Clinical and Translational Radiation Oncology 44 (2024) 100699

Contents lists available at ScienceDirect

Clinical and Translational Radiation Oncology

journal homepage: www.sciencedirect.com/journal/clinical-and-translational-radiation-oncology

Original Research Article

Non-invasive physical plasma for preventing radiation dermatitis in breast cancer: Results from an intrapatient-randomised double-blind placebo-controlled trial

Cas Stefaan Dejonckheere [a], Julian Philipp Layer [a,b], Younèss Nour [a], Katharina Layer [a], Andrea Glasmacher [a], Shari Wiegreffe [a], Arne Fuhrmann [a], Lara Caglayan [a], Franziska Grau [a], Gustavo Renato Sarria [a], Davide Scafa [a], David Koch [a], Martina Heimann [a], Christina Leitzen [a], Mümtaz Ali Köksal [a], Fred Röhner [a], Thomas Müdder [a], Egon Dejonckheere [c,d], Frederic Carsten Schmeel [e], Teresa Anzböck [f], Kira Lindner [g], Anne Bachmann [g], Alina Abramian [g], Christina Kaiser [g], Andree Faridi [g], Alexander Mustea [f], Frank Anton Giordano [h], Matthias Bernhard Stope [f], Leonard Christopher Schmeel [a,*]

[a] Department of Radiation Oncology, University Hospital Bonn, 53127 Bonn, Germany
[b] Institute of Experimental Oncology, University Hospital Bonn, 53127 Bonn, Germany
[c] Faculty of Psychology and Educational Sciences, KU Leuven, 3000 Leuven, Belgium
[d] Department of Medical and Clinical Psychology, Tilburg School of Social and Behavioural Sciences, 5037 Tilburg, the Netherlands
[e] Department of Neuroradiology, University Hospital Bonn, 53127 Bonn, Germany
[f] Department of Gynaecology, Division of Gynaecological Oncology, University Hospital Bonn, 53127 Bonn, Germany
[g] Department of Gynaecology, Division of Senology, University Hospital Bonn, 53127 Bonn, Germany
[h] Department of Radiation Oncology, University Medical Center Mannheim, 68167 Mannheim, Germany

ARTICLE INFO

Keywords:
Radiation dermatitis
Radiation therapy
Breast cancer
Non-invasive physical plasma
Cold atmospheric plasma
Randomised controlled trial

ABSTRACT

Background and Purpose: To investigate the effect of topical non-invasive physical plasma (NIPP), a volatile mix generated out of ambient air, on prevention of acute radiation dermatitis (RD) during and after whole-breast irradiation (WBI).

Materials and Methods: Lateral and medial breast halves were randomised within each patient to receive either 120 s of NIPP or sham treatment daily during WBI. Standard skin care with urea lotion was applied to the whole breast. Blinded acute skin toxicity was assessed weekly for each breast half separately and included clinician- (CTCAE) and patient-reported (modified RISRAS), and objective (spectrophotometry) assessments. As an additional external control, a comparable standard of care (SoC) patient collective from a previous prospective trial was used.

Results: Sixty-four patients were included. There were no significant differences between breast halves. Post-hoc comparison with a similar SoC control collective revealed OR = 0.28 (95% CI 0.11–0.76; $p = 0.014$) for grade ≥ 2 RD upon WBI completion, along with less hyperpigmentation ($p < 0.001$), oedema ($p = 0.020$), dry ($p < 0.001$) and moist desquamation ($p = 0.017$), pain, itching, and burning ($p < 0.001$ for each). Tolerability of NIPP was excellent and side effects were not observed.

Conclusion: Even though there were no differences between intrapatient-randomised breast halves, the overall incidence and severity of acute radiation-induced skin toxicity were considerably lower when compared to a prospectively collected SoC cohort. Our data suggest the potential benefit of NIPP in RD prevention. A randomised trial with a physical control group is warranted to confirm these promising results (DRKS00026225).

* Corresponding author at: Department of Radiation Oncology, University Hospital Bonn, Venusberg-Campus 1, 53127 Bonn, Germany.
E-mail address: christopher.schmeel@ukbonn.de (L.C. Schmeel).

https://doi.org/10.1016/j.ctro.2023.100699
Received 31 October 2023; Accepted 2 November 2023
Available online 4 November 2023
2405-6308/© 2023 Published by Elsevier B.V. on behalf of European Society for Radiotherapy and Oncology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Early breast cancer, one of the most common cancer diagnoses worldwide, involves surgical therapy followed by adjuvant whole-breast irradiation (WBI) in about 70% of cases [1,2]. The most frequent acute side effect of WBI is radiation dermatitis (RD), characterised by erythema, pruritus, pain, and dry or moist desquamation, which occur in up to 85% of patients [3,4]. Severe cases requiring radiation treatment interruption have become rare following advancements in radiation treatment technique (e.g. intensity-modulated radiation therapy [IMRT]) and the exploration of new fractionation regimens (e.g. hypofractionation); however, even mild symptoms are known to impact quality of life and self-image [5–7]. Continuous research efforts are being made, yet potent preventative and therapeutic options are limited, resulting in substantial variation in RD management amongst practitioners and institutions [8–10]. Due to insufficient and sometimes even contradictory evidence, a recommendation can currently only be made for a handful of interventions [11].

