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Research Article

Therapeutic Use of Cold Atmospheric Plasma for the Treatment of Mild Acne Papulopustulosa—A Randomized, Controlled, Double-Blind Pilot Study

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Acne is a common disease worldwide, predominantly occurring in teenagers. Commonly prescribed therapies often cause adverse effects and in case of antibiotics bare the risk of developing resistances. Cold atmospheric plasma (CAP) is a well-tolerable, physical treatment method, which is well established in the treatment of chronic wounds since it provides bactericidal and wound healing properties. Our aim was to evaluate the efficacy and safety of CAP as a potential add-on therapy for mild acne papulopustulosa in a randomized controlled, double-blind pilot study. Forty participants were randomized into two arms of 20 each. Both groups self-applied 0.1% of adapalene cream daily and received weekly skin-cleansing by a physician combined with either CAP verum-device treatments or placebo-device applications instead. The endpoint of the treatment segment was at 6, and follow-up was at 10 weeks. The co-primary endpoints total inflammatory lesion count (TILC) and acne-specific Investigator Global Assessment (IGA) score as well as secondary endpoints sebumetry, total porphyrin count (TPC) reflecting *Cutibacterium acnes* colonization, and occurrence of adverse events (AEs) were measured from baseline until follow-up, while the Acne-specific Quality of Life (AQOL) questionnaire was assessed at baseline and endpoint. TILC decreased greater in the verum versus control group from baseline (45.8 vs. 49.4) toward endpoint (22.1 vs. 38.6; $p = 0.07$) to follow-up (16.5 vs. 28.7; $p = 0.48$), matched by the IGA score with similarly greater improvement in the verum versus control group from baseline (2.3 vs. 2.6) to endpoint (1.3 vs. 2.3; $p < 0.001$) until follow-up (1.0 vs. 1.9; $p = 0.006$). Sebumetry, TPC, and AQOL scores decreased more during treatment and in the verum group. AE occurred less frequently in the verum group without serious AE reported overall (all $p < 0.05$). Conclusively, CAP proved to be an efficient and well-tolerable add-on therapy for the treatment of mild acne papulopustulosa.

Trial Registration: German Registry of Clinical Trials: DRKS00032416

Keywords: acne; cold atmospheric plasma; cutibacterium acnes; quality of life; treatment

1. Introduction

Acne is the most commonly treated inflammatory dermatologic disease globally with a prevalence of 60%–80% in teenagers and a prevalence of about 9.4% in the general population [1–3]. With a multifactorial pathogenesis, the main factors include increased sebum production, abnormal cornification of pilosebaceous ducts, and skin dysbiosis including hypercolonization of the Gram-positive *Cutibacterium acnes* (*C. acnes*), subsequently leading to the formation of inflammatory mediators [4]. Acne can manifest with inflammatory lesions and noninflammatory lesions. "Acne papulopustulosa" presents mainly with pustules and papules (raised inflammatory lesions with or without pus, respectively). Treatment should always be recommended, since according to a meta-analysis, the prevalence of scarring for mild, moderate, and severe acne was 46%, 67%, and 87%, respectively [5]. Because of stigmatizing facial disfigurement, acne often imposes a psycho-social burden on patients and impairs their quality of life [6]. The therapeutic efficacy and clinical outcome are often unfortunately affected by poor treatment adherence of around 50% for general acne treatment and around 40% for topical acne therapy [7]. Therapeutic agents for acne papulopustulosa include topical substances like retinoids, azelaic acid, or benzoyl peroxide. Topical antibiotics like erythromycin are also used for their combined bactericidal and anti-inflammatory effects [8, 9] but bear the risk of resistance formation [10]. New alternative treatments are needed, especially for patients who suffer from adverse events (AEs) by topical agents or are unsuited for systemic therapies. Cold atmospheric plasma (CAP) may provide a novel effective and well-tolerable therapy. Previous studies have shown that it does not damage the skin barrier and is generally regarded as a safe therapy without serious AE being reported so far [11, 12]. In the emerging field of "plasma medicine," the therapeutic value of CAP has been established for its beneficial role in the treatment of chronic wounds like diabetic foot ulcers [13]. By affecting redox pathways, it influences various processes including angiogenesis, cell migration, and proliferation [14–17]. Different types of CAP devices exist, each of them generating a partially ionized gas mixture with highly reactive oxygen and nitrogen species (RONS) and ultraviolet-photons (UV-photons) in an electric field at atmospheric air pressure, not surpassing room temperature (therefore being labeled "cold" and "atmospheric") [18, 19]. The bactericidal properties of CAP are attributed to a combination of emitted UV-photons and a complex mixture of molecules formed in ambient air. More than 600 elementary reactions were described with over 50 reactive species formed including ozone, peroxide, superoxide, and nitric oxide (NO) [20]. This mix of reactive molecules stands out with good bactericidal efficacy, yet remaining more tolerable than high amounts of isolated reactive species themselves for humans [21, 22]. Possible bactericidal mechanisms are the disintegration of DNA, metabolic proteins, and cell wall and membrane oxidation [21, 23]. It has been shown in vitro that CAP is bactericidal to many species like *Escherichia coli* and *Staphylococcus aureus (S. aureus) including* methicillin-resistant *S. aureus,* without resistances being reported so far [19, 24–26]. A recent review indicated that Gram-negative bacteria are more susceptible toward CAP exposure than Gram-positive ones [27]. Even though *C. acnes* is considered a commensal germ of our facial skin flora, the loss of phylotype diversity and predominance of certain phylotypes like IA1 are believed to be strongly associated with the expression of acne symptoms [28, 29]. It was previously demonstrated that CAP can reduce *C. acnes* successfully in vitro, including in a biofilm [30]. Currently, only a few clinical trials exist in which the effects of CAP in moderate to severe acne were evaluated. Two trials investigated the clinical efficacy of CAP alone, whereas another study combined CAP with selective electrothermolysis of sebaceous glands [31–33]. These studies concluded a good clinical outcome regarding total lesion reduction (50%–75%), especially for inflammatory lesions. No data on CAP exist yet to our knowledge in form of a randomized placebo-device controlled, double-blind trial focusing on inflammatory lesions in acne papulopustulosa.

The aim of this study was to evaluate the therapeutic efficacy of CAP application for acne papulopustulosa in the face combined with mechanical acne cleansing and daily topical application of 0.1% adapalene. Clinical improvement was assessed via two co-primary endpoints: reduction of total inflammatory lesion count (TILC) and acne-specific Investigator Global Assessment (IGA) score. Secondary endpoints were the reduction of sebum levels, reduction of total porphyrin count (TPC) reflecting *C. acnes* metabolic activity, and improvement of acne-specific Quality of Life (AQOL) questionnaire score. The safety endpoint was reported as AE.

2. Materials and Methods

2.1. Subjects. Forty participants were recruited in a medical practice in Potsdam (Germany) from August until November 2023 and randomly assigned in a 1:1 ratio in two parallel treatment arms for this randomized, controlled, double-blind trial. All subjects gave their written consent (including their parents when underage).

