($p = 0.007$; Fig. 3b). Accordingly, participants evaluated CAP treatment very positively in their weekly diary. Consistent with the excellent tolerability of CAP in other studies, AEs in our pilot trial were rare, mild, and only partly related to CAP [49, 59, 60].

An ≥1 IGA improvement of CAP-treated compared to control side after 6 weeks was another co-primary endpoint. On both sides, the IGA score declined by a median of 1.0 grade, and no significant differences were detected between the two sides ($p = 0.2$). Importantly, IGA scoring included a combined assessment of erythema and inflammatory lesions and could therefore not distinguish between relief of vascular symptoms or improvement of inflammatory lesions. The rosacea area and severity index is a newly developed scoring system for clinical assessment of rosacea severity that allows for a more nuanced evaluation of rosacea subtypes/phenotypes, and its use might be favorable in future trials [61].

In our pilot study, CAP was used as a monotherapy. However, different studies have revealed a great potential of CAP as a combination partner with other, in particular

oncological, treatments [62–64]. In rosacea, using CAP in conjunction with other treatments might also be advantageous due to different aspects: On the one hand, CAP combination with topical drugs might increase their efficacy due to its enhancement of stratum corneum permeabilization [65, 66]. On the other hand, combined systemic and local treatment is the common standard for difficult-to-treat rosacea. CAP is particularly useful for combination with systemic treatment due to its antimicrobial properties, which are neglectable for systemic low-dose antibiotics like doxycycline [17, 67].

Taieb et al. [68] evaluated the costs of ivermectin, metronidazole, and azelaic acid for the topical treatment of PPR in the USA in 2014, which varied from $5.08–$6.60 per day. At present, CAP treatment is not reimbursed by the statutory health insurance in Germany and cost estimations derived from the current configuration of the utilized PlasmaDerm® device would be without reliable accuracy. Nevertheless, a once purchased device, which can be repetitively utilized, might be an attractive and cost-effective treatment alternative, in particular considering the long-term therapy of rosacea.

Our study has several limitations. First, because of its design as a monocentric pilot study, the number of participants was low. Second, we utilized the "old" rosacea classification into subtypes, although the National Rosacea Society Expert Committee now recommends the use of phenotypes and the differentiation according to diagnostic, major, and minor (secondary) symptoms [1]. This updated classification reflects the clinical reality better. However, the simple distinction of ETR and PPR was more convenient for our study since some of the phenotypes were either not present (e.g., phymatous changes) or not specifically considered (e.g., transient vs. persistent erythema). Third, DLQI is a general dermatological QOL questionnaire, which did not distinguish between the two sides of this split-face study. That is why a diary was additionally filled by participants that consistently revealed a positive perception of CAP treatment. The complementary use of the rosacea-specific QOL questionnaire RosaQoL might be beneficial in the future [69, 70].

Despite all these limitations, our study provides significant new findings: CAP was effective in the reduction of papulopustular lesions and erythema size in rosacea. Furthermore, we observed an improved QOL and the half-sided CAP treatment was well tolerated. Further studies with a larger sample size are needed to confirm our results and should put a special focus on treatment of inflammatory lesions and combination with other agents.

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Statement of Ethics

This study has been approved by the Ethics Committee of the Medical Faculty, University of Rostock (study reference: A 2021-0275) and was conducted adhering to the principles of the Declaration of Helsinki. Written informed consent was obtained from each participant before study participation for publication of their images.

Conflict of Interest Statement

SE received research funding and speakers' honoraria from CINOGY GmbH and research funding from Teion Medizinprodukte GmbH. The other authors have no conflicts of interest to declare.

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Author Contributions

The study was concepted and designed by S.H. and A.T.; S.H. performed the study. S.G. and A.T. evaluated the photos. S.H. and A.T. were responsible for the data analysis and interpretation. S.H. performed the statistical analysis, with methodological support by F.W. Administrative, technical, and material support was provided by S.E. The first manuscript draft was provided by S.H.; A.T. reviewed and edited the script. All authors have participated in the critical revision of the manuscript regarding important intellectual content.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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