Non-invasive physical plasma (NIPP) is an emerging treatment modality for various skin conditions, such as psoriasis, eczema, diabetic ulcers, and different types of dermatitis [12,13]. Physical plasma, the fourth state of matter, is characterised by free electrons and derived from the other states of matter (gas, liquid, solid) by altering temperature or pressure [14]. Contrary to thermal plasma, NIPP is created using a high-frequency alternating field under atmospheric pressure, thereby only reaching body temperatures, rendering it safe for clinical use. Dielectric barrier discharges (DBD) are a type of NIPP, generated out of ambient air, without the need for a carrier gas [15]. The reactive mix of electrons, ions, excited atoms, reactive oxygen species (ROS), and ultraviolet radiation has been shown to positively affect tissue healing in a dose-dependent manner [16]. NIPP treatment is well-tolerated and multiple trials have confirmed its safety [17–19].

ROS play a crucial role in tissue damage mediated by ionising radiation, yet are also significantly involved in NIPP-associated tissue healing and regeneration [20]. A first-in-human benchmarking trial previously reported safety and feasibility of a topical DBD-generated NIPP-based prevention of acute RD [21]. In the current prospective, intrapatient-randomised, double-blind, placebo-controlled trial, we evaluate the effect of NIPP on the incidence and severity of RD and associated symptoms in breast cancer patients undergoing WBI.

Materials and methods

Participants

From March 2022 through September 2023, we conducted a monocentric phase 2 study enrolling breast cancer patients that were scheduled for adjuvant WBI. Inclusion criteria were age $\geq$ 18 years, breast-conserving surgery, and a moderately hypofractionated radiation regimen. A normofractionated sequential boost to the tumour bed was allowed. To avoid bias in RD grading, the following exclusion criteria were defined: synchronous metastatic disease, mastectomy, reconstruction with breast implant, alternative fractionation regimens, history of ipsilateral breast irradiation, any pre-existing dermatological disorder, active dermatitis, current treatment with topical or oral corticosteroids, and patient refusal to participate. Written informed consent was obtained from all included patients. This study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Institutional Review Board of the University Bonn in September 2021 (210/21).

Radiation protocol

All patients received 40.05 Gy in 15 fractions of 2.67 Gy each. Patients $\leq$ 50 years or those with risk factors ($\geq$pT2, HER2/neu positive, triple-negative, or poor cell differentiation) regardless of age received a sequential boost to the tumour bed of 16 Gy in 8 fractions of 2 Gy. Target volume delineation followed international standards [22]. The treatment technique used was 6 MV sliding window IMRT or hybrid 6 and 10 MV volumetric modulated (partial) arc therapy and the International Commission on Radiation Units and Measurements recommendations for dose limits of 95–107% were followed. All patients were treated on a TrueBeam STx (Varian Medical Systems, Palo Alto, CA, USA) linear accelerator in a supine position on a breast board. Left-sided WBI was performed in deep inspiration breath-hold (DIBH) for compliant patients. Post-hoc assessment of skin dose to lateral and medial breast halves is described in *Supplementary Fig. 1*.

Intrapatient control and standard skin care

The irradiated breast of each patient was evenly divided into a lateral and medial half. Using computer-generated random permuted blocks with sequentially numbered containers, one half was randomly assigned to receive NIPP treatment, while the other half served as an intrapatient control and received placebo. This design omits the need for stratification based on established risk factors influencing RD incidence and severity, such as breast size or Fitzpatrick skin type [23]. In those requiring a sequential boost, another acknowledged risk factor, breast halves were stratified accordingly (i.e. based on the primary breast half in which the boost area was located). Patients were blinded to their breast half assignment.

Institutional standard skin care with urea-based lotion (UreaRepair PLUS 5%, Eucerin, Beiersdorf, Hamburg, Germany) was applied to the whole breast. All patients were given oral and written information to apply it twice daily from the first day of treatment onwards and were encouraged not to use any complementary topical treatments. Compliance was checked on the scheduled patient visits. At the discretion of the principal investigator, those presenting with grade $\geq$ 2 RD, moist desquamation, or intense pain could be prescribed topical corticosteroids until symptoms resolved.

NIPP protocol

To generate and apply the NIPP, a wireless topical physical plasma device (plasma care, terraplasma medical, Garching, Germany) was employed. The device was pressed loosely on the patient's breast skin with a separate 4 $\times$ 4 cm spacer for each patient and each visit, to achieve an optimal and reproducible distance to the skin (Fig. 1). Based on the initial feasibility trial, the treatment time was set at 120 s, balancing the dose-dependent effect of NIPP with treatment time and using the device's preset program to ensure a constant dose of NIPP. This process was repeated until the entire assigned breast half had received treatment [24]. This process was repeated for the other (control) breast half, using an identical yet non-functional sham device. NIPP was applied daily following every radiation fraction by a trained radiation oncology nurse.

Patient evaluation

a *Clinician-reported outcome*

Patients were evaluated during the standard weekly on-treatment visits and any additional visits requested by the participants were also documented. RD was assessed for each breast half separately, according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE; version 5.0) [25]. Furthermore, hyperpigmentation, breast oedema, and desquamation (dry or moist) were assessed and recorded (yes–no) for each breast half separately. Upon radiation treatment completion, as well as two and six weeks later, acute toxicity was reassessed. The experienced breast radiation oncologists who performed the clinical assessments were blinded to the initial breast half assignment.