2.2. Study Outline. Visits were planned weekly for 6 weeks consisting of the "treatment segment" including daily topical self-therapy with adapalene 0.1% cream and weekly acne lesion cleansing combined with either a CAP verum- or placebo-device application. The "diagnostic segment" included photo-documentation, sebumetry, and porphyrin measurement (see Figure 1). The AQOL questionnaire was assessed at baseline and endpoint. Initially, the permission to utilize AQOL was requested to the authors who developed the questionnaire. The follow-up was for 4 weeks after endpoint, with two visits (weeks 8 and 10) including photo-documentation, sebumetry, and porphyrin measurements. The inclusion criteria were written consent, confirmed diagnosis of acne papulopustulosa for at least 6 months by a physician (≤ IGA score 3), and participant´s agreement not to manipulate lesions by themselves or others during the

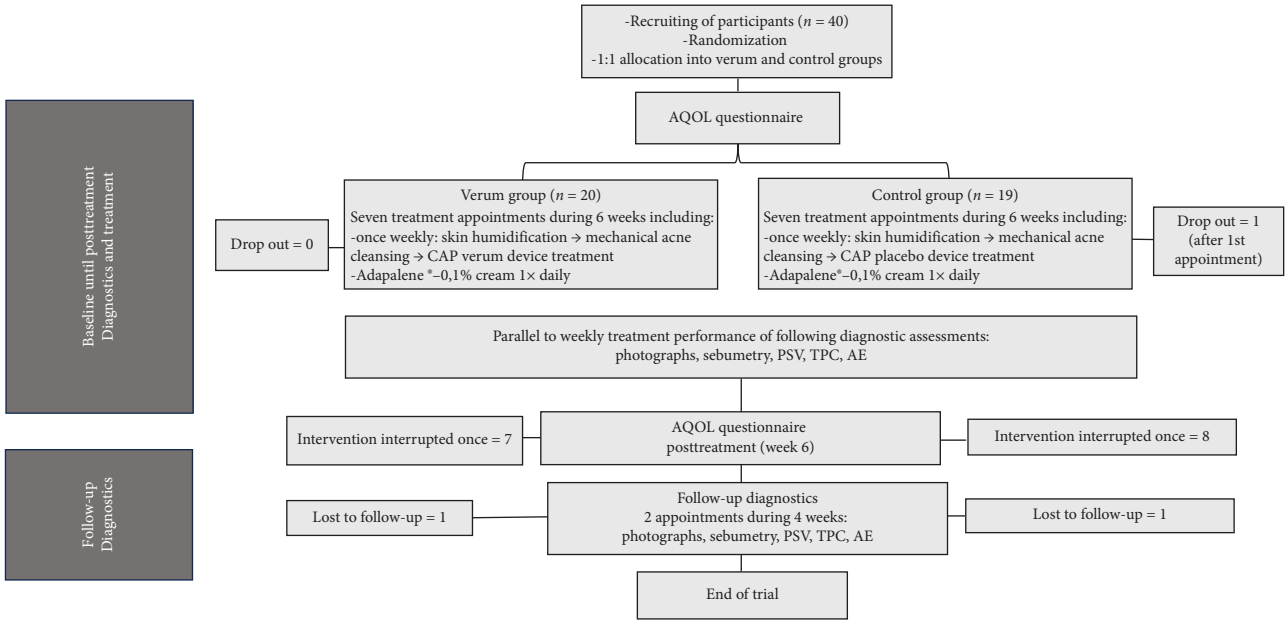
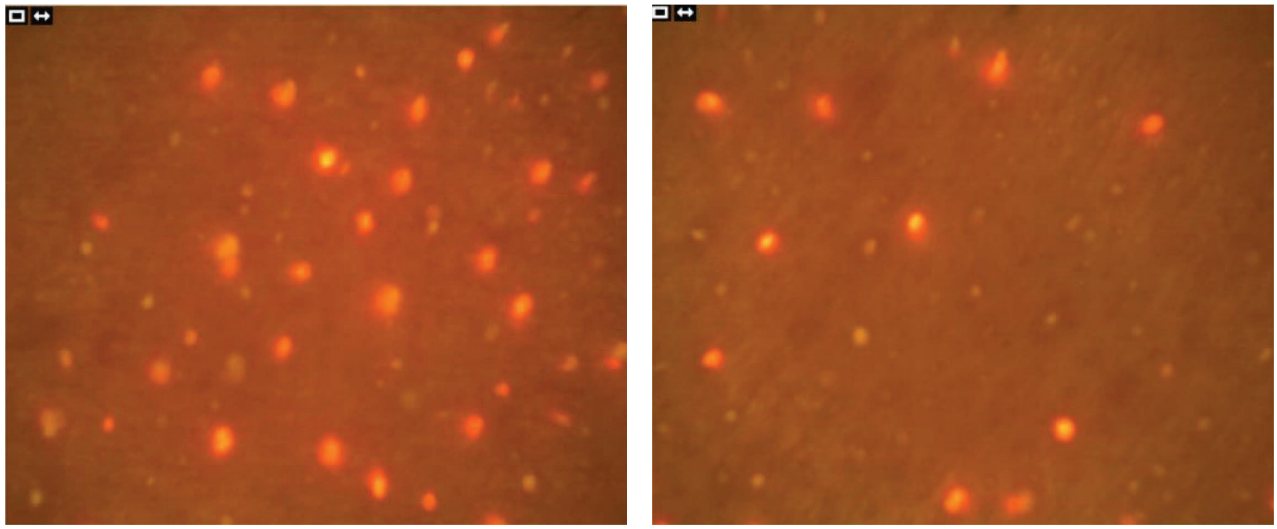


FIGURE 1: Study phase flowchart (AEs = adverse events, AQOL questionnaire = acne-specific Quality of Life Questionnaire, CAP = cold atmospheric plasma, IGA score = Investigator Global Assessment score, PSV = porphyrin surface value, TILC = total inflammatory lesion count, TPC = total porphyrin count).

study. The exclusion criteria included age 13 or younger, smoking, systemic or local treatment for acne in the previous 4 weeks (planned) pregnancy, open facial wounds, and an intolerability against adapalene cream. Patients were allowed only one absence during the treatment segment, excluding baseline and endpoint visits. Sebumetry and TPC were performed on five representative facial regions in repeating clockwise order: chin, right cheekbone, right forehead, left forehead, and left cheekbone. Permuted block randomization was done electronically by the nonblinded study physician in a ratio of 1:1 into two groups with alternating block sizes of 2, 4, and 6 [34]. The allocation document was kept inaccessible during the whole trial for the blinded physician who assessed TILC and IGA scores. Participants were blinded regarding whether they received the CAP verum- or placebo-device treatment during the trial.