Fig. 1. Generation and application of NIPP on the breast skin. The device is pressed loosely on the skin with a separate 4 × 4 cm spacer for each patient and each visit. NIPP = non-invasive physical plasma. Image from Dejonckheere et al., with permission [21].

b *Patient-reported outcome*

At the end of the radiation course and during the two follow-up visits, the patient-assessed modified Radiation-Induced Skin Reaction Assessment Scale (RISRAS) was recorded [26]. All patients reported their maximum breast-related experience of pain, itching, burning, and limitations in daily activities, for each breast half separately. All items were scored on a 4-point Likert scale: 0 = not at all; 1 = a little; 2 = quite a bit; 3 = very much. During the last follow-up visit, patient-reported experiences were captured with four yes–no statements (*Supplementary Table 1*).

c *Objective assessment*

At baseline, during every visit, on the last day of radiation treatment, as well as during both follow-up visits, five erythema readings were performed with a reflectance spectrophotometer (CR-10 Plus, Konica Minolta, Tokyo, Japan), across both the NIPP-treated and control breast half (*Supplementary Fig. 2*). This compact device has previously been validated to objectively assess acute and chronic RD [23,27–29]. Its automatically performed measurements are based on the Commission Internationale de l'Éclairage system of tristimulus values and describe a measured colour in three coordinates using the L*a*b* system: lower L* values describe darker skin (hyperpigmentation) and higher a* values indicate redness (RD), whereas b* values describe the position on a scale from blue to yellow (of secondary importance in the acute setting).

Trial endpoints

The primary endpoint was the difference in clinician-assessed RD between NIPP and placebo breast halves upon treatment completion. Secondary endpoints were differences in grade ≥ 2 RD, hyperpigmentation, oedema, dry and moist desquamation, patient-reported outcomes, and spectrophotometric values. The evaluation of said endpoints compared to an identical standard of care (SoC) control collective from a previously published prospective trial on acute toxicity was added as a secondary endpoint upon completion of the study [4]. The latter trial had very similar inclusion and exclusion criteria, identical fractionation and radiation treatment technique, and outcome assessment.

Statistical analysis and visualisation of results

As prior studies on this topic are lacking, we estimated a meaningful reduction in clinician-assessed RD severity upon radiation treatment completion of 33%. Assuming at least 80% statistical power and a type I error rate of 5% for the two-tailed *t*-test, a minimum sample size of $n = 63$ was needed for this intrapatient-randomised trial design. Mean, median, standard deviation (SD), and range were calculated for all applicable clinical data. For the pairwise comparison of categorical variables (clinician- and patient-assessed outcomes), the Mann-Whitney-*U* test was used and odds ratios (OR) with 95% confidence intervals (CI) were calculated. Objective spectrophotometric data were compared with Welch's *t*-test, after ensuring homoscedasticity with Levene's test. The statistical significance level was defined as $p < 0.05$. Microsoft Excel (version 16; Microsoft, Redmond, WA, USA), SPSS Statistics (version 27; IBM, Armonk, NY, USA), and GraphPad Prism (version 10; GraphPad Software, San Diego, CA, USA) were used to perform the analyses. GraphPad Prism and Adobe Illustrator 2023 (Adobe Inc., Mountain View, CA, USA) were used to generate graphs. The graphical abstract was created with BioRender.com.

Results

Patient and treatment characteristics

A Consolidated Standards of Reporting Trials (CONSORT) flowchart of patient selection and inclusion is shown in Fig. 2. Sixty-four patients completed the trial per protocol, with a median age (range) of 58 (29–83) years. All patients were female, 97% Caucasian, and Fitzpatrick II was the most common skin type (70%). A sequential boost to the tumour bed was delivered in 59% and 9% received topical corticosteroids to alleviate RD-induced symptoms. The generalised equivalent uniform dose (gEUD) to NIPP and placebo breast skin was similar ($p = 0.734$), implying a homogeneous dose distribution with a subsequent similar probability to develop acute RD.

When compared to the SoC collective, baseline characteristics were very similar. In the SoC collective, fewer patients received a sequential boost (44% versus 59%; $p = 0.081$), the mean breast size was significantly smaller ($p = 0.007$), and fewer patients received neoadjuvant systemic therapy ($p < 0.001$), implying that these patients were at an overall lower risk of developing RD. Patient and radiation treatment characteristics for both collectives are presented in Table 1.

Clinician-reported outcome

Mean ± SD graded severity of RD upon radiation treatment completion was 0.79 ± 0.60 for NIPP halves versus 0.83 ± 0.52 for placebo halves ($p = 0.644$). There were no statistically significant differences between NIPP and placebo halves in terms of physician-assessed RD, hyperpigmentation, oedema, and dry and moist desquamation over the course of treatment and follow-up.

The mean ± SD RD severity upon radiation treatment completion was 1.06 ± 0.71 in the SoC control collective ($p = 0.004$; OR = 0.28 [95% CI 0.11–0.76; $p = 0.014$] for grade ≥ 2 RD). After two weeks, this had dropped to 0.27 ± 0.55 in NIPP patients, however, remained at 1.05 ± 0.70 in SoC controls ($p < 0.001$). At six weeks, there was no significant difference (0.04 ± 0.19 versus 0.10 ± 0.30; $p = 0.156$). Upon treatment completion, there was an overall lower maximum toxicity ($p < 0.001$) with less hyperpigmentation ($p < 0.001$), oedema ($p = 0.020$), and dry and moist desquamation ($p < 0.001$ and $p = 0.017$, respectively) in patients receiving NIPP. Results are summarised in Fig. 3a and Fig. 4*a–c*.

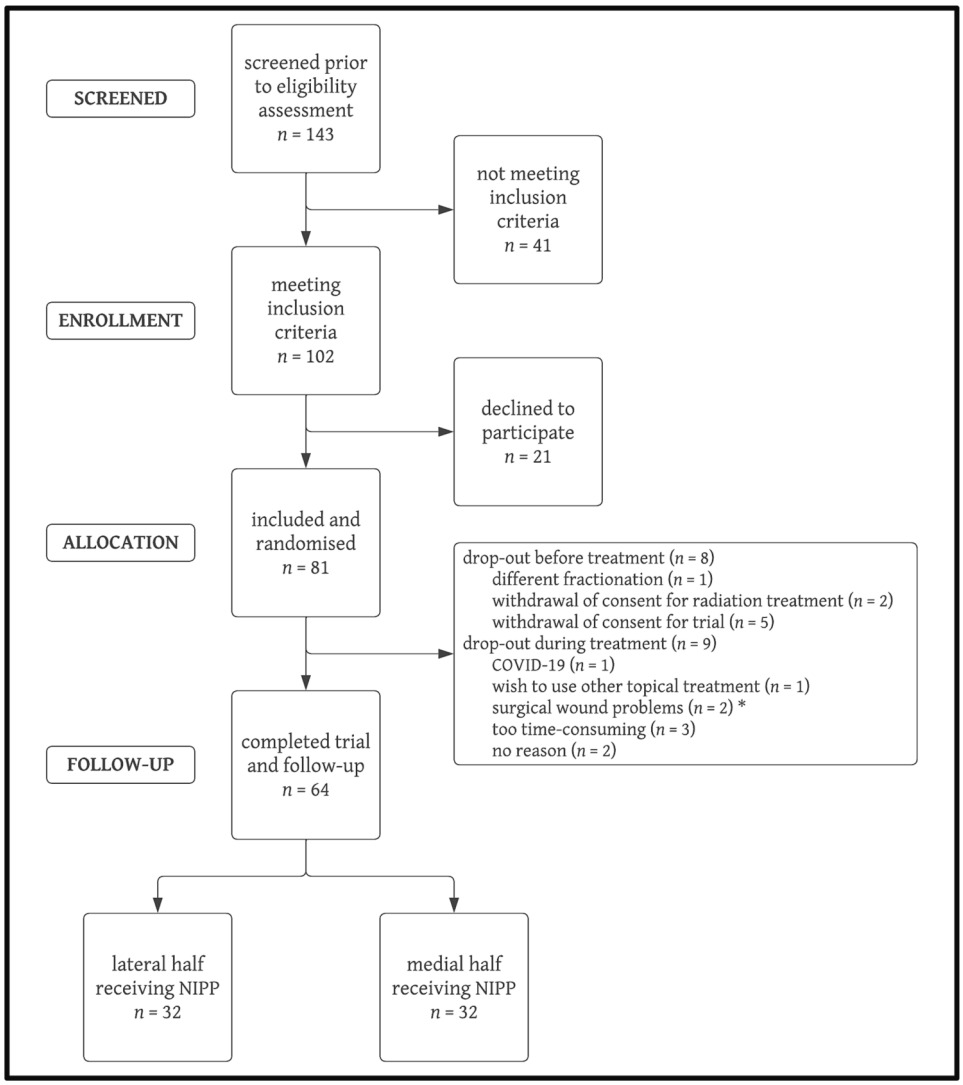


Fig. 2. Flowchart of patient selection and inclusion. NIPP = non-invasive physical plasma. * not related to NIPP use.

Patient-reported outcome

There were no statistically significant differences between NIPP and placebo breast halves in terms of the maximum patient-assessed RD symptoms.

In comparison with the SoC control collective, however, NIPP patients reported significantly less pain, itching, and burning ($p < 0.001$). There was no difference in limitations in daily activities ($p = 0.130$). Results are summarised in Fig. 3b and Fig. 4d.

Objective assessment

Fig. 5a–b shows the evolution of L* and a* values of the irradiated skin over the course of treatment and follow-up. There were no statistically significant differences between NIPP and placebo halves.

On comparison with the SoC control collective, the differences in clinician-reported RD assessment were confirmed: lower a* values for NIPP patients upon treatment completion and at two weeks ($p < 0.001$), indicating less erythema.

Safety and satisfaction

In accordance with the initial feasibility trial, the tolerability of NIPP was excellent and no adverse events or discontinuations due to side effects of NIPP or its combination with WBI were recorded. This was reflected by the positive patient-reported experiences, as shown in *Supplementary Table 1*.

Discussion

Progress in the development of new RD prophylactic and therapeutic agents has been slow, leading to a considerable physical and psychological impact on numerous patients. Topical corticosteroids (mometasone, betamethasone) are effective in reducing RD-related symptoms, however, their widespread and prolonged use remains limited due to the associated side effect profile [30–32]. Care should also be taken if moist desquamation is present, as topical corticosteroids might delay wound healing or promote infection.

Despite some experimental products showing promise in the context of RD prevention or management, few of them proved consistently useful in sufficiently powered randomised trials [11,33]. Furthermore, such trials often recruit heterogeneous patient collectives with varying dose-fractionation regimens and treatment sites, and most lack a uniform control group or adequate placebo control. Another issue is the subjectivity of physician-assessed RD gradings such as CTCAE, with considerable inter- and intra-observer variability, accompanied by significant discrepancies with patient-reported outcomes [34,35]. To accurately investigate prevention and treatment strategies, there is a

Table 1
Overview of patient and radiation treatment characteristics (n = 64) and comparison of baseline characteristics between the current trial (NIPP + SoC) and a similar control trial (identical SoC) [4]. NIPP = non-invasive physical plasma; SoC = standard of care; BMI = body mass index; pT = pathological stage of the primary tumour; Tis = carcinoma in situ; pN = pathological stage of the regional lymph nodes; PTV = planning target volume; SD = standard deviation; gEUD = generalised equivalent uniform dose.