2.3. Skin Parameter Analysis. Orange/red fluorescent signals representing bacterial porphyrin metabolites were detected in a 6 × 8 mm camera field combined with a 375-nm UVA light ("Visiopor PP 34" by Courage + Khazaka electronic GmbH, Germany) (see Figure 2). The obtained TPC consists of the summed-up five values. Afterward, sebum levels were measured photometrically ("Sebumeter-SM 815" by Courage + Khazaka electronic GmbH, Germany). The measurement area of the probe was 64 mm^2, and results were given in $\mu g/cm^2$, with the maximum measurement inaccuracy of 5% according to the developer. The mean of five values per appointment was used. All measurements were performed in 22C° and 35% average air humidity with at least a 5-h delay after the last facial wash. Furthermore, the AQOL questionnaire was performed at baseline and endpoint. It consists of 19 acne-specific framed items in total, with a possible score of 6 points for each question and a maximum score of 114. The first 14 questions addressed sub-scales of self-perception and psycho-social matters with answering options including "0" = not at all, "1" = very little, "2" = little bit, "3" = medium, "4" = strong, "5" = very strong, and "6" = extreme. The remaining five questions referred to the participants' personal assessment of their skin symptoms with the options "0" = none, "1" = very little, "2" = little, "3" = medium, "4 = many, "5" = very many, and "6" = extreme amount [35]. Participants were also routinely asked on each appointment if any AE occurred previously. AEs were categorized as itching, burning, pain, and erythema of the skin ranging from "0" = none, "1" = mild, "2" = medium, "3" = severe, and "4" = very severe. A blinded physician who was neither present during the diagnostic nor treatment segments assessed TILC and IGA scores on the participants' five facial regions via standardized photographs (see Figure 3) at baseline, endpoint, and follow-up. TILC consists of the sum of papules and pustules, while IGA is a global score reaching from levels "0" = unaffected, "1" = minimally, "2" = mildly, "3" = medium, and "4" = very severely affected.

2.4. Treatment. Both groups received a weekly mechanical acne lesion cleansing by the study physician through opening pustules with a sterile needle and extracting pus through a comedone-compressor. The facial skin was humidified before treatment for 1 min with a lukewarm towel of approximately 45C°. One group later received a CAP verum-device application for 6 min in total, while the other group received treatment with a resembling CAP placebo-device instead. The handheld Surface Micro-Discharge (SMD) CAP-device "Plasma care" (Terraplasma medical



(a) (b)

Figure 2: Exemplary images of porphyrin measurement in the Visiopor software of total porphyrin count (TPC) (a) at baseline in one verum group participant, indicating the metabolic activity of *C. acnes* on the left forehead. (b) TPC decreased significantly in the same participant's facial region after 6 weeks of treatment.

GmbH, Germany) was used with a voltage of 3.5 kV at a frequency of 4 kHz and a power consumption of 0.4–1.5 W. Skin contact was made through a sterile, disposable spacer with an area of 13 cm^2 and an 18.4-cm^3 reaction chamber with ambient air. The treatment was applied on standardized spacer contact zones for 1 min each on the mentalis region, both cheekbones (anatomic triangle of zygomatic, mandibular, and infraorbital regions) and bilateral and medial thirds of the forehead (Figure 4). All participants were instructed how to self-apply adapalene 0.1% cream at home in the evening. They were handed out 20 g each in neutral containers every 2 weeks until reaching 60 g in total by endpoint at week 6. Participants were told to start with roughly a pea-sized amount of cream for the whole face and increase daily, until reaching approximately a hazelnut-sized daily dose after 1 week, maintaining that dosage until the end of the treatment segment. In the case of severe AE, participants were instructed to interrupt self-application and report back to the physician.

2.5. Statistical Analysis. To assess treatment effects, we conducted for each variable a mixed analysis of variance (ANOVA) with condition (verum vs. control) as between-subjects factor and measurement point as within-subjects factor. We performed separate ANOVAs for assessing short-term effects (baseline vs. endpoint) and long-term effects (baseline vs. follow-up). The primary effect of interest concerns the interaction between condition (verum vs. control) and measurement point (baseline vs. endpoint and baseline vs. follow-up, respectively), which indicates whether the change in the treatment group is different from the change in the control group.

3. Results

Demographic characteristics are shown in Table 1. One participant dropped out after baseline and was excluded from all analyses. Two further participants were lost during follow-up (all of the above due to time-management-related issues). From nine total visits, 15 participants skipped one visit (7 verum, 8 control). Regarding baseline, endpoint, and follow-up outcomes, the relevant statistical effects concern the interaction between group and measurement, indicating whether the improvement in the treatment condition was larger than in the control condition (see the supplement for a complete set of analyses and additional linear mixed effect model analyses, which yielded comparable results). TILC as one of the co-primary endpoints consisting of papule and pustule count decreased more in the verum group from baseline to endpoint versus the control group ($p = 0.07$) (see Figure 3). This difference was not significant between baseline and follow-up ($p = 0.48$). The IGA score as the second co-primary endpoint showed accordingly a greater and significant reduction in the verum versus the control group from baseline to endpoint ($p < 0.001$). This trend also continued from baseline until follow-up ($p = 0.006$). Sebumetry mean levels decreased from baseline greater toward endpoint in the verum versus control group ($p < 0.001$). During follow-up, a slight increase was observed compared to the control group, which itself showed a slight decline ($p = 0.01$).

In the control group, the mean baseline values were higher for sebumetry as well as TPC. The latter showed similar progression like sebumetry with greater reduction in the verum group compared to the slight increase in the control group toward endpoint ($p < 0.001$). During follow-up, this trend was reversed with a slight incline of the verum versus a decline in the control group ($p = 0.013$). Porphyrin surface value was also assessed, but since it did not provide additional insights beyond the information provided by TPC, we reported it in only the supplement materials. Even though AQOL was on average worse at baseline compared to the verum group, it improved beyond levels of control

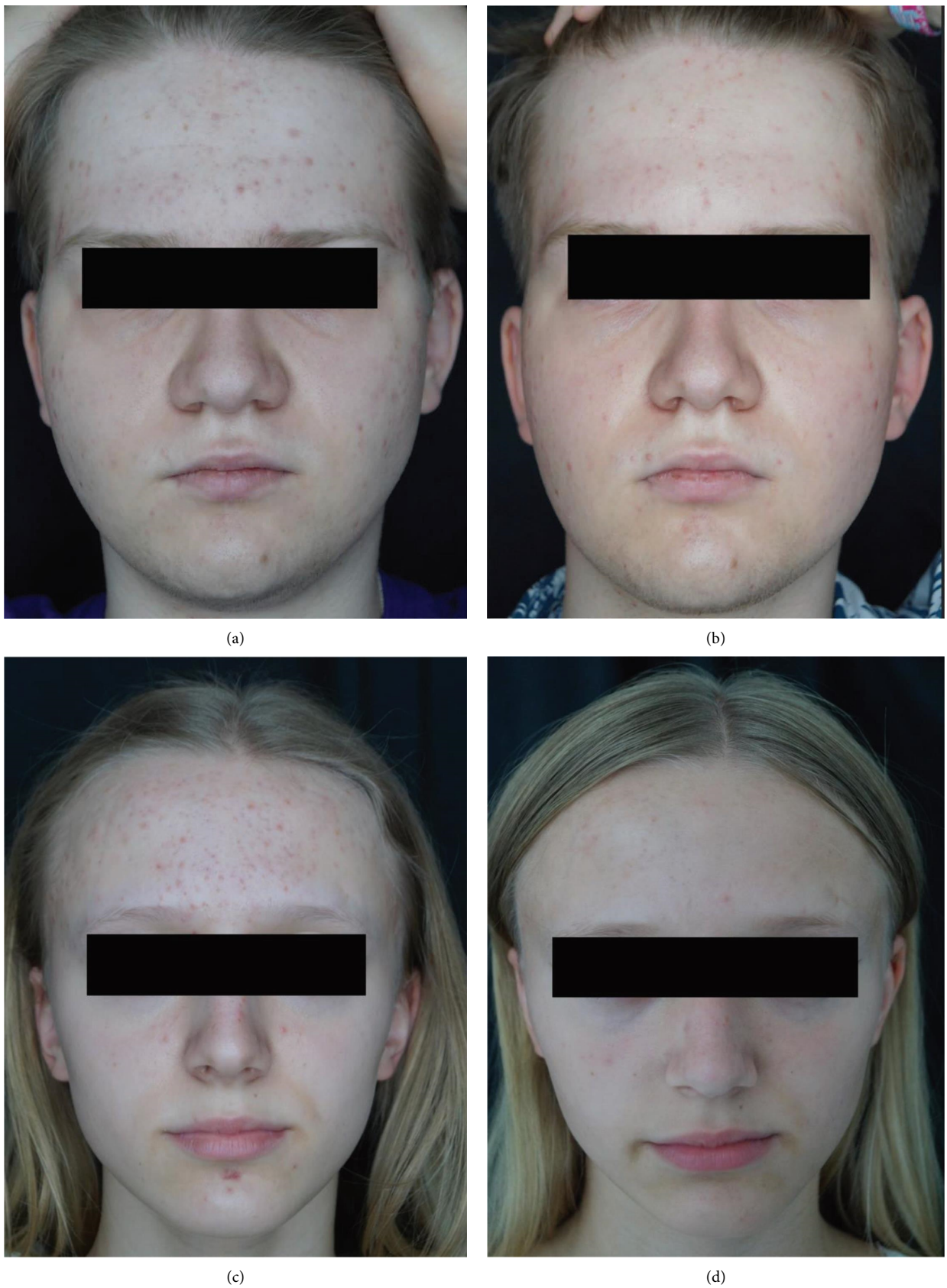


Figure 3: (a) Exemplary photos of one participant of the control group treated at baseline and (b) 6 weeks after being treated with a placebo cold atmospheric (CAP) device. (c) Another participant of the verum group at baseline and (d) 6 weeks after being treated with a verum CAP device.

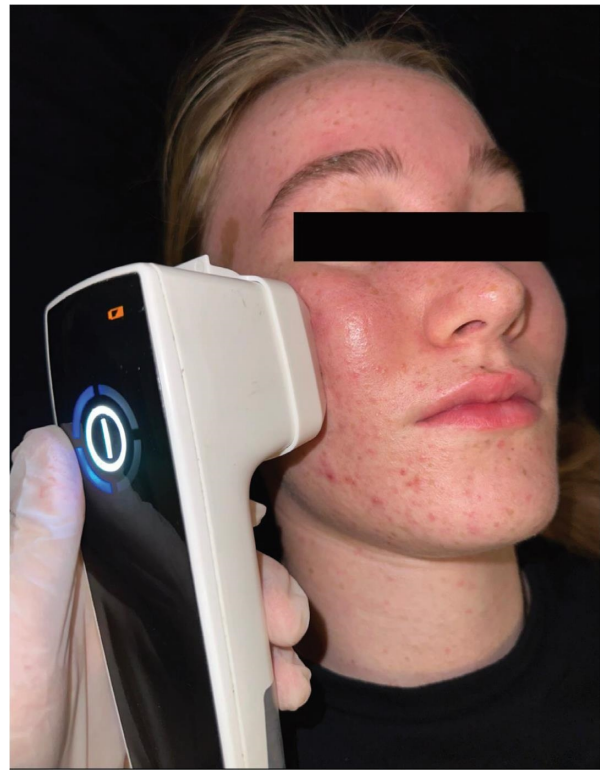


Figure 4: Application of the handheld cold atmospheric plasma generator ("plasma care" by Terraplasma medical GmbH, Germany) with a sterile spacer attached for the six facial regions during each treatment cycle.

Table 1: Participant characteristics at baseline.

	Verum	Control	Total
Randomized participants, *n* (%)	20	20	40
Drop out from study, *n* (weeks)	0	1 (1)	1 (1)
Loss to follow-up, *n* (weeks)	1 (8)	1 (8)	2 (8.8)
Mean age (±SD)	19.05 (3.24)	18.74 (4.79)	18.89 (4.06)
Min., max	14, 26	13, 31	13, 31
Sex			
Male, *n* (%)	5 (12.8)	8 (20.5)	13 (33.3)
Female, *n* (%)	15 (38.4)	11 (28.2)	26 (66.6)
Skin type			
FST I, *n* (%)	0	1 (5.2)	1 (2.5)
FST II, *n* (%)	1 (5)	0	1 (2.5)
FST III, *n* (%)	18 (90)	18 (94.7)	36 (92.3)
FST IV, *n* (%)	1 (5)	0	1 (2.5)

Abbreviations: FST = Fitzpatrick skin type, SD = standard deviation.

Table 2: Adverse events.

	Verum (*n* = 20)	Control (*n* = 19)	Total (*n* = 39)
Total participants with AE, *n* (%)	5 (25)	16 (84.2)	21 (53.8)
Total AE occurrence, *n* (%)	6 (13.6)	38 (86.3)	44
Itch	2 (4.5)	8 (18.1)	10 (22.7)
Pain	0	4 (9.1)	4 (9.1)
Erythema	1 (2.2)	6 (13.6)	7 (15.9)
Burning	3 (6.8)	20 (45.4)	23 (52.3)

Abbreviation: AE = adverse events.

toward endpoint ($p < 0.001$). In total, 44 AEs were observed in 21 participants, with a total of six AEs in the verum group in 5 out of 20 participants versus a total of 38 in the control group in 16 out of 19 participants ($p = 0.002$) (see Table 2). On average, less AEs from each subtype including burning, erythema, pain, and itch were observed in the verum group $M = 0.3$, 95% CI (0, 0.6) versus the control group $M = 2$ (95% CI [1, 3], [$p = 0.0009$]).

4. Discussion

This study demonstrates for the first time in a randomized controlled, double-blind study that adapalene treatment and skin cleansing improve acne symptoms more efficiently when combined with CAP as an add-on therapy. CAP application was planned once weekly since other protocols, for example, thrice weekly did not prove to be superior in the past for wound healing [36]. Next to SMD generators, other types exist like direct Dielectric Barrier Discharge (DBD) systems or indirect sources like plasma-needles, -jets, or-torches, each of which can potentially generate a different plasma "cocktail" depending on factors like the type of gas or the amount of power consumption [21]. Whereas in the few previous acne trials a plasma torch or DBD systems were used [31–33], the latter of which relies for the planar or rounded electrode to contact the human skin with its nonuniform surface as a grounded electrode, the handheld "Plasma care" SMD device has a planar-powered electrode connected to the grounded mesh electrode, thus not relying on direct contact with the skin. While for SMD devices a good bactericidal effect was demonstrated [19], it was furthermore utilized in this trial because of its easy handleability to cover the predefined facial zones during each treatment cycle, allowing the application of a reproducible dosage and therefore in combination with its low power consumption a good tolerability overall. Currently, the specific mechanisms of CAP application on skin physiology remain unclear. Some authors observed though that after CAP application, human sebum-resembling substrates expressed changes in NO-containing and acidic functional groups in vitro, suggesting a regenerative and bactericidal effect in the stratum corneum where CAP typically exerts its effects [37–39]. We included adapalene as the basic underlying treatment because it offers early effect onset and better tolerance than other retinoids and prevents microcomedone formation effectively [40]. This was beneficial for our comparative study since we needed to prevent scar formation effectively for both groups. Whereas acne lesion counting often includes next to inflammatory lesions like papules and pustules also noninflammatory lesions like comedones, we excluded the latter as mainly inflammatory lesions occur in acne papulopustulosa, thereby increasing the focus on TILC and avoiding result distortion. This was also beneficiary to elucidate the possible anti-inflammatory role of CAP by the greater reduction of TILC in the verum group and its possible reduction in inflammatory-related AE. Nevertheless, IGA score, which additionally included comedones, showed an identical trend of reduction for both groups compared to TILC, most probably because mainly papules and pustules were present in the first place.