	Current trial	Control trial [a]	*p* [b]
n	64	70	
Median age (range) in years	58 (29–83)	59 (37–84)	0.592
	***n* (%)**		
Female	64 (100)	70 (100)	
Caucasian	62 (97)	70 (100)	0.136
Fitzpatrick skin type			
I	12 (19)		
II	45 (70)		
III	7 (11)		
Median BMI (range) in kg/m^2	25 (18–35)	27 (18–40)	**< 0.001**
Diabetes mellitus	4 (6)	2 (3)	0.343
Active smoking	3 (5)	0 (0)	0.067
Neoadjuvant chemo- or immunotherapy	24 (38)	0 (0)	**< 0.001**
pT-stage			
Tis	20 (31)	6 (9)	
T1	29 (45)	48 (69)	
T2	14 (22)	16 (23)	
T3	1 (2)	0 (0)	**0.004**
pN-stage			
N0	55 (86)		
N1	8 (13)		
N2	1 (2)		
Sequential boost to the tumour bed	38 (59)	31 (44)	0.081
Boost compartment = NIPP half	19 (50)		
Boost compartment = placebo half	19 (50)		
Median PTV Breast (range) in mL	892 (303–2154)	720 (134–1771)	**0.007**
Median PTV Boost (range) in mL	110 (46–291)	192 (50–413)	**< 0.001**
Mean ± SD gEUD in %			0.734[c]
NIPP halves	74.1 ± 4.2		
Placebo halves	74.4 ± 4.9		

[a] Patients in the moderately hypofractionated trial arm.
[b] Difference assessed using Fisher's exact test, Pearson's χ^2, or Student's unpaired *t*-test, as appropriate.
[c] Comparison between NIPP and placebo halves in the current trial.

need for objective RD assessment methods and inclusion of the patient's perspective into clinical trial protocols.

In this prospective, double-blind, placebo-controlled trial, we evaluated the effect of topical NIPP on the incidence and severity of acute RD in a homogeneous patient collective undergoing WBI for breast cancer, using clinician- and patient-reported as well as objective assessment methods.

NIPP shows promising results in the context of several skin conditions and positively affects tissue healing without side effects. In vitro and in vivo studies have revealed that its application to human skin cells does not result in any impairment of cell physiology, cytology, nor DNA integrity, making it safe for clinical use [19,36–39]. A preclinical placebo-controlled trial in irradiated mice showed delayed onset and reduced severity of RD and the only case report published so far showed successful treatment of acute RD following head and neck irradiation [40,41].

When comparing NIPP and placebo breast halves, no significant differences in either clinician- or patient-reported outcomes nor objective assessments were observed. However, the overall incidence and severity of acute skin toxicity were low. A comparison with a very similar patient collective in terms of radiation dose and treatment technique as well as identical SoC supports this: even though patients in the NIPP cohort were at an overall higher risk of developing RD (more sequential boosts and larger breast volumes; both risk factors for RD development), they still developed less frequent and milder acute toxicity [23,42]. An additional comparison with international landmark trials on acute radiation-induced toxicity following hypofractionated WBI with modern treatment techniques further supports the apparent benefit of NIPP in this context (*Supplementary Table 2*). Also in accordance with previous reports on the use of NIPP in comparable clinical settings, side effects were not reported and patient tolerability and acceptance were excellent [18,19]. Treatment time and cost are discussed in more detail in the initial feasibility trial [21].

We reason that the trial design with an intrapatient control breast half may be responsible for the discrepancy between the placebo and SoC controls. Although NIPP was applied to one breast half only, diffusion to the other (placebo) breast half cannot be ruled out, which is the main limitation of this trial. Said design proved useful in previous trials investigating topical therapies for RD prevention [43–45]: each patient acting as their own control promotes patient accrual and omits the need for stratification based on other risk factors for RD development such as breast volume or skin type, limiting confounding in RD grading [23]. To confirm this hypothesis and to accurately investigate the effect of NIPP on RD development (or radiation injury in general), an interpatient-randomised design with a physical control group receiving SoC and sham treatment will be needed [46].

The pathophysiology of RD is complex and the signalling pathways and mechanisms through which NIPP positively affects tissue healing are not yet fully understood. We reason that its primary mode of action in the context of RD mainly involves a reduction of the bacterial load on the irradiated breast skin. Ionising radiation disrupts the skin barrier function and microorganisms or microbial antigens may subsequently trigger an inflammatory response, enhancing the clinical appearance of RD through an increased immune reaction [47]. In this context, patients with *Staphylococcus aureus* colonisation before initiation of radiotherapy are more prone to severe RD development [48]. A recent study supporting these findings investigated the use of chlorhexidine body wash once daily in patients undergoing WBI and observed a significant reduction of RD incidence and severity when compared to standard skin care [49]. NIPP also achieves bacterial decolonisation, both on working surfaces and humans, among other by promoting macrophage ability to eliminate internalised *Staphylococcus* [18,50–52]. Its beneficial effects on diabetic foot ulcers is, among other factors, attributed to the observed immediate reduction of the bacterial load on the damaged skin [13,19,53].

A proposed additional mechanism is that NIPP promotes proliferation and migration of keratinocytes, fibroblasts, and endothelial cells, which facilitates tissue recovery [54]. Accelerated endothelial tube formation improves vascular shear stress which contributes to angiogenesis, in turn enhancing capillary blood flow and increasing local