Lesion counting including TILC was reportedly more sensitive for the evaluation of acne severity compared to TPC alone, which is why we combined both for a stronger correlation [41]. The authors observed for adapalene 0.1% application after 12 weeks, a 15% reduction of TILC in the verum group ($n = 162$) versus a 5% reduction in the placebo group ($n = 167$) Reference [42]. These mentioned verum group results are comparable with the mean TILC reduction in our control group from baseline to endpoint (21.9%). Since we combined adapalene therapy with skin cleansing, this suggests a sufficient basic self-treatment adherence of participants throughout the study. Even though weekly pustule cleansing is uncommon during acne treatment with adapalene, it was performed in this study prior to CAP application in order to allow next to the diffusion of longer-lived RONS like H_2O_2 or NO_3^- into the skin additionally the unhindered penetrance of shorter-lived species like $^{\bullet}OH$ and $O_2^{\bullet-}$ into the opened, inflamed pustular cavities. Although sebum overproduction plays a key role for acne development and sebum levels were generally higher in individuals with acne symptoms compared to healthy controls [43], we registered a slight divergence between the verum versus control group at baseline even though both groups had comparable TILC and IGA scores on average at baseline. This confirms the previously made observation that in general, the degree of facial sebum levels cannot be correlated with precision topographically with the extent of acne lesion [43]. In the past, it was demonstrated that the sebum production in two treatment-refractory acne participants was reduced markedly after CAP exposure [33], which correlates also with our findings. Also, it was found that *C. acnes* was highly sensitive to NO in nanoparticles (NPs), which were able to suppress TNF alpha, IL-1b, IL6, and IL8 in human monocytes. While NO is also formed in CAP, this suggests that, on one hand, NO-NP can effectively reduce *C. acnes* colonization directly and, on the other hand, reduce the subsequently induced release of inflammatory mediators [44]. Although it remains unclear which exact role *C. acnes* plays in the development of inflammatory lesions during acne pathogenesis [26], a recent trial by Jahns et al. suggested that improper sampling may have falsely disregarded its role. Through histological in vivo tissue sampling of acne lesions, they showed for the first time to our understanding that *C. acnes* is able to form macrocolonies and biofilms in sebaceous follicles and *C. acnes* also occurred more frequently in samples of acne lesions than in control samples [45]. To avoid invasive tissue sampling in our study, TPC was performed, which correlates proportionally to the follicular colonization of *C. acnes*, sebum levels, and clinical improvement overall [41, 46, 47]. It is important to mention though that different mechanisms exist, which may interfere with porphyrin signal detection. These include the reduction of *C. acnes* colonization directly or indirectly via inhibited sebum production, the suppression of porphyrin production by interfering with intracellular pathways, or chemical altercation of porphyrins through therapeutic agents [48]. As

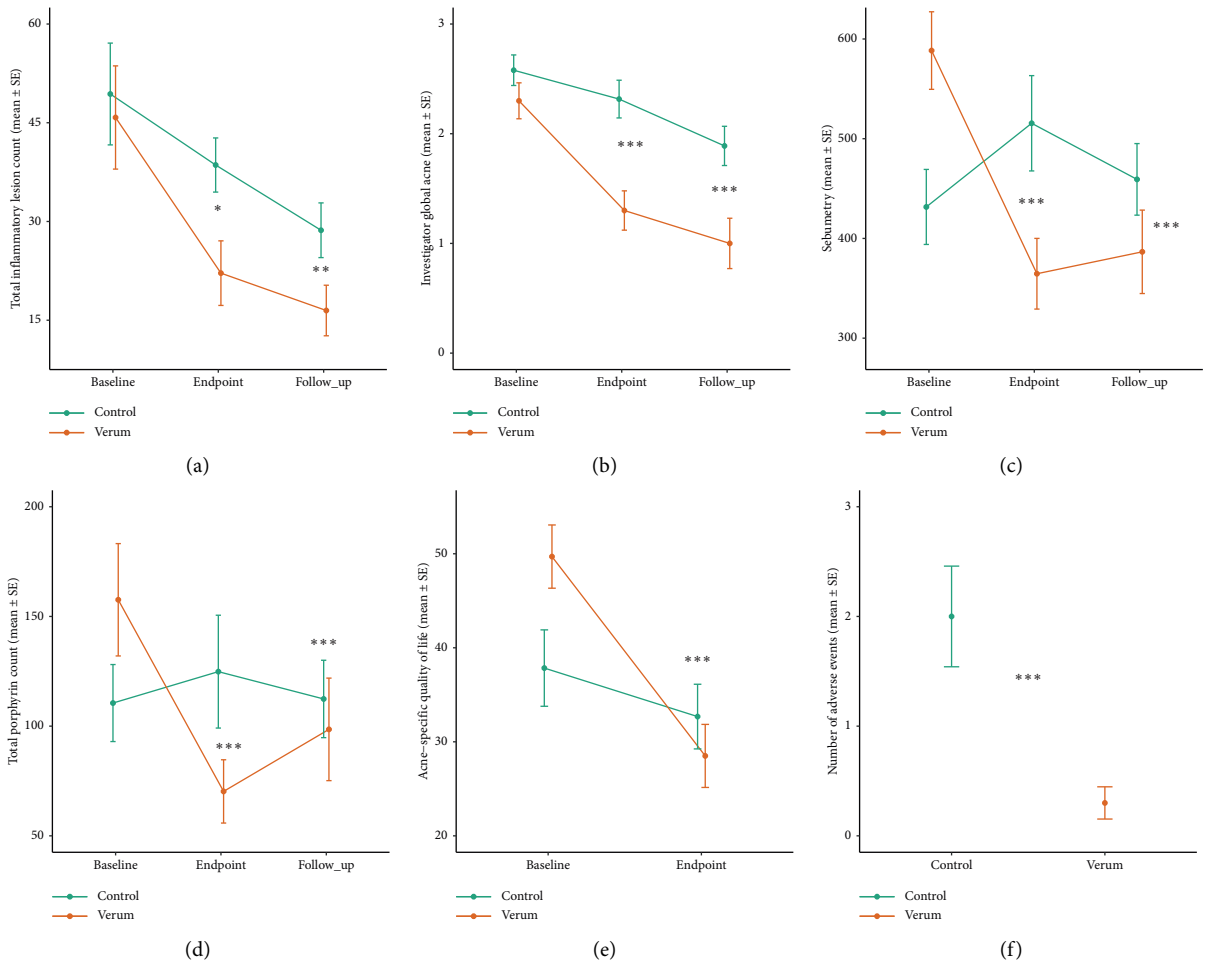


FIGURE 5: Panels showing means with the error bars representing ± standard error of measurements at baseline, endpoint at week 6, and follow-up at week 10 of primary endpoints (a) and (b) and secondary endpoints (c–f) ($n = 39$). $^{*}p = 0.07$; $^{**}p = 0.48$; $^{***}p < 0.05$. (a) Total inflammatory lesion count (TILC), (b) mean investigator global acne (IGA), (c) sebumetry, (d) total porphyrin count (TPC), (e) acne-specific quality of life (acne−QoL), and (f) adverse events.

expected without integrated antiseptic therapy component in the control group, TPC remained fairly constant. On the contrary, we observed a significant reduction of TPC in the verum group at endpoint; see Figure 5. However, this effect reversed during follow-up, which indicates a bacterial recolonization once CAP treatment is stopped. The phenomenon of *C. acnes* recolonization after treatment cessation was also described by Chien et al. who observed a 1.4-fold reduction after 4 weeks of minocycline therapy and an increase during follow-up to baseline value 8 weeks after discontinuation of the treatment [49]. AQOL was chosen since it proved to be a sensitive tool to correlate specifically acne-treatment-related changes regarding participants' quality of life [35]. We observed that greater clinical improvement at endpoint in the verum group also resulted in greater improvement in the quality of life. The type of AE and their progression during treatment-segment resemble the well-known, expected AE associated with adapalene application. These include burning, itching, and erythema, which typically peak in the first 2–4 weeks of topical treatment, followed then by a decline of symptoms [42, 50]. AE especially burning and erythema occurred less frequently in the verum group overall with none higher than 2 out of 4 hence labeled "nonserious" and not leading to any trial withdrawal. Thus, we conclude that CAP treatment offers an overall good tolerability, which also correlates with findings of previous studies [11, 12]. Since AE occurred less frequently in the verum group, this may indicate a potential beneficiary role of CAP to reduce AE including adapalene-associated ones, which could potentially improve the general treatment adherence overall [51]. This finding should be further investigated but correlates well with the previously described effect of CAP on inducing macrophages, M2-phenotype differentiation, and migration, which are associated with anti-inflammatory and wound-healing properties [52].

Possible limitations of our study are the small cohort, the short study duration, and a mainly Caucasian skin type. Selection and detection bias were both circumvented to our best knowledge through envelope-sealed allocation and

blinding. Manual lesion manipulation by participants could potentially cause performance bias and distort the measured clinical improvement in any acne trial, but participants were educated before the trial to avoid this consequently. This was confirmed by participants weekly. The sensitivity of TILC depends greatly on the expertise of the analyzing professional and the quality of the photographs. Other limitations are the small contact zones of the Visiopor-camera and the Sebumeter, which represent only small facial skin areas, making it challenging to aim for the exact anatomic location during the appointments. Thus, we implemented five standardized measurement areas and six standardized treatment zones independently from the varying acne distribution of the participants' facial areas to maintain good reproducibility.

5. Conclusion

Our study indicates that CAP may provide a new efficient and well-tolerable add-on therapy for acne papulopustulosa. Since this is a pilot study testing a new treatment approach with a small cohort, further research should be pursued with greater cohorts and longer trial duration, comparing CAP with adapalene directly or with a true placebo group, which may provide further insight into the emerging field of applied plasma medicine, with the focus on the treatment of acne.

Nomenclature

CAP	Cold atmospheric plasma.
IGA score	Investigator Global Assessment score
TPC	Total porphyrin count
TILC	Total inflammatory lesion count
AQOL	Acne-specific Quality of Life
questionnaire	Questionnaire
AE	Adverse events

Data Availability Statement

The data are not publicly available due to privacy or ethical restrictions. The supplementary, the study protocol, and the supporting information will be provided upon reasonable request by the corresponding author.

Ethics Statement

The trial was conducted under the ethical principles of the Declaration of Helsinki and was submitted and approved by the ethics commission of the "Landesärztekammer Brandenburg", Germany (2021-2120-BO-ff) on July 19, 2023.

Conflicts of Interest

Melvin Bae received a solitary honorarium as a congress-speaker by Terraplasma medical GmbH, the producer of the medical device under study. The other authors declare no conflicts of interest.

Author Contributions

Melvin Bae, Jürgen Lademann, Martina Meinke, and Björn Meder had full access to all of the data in this study, while Christoph Geilen was additionally blinded from the allocation sequence for neutral TILC and IGA assessments. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published. Melvin Bae wrote the manuscript, and the co-authors have read and approved the final manuscript.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. *(Supporting Information)*

"Therapeutic use of cold atmospheric plasma for the treatment of mild acne papulopustulosa-supporting material.html" containing raw data of measurements, *R*-codes, and graphs.

References

[1] B. Rzany and C. Kahl, "Epidemiology of Acne Vulgaris," *Journal der Deutschen Dermatologischen Gesellschaft* 4, no. 1 (2006): 8–9, https://doi.org/10.1111/j.1610-0387.2005.05876.x.

[2] T. Vos, A. D. Flaxman, M. Naghavi, et al., "Years Lived With Disability (YLDs) for 1160 Sequelae of 289 Diseases and Injuries 1990-2010: A Systematic Analysis for the Global Burden of Disease Study 2010," *The Lancet* 380, no. 9859 (2012): 2163–2196, https://doi.org/10.1016/S0140-6736(12)61729-2.

[3] A. M. Layton, D. Thiboutot, and J. Tan, "Reviewing the Global Burden of Acne: How Could We Improve Care to Reduce the Burden?" *British Journal of Dermatology* 184, no. 2 (2021): 219–225, https://doi.org/10.1111/bjd.19477.

[4] J. W. Lee, B. J. Kim, M. N. Kim, G. Y. Ahn, and H. Aso, "Selective Sebaceous Gland Electrothermolysis as a Treatment for Acne: A Prospective Pilot Study," *International Journal of Dermatology* 51, no. 3 (2012): 339–344, https://doi.org/10.1111/j.1365-4632.2011.05255.x.

[5] L. Liu, Y. Xue, Y. Chen, et al., "Prevalence and Risk Factors of Acne Scars in Patients With Acne Vulgaris," *Skin Research and Technology* 29, no. 6 (2023): e13386, https://doi.org/10.1111/srt.13386.

[6] O. Revol, N. Milliez, and D. Gerard, "Psychological Impact of Acne on 21st-Century Adolescents: Decoding for Better Care," *British Journal of Dermatology* 172, no. 1 (2015): 52–58, https://doi.org/10.1111/bjd.13749.

[7] B. Dreno, D. Thiboutot, H. Gollnick, et al., "Large-Scale Worldwide Observational Study of Adherence With Acne Therapy," *International Journal of Dermatology* 49, no. 4 (2010): 448–456, https://doi.org/10.1111/j.1365-4632.2010.04416.x.