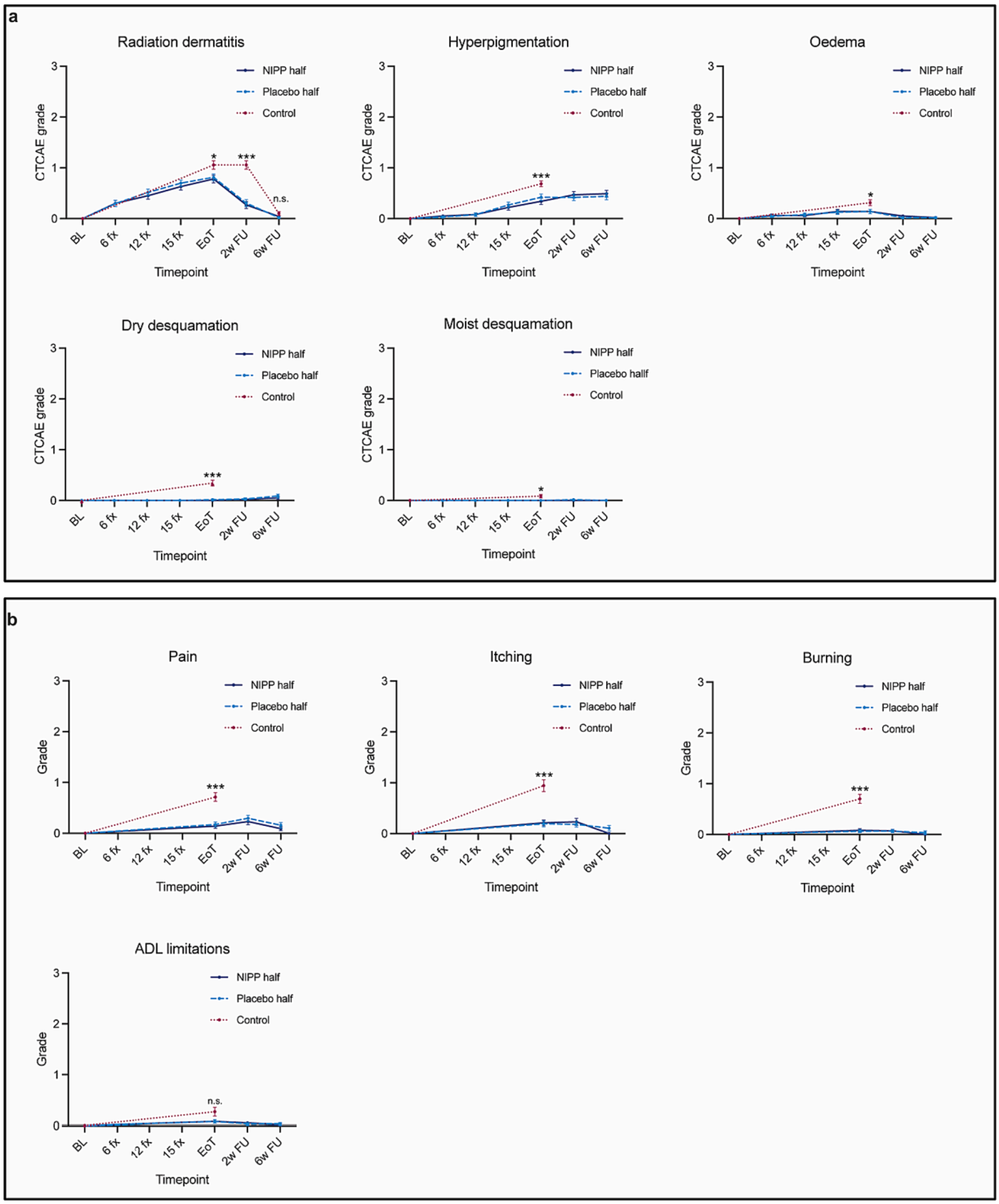


Fig. 3. a–b. Blinded clinician- and patient-reported outcomes over the course of treatment and follow-up. (a) Assessment of clinician-reported acute skin toxicity in terms of radiation dermatitis, hyperpigmentation, breast oedema, dry and moist desquamation, for NIPP (dark blue; full) and placebo (light blue; dashed) breast halves separately, mean grading according to CTCAE (version 5.0) [25]. (b) As in (a), but for patient-reported outcomes in terms of pain, itching, burning, and limitations in daily activities, according to the modified Radiation-Induced Skin Reaction Assessment Scale [25]. The dotted line (purple) represents a comparable standard of care control collective, not receiving NIPP. Error bars represent standard error of the mean. * $p < 0.05$; *** $p < 0.001$; n.s. = not significant. NIPP = non-invasive physical plasma; CTCAE = Common Terminology Criteria for Adverse Events; BL = baseline; fx = fractions; EoT = end of treatment; w = weeks; FU = follow-up. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

oxygen saturation and nutrient supply [55,56]. The expression of wound healing gene signatures alongside significant changes to the human skin barrier lipid stoichiometry are two additional modes of action, which are, however, still poorly understood [57,58].

The main limitation of the current trial is its intrapatient-randomised design, which cannot rule out diffusion of NIPP to the other (placebo) breast half. In order to unequivocally assess the benefit of NIPP in the context of adjuvant WBI, an interpatient-randomised design with a

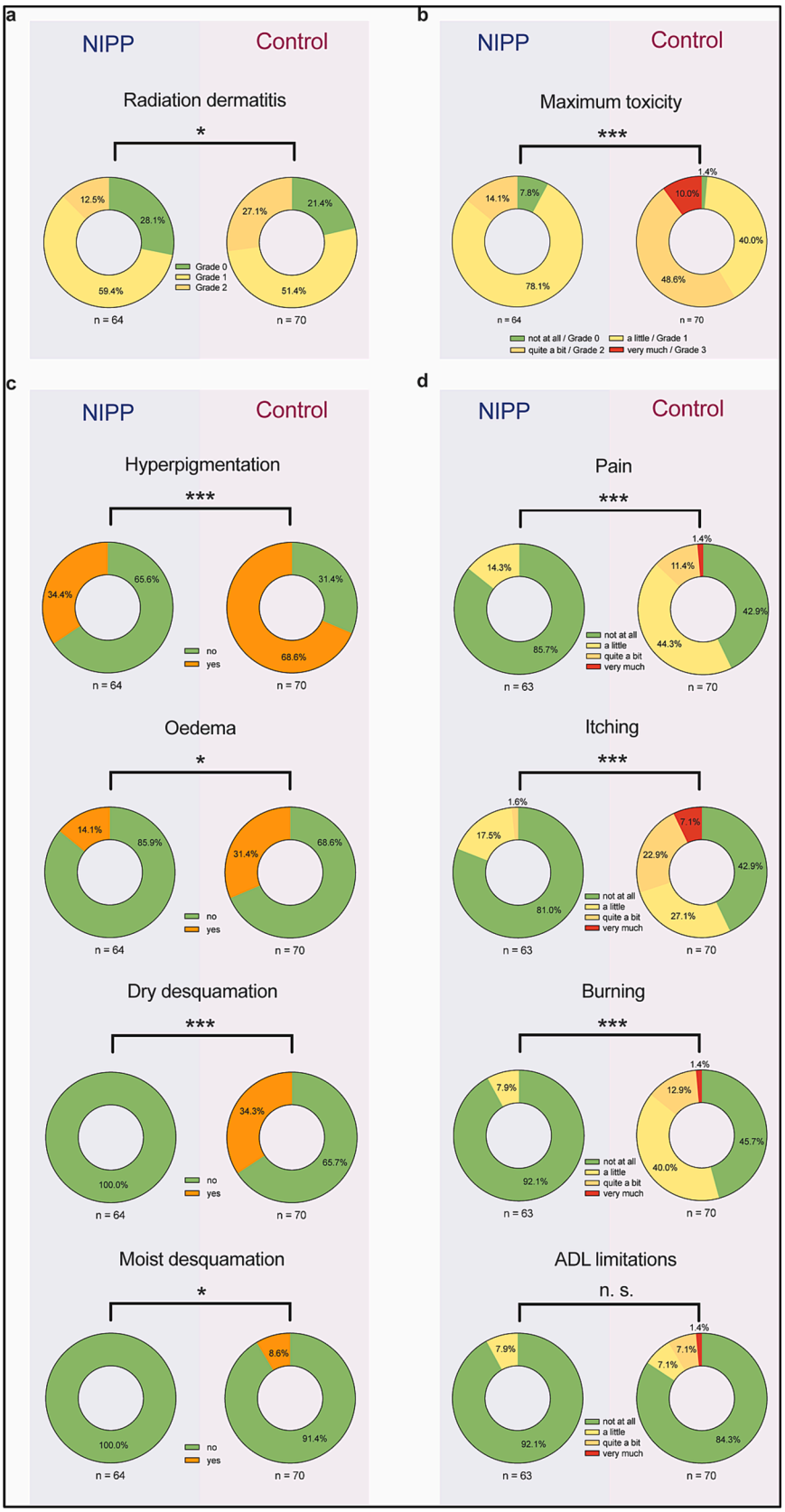


Fig. 4. a–d. Maximum clinician- and patient-reported toxicity. Frequency of radiation dermatitis CTCAE gradings (a), overall maximum observed toxicity grade (b), clinician-reported (c) and patient-reported (d) outcomes, with NIPP (blue; left) and in a comparable SoC control collective (purple; right). Mann-Whitney-*U* test: * $p < 0.05$; *** $p < 0.001$; n.s. = not significant. CTCAE = Common Terminology Criteria for Adverse Events; NIPP = non-invasive physical plasma; SoC = standard of care; ADL = activities of daily living. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

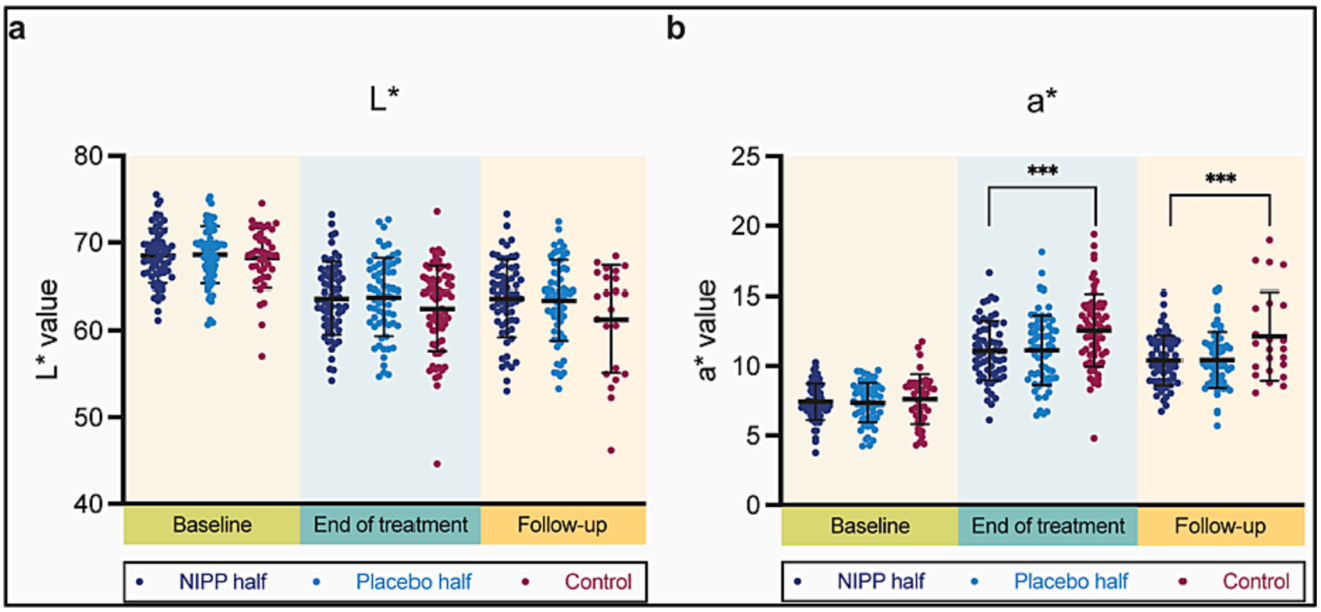


Fig. 5. a–b. Objective spectrophotometric readings (n = 4,600), for NIPP (dark blue) and placebo (light blue) breast halves separately. The purple dots represent a comparable SoC collective, not receiving NIPP. Lower L* values (a) indicate darker skin (hyperpigmentation) and higher a* values (b) imply erythema (radiation dermatitis). Black line and error bars represent mean ± standard deviation. Mann-Whitney-*U* test: *** $p < 0.001$. SoC = standard of care; NIPP = non-invasive physical plasma. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