[8] J. Kraft and A. Freiman, "Management of Acne," *Canadian Medical Association Journal* 183, no. 7 (2011): E430–E435, https://doi.org/10.1503/cmaj.090374.

[9] C. Y. Huang, I. J. Chang, N. Bolick, et al., "Comparative Efficacy of Pharmacological Treatments for Acne Vulgaris: A Network Meta-Analysis of 221 Randomized Controlled Trials," *The Annals of Family Medicine* 21, no. 4 (2023): 358–369, https://doi.org/10.1370/afm.2995.

[10] J. I. Ross, A. M. Snelling, E. Carnegie, et al., "Antibiotic-Resistant Acne: Lessons From Europe," *British Journal of Dermatology* 148, no. 3 (2003): 467–478, https://doi.org/10.1046/j.1365-2133.2003.05067.x.

[11] G. Daeschlein, S. Scholz, R. Ahmed, et al., "Cold Plasma is Well-Tolerated and Does Not Disturb Skin Barrier or Reduce Skin Moisture," *Journal der Deutschen Dermatologischen Gesellschaft* 10, no. 7 (2012): 509–515, https://doi.org/10.1111/j.1610-0387.2012.07857.x.

[12] R. Rutkowski, G. Daeschlein, T. von Woedtke, R. Smeets, M. Gosau, and H. R. Metelmann, "Long-Term Risk Assessment for Medical Application of Cold Atmospheric Pressure Plasma," *Diagnostics* 10, no. 4 (2020): 210, https://doi.org/10.3390/diagnostics10040210.

[13] T. Von Woedtke, A. Schmidt, S. Bekeschus, K. Wende, and K. D. Weltmann, "Plasma Medicine: A Field of Applied Redox Biology," *In Vivo* 33, no. 4 (2019): 1011–1026, https://doi.org/10.21873/invivo.11570.

[14] S. Rupf, A. Lehmann, M. Hannig, et al., "Killing of Adherent Oral Microbes by a Non-Thermal Atmospheric Plasma Jet," *Journal of Medical Microbiology* 59, no. 2 (2010): 206–212, https://doi.org/10.1099/jmm.0.013714-0.

[15] S. Kalghatgi, G. Friedman, A. Fridman, and A. M. Clyne, "Endothelial Cell Proliferation is Enhanced by Low Dose Non-Thermal Plasma Through Fibroblast Growth Factor-2 Release," *Annals of Biomedical Engineering* 38, no. 3 (2010): 748–757, https://doi.org/10.1007/s10439-009-9868-x.

[16] J. W. Choi, S. U. Kang, Y. E. Kim, et al., "Novel Therapeutic Effects of Non-Thermal Atmospheric Pressure Plasma for Muscle Regeneration and Differentiation," *Scientific Reports* 6, no. 1 (2016): 28829, https://doi.org/10.1038/srep28829.

[17] S. Hasse, T. Duong Tran, O. Hahn, et al., "Induction of Proliferation of Basal Epidermal Keratinocytes by Cold Atmospheric-Pressure Plasma," *Clinical and Experimental Dermatology* 41, no. 2 (2016): 202–209, https://doi.org/10.1111/ced.12735.

[18] B. Boekema, M. Stoop, M. Vlig, et al., "Antibacterial and Safety Tests of a Flexible Cold Atmospheric Plasma Device for the Stimulation of Wound Healing," *Applied Microbiology and Biotechnology* 105, no. 5 (2021): 2057–2070, https://doi.org/10.1007/s00253-021-11166-5.

[19] T. G. Klampfl, G. Isbary, T. Shimizu, et al., "Cold Atmospheric Air Plasma Sterilization Against Spores and Other Microorganisms of Clinical Interest," *Applied and Environmental Microbiology* 78, no. 15 (2012): 5077–5082, https://doi.org/10.1128/AEM.00583-12.

[20] Y. Sakiyama, D. B. Graves, H.-W. Chang, T. Shimizu, and G. E. Morfill, "Plasma Chemistry Model of Surface Microdischarge in Humid Air and Dynamics of Reactive Neutral Species," *Journal of Physics D: Applied Physics* 45, no. 42 (2012): 425201, https://doi.org/10.1088/0022-3727/45/42/425201.

[21] G. Daeschlein, "Antimicrobial Activity of Plasma," in *Comprehensive Clinical Plasma Medicine*, eds. T. v. Metelmann HRW and K.-D. Weltmann (Cham, Switzerland: Springer International Publishing, 2018), 114.

[22] D. Brany, D. Dvorska, E. Halasova, and H. Skovierova, "Cold Atmospheric Plasma: A Powerful Tool for Modern Medicine," *International Journal of Molecular Sciences* 21, no. 8 (2020): 2932, https://doi.org/10.3390/ijms21082932.

[23] I. Niedzwiedz, A. Wasko, J. Pawlat, and M. Polak-Berecka, "The State of Research on Antimicrobial Activity of Cold Plasma," *Polish Journal of Microbiology* 68, no. 2 (2019): 153–164, https://doi.org/10.33073/pjm-2019-028.

[24] J. L. Zimmermann, T. Shimizu, H. U. Schmidt, Y. F. Li, G. E. Morfill, and G. Isbary, "Test for Bacterial Resistance Build-Up Against Plasma Treatment," *New Journal of Physics* 14, no. 7 (2012): 073037, https://doi.org/10.1088/1367-2630/14/7/073037.

[25] R. Matthes, O. Assadian, and A. Kramer, "Repeated Applications of Cold Atmospheric Pressure Plasma Does Not Induce Resistance in *Staphylococcus aureus* Embedded in Biofilms," *GMS Hygiene and Infection Control* 9, no. 3 (2014): Doc17, https://doi.org/10.3205/dgkh000237.

[26] J. J. Leyden, K. J. McGinley, and B. Vowels, "Propionibacterium Acnes Colonization in Acne and Nonacne," *Dermatology* 196, no. 1 (1998): 55–58, https://doi.org/10.1159/000017868.

[27] V. Scholtz, E. Vankova, P. Kasparova, R. Premanath, I. Karunasagar, and J. Julak, "Non-Thermal Plasma Treatment of ESKAPE Pathogens: A Review," *Frontiers in Microbiology* 12 (2021): 737635, https://doi.org/10.3389/fmicb.2021.737635.

[28] M. A. Dagnelie, S. Corvec, M. Saint-Jean, et al., "Decrease in Diversity of Propionibacterium Acnes Phylotypes in Patients With Severe Acne on the Back," *Acta Dermato-Venereologica* 98, no. 2 (2018): 262–267, https://doi.org/10.2340/00015555-2847.

[29] M. P. Cros, J. Mir-Pedrol, L. Toloza, et al., "New Insights Into the Role of Cutibacterium Acnes-Derived Extracellular Vesicles in Inflammatory Skin Disorders," *Scientific Reports* 13, no. 1 (2023): 16058, https://doi.org/10.1038/s41598-023-43354-w.

[30] A. Ali, Y. H. Kim, J. Y. Lee, et al., "Inactivation of Propionibacterium Acnes and its Biofilm by Non-Thermal Plasma," *Current Applied Physics* 14 (2014): S142–S148, https://doi.org/10.1016/j.cap.2013.12.034.

[31] C. Chutsirimongkol, D. Boonyawan, N. Polnikorn, W. Techawatthanawisan, and T. Kundilokchai, "Non-Thermal Plasma for Acne and Aesthetic Skin Improvement," *Plasma Medicine* 4, no. 1-4 (2014): 79–88, https://doi.org/10.1615/PlasmaMed.2014011952.

[32] X. Wu, Y. Yang, Y. Wang, et al., "Treatment of Refractory Acne Using Selective Sebaceous Gland Electro-Thermolysis Combined With Non-Thermal Plasma," *Journal of Cosmetic and Laser Therapy* 23, no. 7-8 (2021): 188–194, https://doi.org/10.1080/14764172.2022.2050760.

[33] A. Mariachiara, V. Anna, G. Alessandra, et al., "Cold Atmospheric Plasma (CAP) as a Promising Therapeutic Option for Mild to Moderate Acne Vulgaris: Clinical and Non-Invasive Evaluation of Two Cases," *Clinical Plasma Medicine* 19-20 (2020): 100110, https://doi.org/10.1016/j.cpme.2020.100110.

[34] "Sealed Envelope Ltd. 2022. Create a Blocked Randomisation List," (2023), https://www.sealedenvelope.com/simple-randomiser/v1/lists.

[35] S. E. Fehnel, L. D. McLeod, J. Brandman, et al., "Responsiveness of the Acne-Specific Quality of Life

Questionnaire (Acne-QoL) to Treatment for Acne Vulgaris in Placebo-Controlled Clinical Trials," *Quality of Life Research: An International Journal of Quality of Life Aspects of Treatment, Care and Rehabilitation* 11, no. 8 (2002): 809–816, https://doi.org/10.1023/a:1020880005846.

[36] M. Moelleken, F. Jockenhofer, C. Wiegand, J. Buer, S. Benson, and J. Dissemond, "Pilot Study on the Influence of Cold Atmospheric Plasma on Bacterial Contamination and Healing Tendency of Chronic Wounds," *Journal der Deutschen Dermatologischen Gesellschaft* 18, no. 10 (2020): 1094–1101, https://doi.org/10.1111/ddg.14294.

[37] K. Kartaschew, M. Mischo, S. Baldus, E. Brundermann, P. Awakowicz, and M. Havenith, "Unraveling the Interactions Between Cold Atmospheric Plasma and Skin-Components With Vibrational Microspectroscopy," *Biointerphases* 10, no. 2 (2015): 029516, https://doi.org/10.1116/1.4919610.

[38] A. Schmidt, G. Liebelt, J Striesow, et al., "The Molecular and Physiological Consequences of Cold Plasma Treatment in Murine Skin and its Barrier Function," *Free Radical Biology and Medicine* 161 (2020): 32–49, https://doi.org/10.1016/j.freeradbiomed.2020.09.026.

[39] F. Tan, Y. Wang, S. Zhang, R. Shui, and J. Chen, "Plasma Dermatology: Skin Therapy Using Cold Atmospheric Plasma," *Frontiers in Oncology* 12 (2022): 918484, https://doi.org/10.3389/fonc.2022.918484.

[40] L. Tolaymat, H. Dearborn, and P. M. Zito, in *Adapalene* (Treasure Island, FL: StatPearls, 2023).

[41] C. Richter, C. Trojahn, G. Dobos, U. Blume-Peytavi, and J. Kottner, "Follicular Fluorescence Quantity to Characterize Acne Severity: A Validation Study," *Skin Research and Technology* 22, no. 4 (2016): 451–459, https://doi.org/10.1111/srt.12286.

[42] "Product Monograph Pr Differin Adapalene Topical Cream 0.1% W/w, Adapalene Topical Gel 0.1% W/w, Adapalene Topical Lotion 0.1% W/w, Pr DIFFERIN XP Adapalene Topical Gel 0.3% W/w, Acne Therapy," (2023), https://www.galderma.com/sites/default/files/2021-10/Differin%20adapalene_PM_E_20210928.pdf.

[43] S. W. Youn, E. S. Park, D. H. Lee, C. H. Huh, and K. C. Park, "Does Facial Sebum Excretion Really Affect the Development of Acne?" *British Journal of Dermatology* 153, no. 5 (2005): 919–924, https://doi.org/10.1111/j.1365-2133.2005.06794.x.

[44] M. Qin, A. Landriscina, J. M. Rosen, et al., "Nitric Oxide-Releasing Nanoparticles Prevent Propionibacterium Acnes-Induced Inflammation by Both Clearing the Organism and Inhibiting Microbial Stimulation of the Innate Immune Response," *Journal of Investigative Dermatology* 135, no. 11 (2015): 2723–2731, https://doi.org/10.1038/jid.2015.277.

[45] A. C. Jahns, B. Lundskog, R. Ganceviciene, et al., "An Increased Incidence of Propionibacterium Acnes Biofilms in Acne Vulgaris: A Case-Control Study," *British Journal of Dermatology* 167, no. 1 (2012): 50–58, https://doi.org/10.1111/j.1365-2133.2012.10897.x.

[46] C. Borelli, K. Merk, M. Schaller, et al., "In Vivo Porphyrin Production by P. Acnes in Untreated Acne Patients and Its Modulation by Acne Treatment," *Acta Dermato-Venereologica* 86, no. 4 (2006): 316–319, https://doi.org/10.2340/00015555-0088.

[47] L. C. Lucchina, N. Kollias, R. Gillies, et al., "Fluorescence Photography in the Evaluation of Acne," *Journal of the American Academy of Dermatology* 35, no. 1 (1996): 58–63, https://doi.org/10.1016/S0190-9622(96)90497-1.

[48] H. Dobrev, "Fluorescence Diagnostic Imaging in Patients With Acne," *Photodermatology, Photoimmunology & Photomedicine* 26, no. 6 (2010): 285–289, https://doi.org/10.1111/j.1600-0781.2010.00541.x.

[49] A. L. Chien, J. Tsai, S. Leung, et al., "Association of Systemic Antibiotic Treatment of Acne With Skin Microbiota Characteristics," *Journal of the American Medical Association Dermatol* 155, no. 4 (2019): 425–434.

[50] J. H. Herndon, T. J. Stephens, N. S. Trookman, et al., "A Comparison of the Tolerability of Adapalene 0.1% Cream and Adapalene 0.1% Lotion in Healthy Individuals," *Skinmed* 10, no. 3 (2012): 136–142.

[51] S. Arndt, M. Landthaler, J. L. Zimmermann, et al., "Effects of Cold Atmospheric Plasma (CAP) on SS-Defensins, Inflammatory Cytokines, and Apoptosis-Related Molecules in Keratinocytes In Vitro and In Vivo," *PLoS One* 10, no. 3 (2015): e0120041, https://doi.org/10.1371/journal.pone.0120041.

[52] Y. Zhang, S. Choksi, K. Chen, Y. Pobezinskaya, I. Linnoila, and Z. G. Liu, "ROS Play a Critical Role in the Differentiation of Alternatively Activated Macrophages and the Occurrence of Tumor-Associated Macrophages," *Cell Research* 23, no. 7 (2013): 898–914, https://doi.org/10.1038/cr.2013.75.