physical control group receiving placebo will be needed. Furthermore, an additional NIPP dose-escalation should be investigated, as it might yield an even better outcome. Longer treatment times generate more ozone, enabling bacterial decolonisation and increasing the secretion of anti-inflammatory and regenerative signalling molecules, which might improve outcome even further [13,49,59]. The subsequent additional treatment time and costs generated with the device used in this trial might delineate a subset of high-risk patients (i.e. those with risk factors for RD development), in which NIPP is most cost-effective. Lastly, the long-term effects of daily NIPP application will have to be investigated in future trials.

Conclusion

Even though there were no significant differences between intrapatient-randomised breast halves receiving NIPP versus placebo, the overall incidence and severity of acute radiation-induced skin toxicity was considerably lower in comparison with an independent, prospectively-recruited control collective receiving identical SoC. Diffusion of the volatile NIPP towards the placebo half is the most likely explanation for this discrepancy. We provide first evidence supporting the benefits of NIPP as an add-on RD prevention method in the context of adjuvant WBI for breast cancer, while confirming the excellent safety and tolerability from the feasibility trial. A randomised controlled trial with a physical control group is needed to further investigate this promising approach for RD prevention.

Author Contributions

Cas Stefaan Dejonckheere: Investigation, Methodology, Data curation, Formal analysis, writing – original draft. **Julian Philipp Layer:** Formal analysis, Visualization, Writing – review and editing. **Younèss Nour:** Data curation. **Katharina Layer:** Data curation. **Andrea Glasmacher:** Data curation. **Shari Wiegreffe:** Data curation. **Arne Fuhrmann:** Data curation. **Lara Caglayan:** Data curation. **Franziska Grau:** Data curation. **Gustavo Renato Sarria:** Writing – review and editing. **Davide Scafa:** Data curation. **David Koch:** Data curation. **Martina Heimann:** Data curation. **Christina Leitzen:** Data curation. **Mümtaz Ali Köksal:** Data curation. **Fred Röhner:** Data curation. **Thomas Müdder:** Data curation. **Egon Dejonckheere:** Data curation. **Frederic Carsten Schmeel:** Data curation. **Teresa Anzböck:** Data curation. **Kira Lindner:** Data curation. **Anne Bachmann:** Data curation. **Alina Abramian:** Data curation. **Christina Kaiser:** Data curation. **Andree Faridi:** Data curation. **Alexander Mustea:** Data curation. **Frank Anton Giordano:** Data curation. **Matthias Bernhard Stope:** Writing – review and editing. **Leonard Christopher Schmeel:** Conceptualization, Project administration, Investigation, Methodology, Data curation, Writing – review and editing.

Patient Consent Statement

Written informed consent was obtained from all included patients. This study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Institutional Review Board of the University Bonn in September 2021 (210/21).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Katja Klever and Monika Brüggemann for their technical expertise and assistance in conducting this trial, as well as Jens Kirsch, Stefanie Ascher, Lisa Gebhardt, Ute Gratzfeld, and Mara Murgia from terraplasma medical for kindly providing the plasma care device free of charge.

Funding and Conflict of Interest

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript. There are no relevant financial or non-financial interests to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ctro.2023.100699.

References

[1] Schreuder K, Middelburg JG, Aarts MJ, Merkus JWS, Poortmans PMP, Jobsen JJ, et al. An actualised population-based study on the use of radiotherapy in breast cancer patients in the Netherlands. Breast J 2019;25:942–7. https://doi.org/10.1111/tbj.13376.

[2] Katsura C, Ogunmwonyi I, Kankam HK, Saha S. Breast cancer: presentation, investigation and management. Br J Hosp Med (Lond) 2022;83:1–7. 10.12968/hmed.2021.0459.

[3] Shaitelman SF, Schlembach PJ, Arzu I, Ballo M, Bloom ES, Buchholz D, et al. Acute and short-term toxic effects of conventionally fractionated vs. hypofractionated whole-breast radiation: a randomized clinical trial. JAMA Oncol 2015;1:931–41. https://doi.org/10.1001/jamaoncol.2015.2666.

[4] Schmeel LC, Koch D, Schmeel FC, Röhner F, Schoroth F, Bücheler BM, et al. Acute radiation-induced skin toxicity in hypofractionated vs. conventional whole-breast irradiation: An objective, randomized multicenter assessment using spectrophotometry. Radiother Oncol J Eur Soc Ther Radiol Oncol 2020;146:172–9. https://doi.org/10.1016/j.radonc.2020.02.018.

[5] Buwenge M, Cammelli S, Ammendolia I, Tolento G, Zamagni A, Arcelli A, et al. Intensity modulated radiation therapy for breast cancer: current perspectives. Breast Cancer 2017;9:121–6. https://doi.org/10.2147/BCTT.S113025.

[6] de Fuzissaki MA, Paiva CE, de Oliveira MA, Lajolo Canto PP, de Paiva Maia YC. The impact of radiodermatitis on breast cancer patients' quality of life during radiotherapy: A prospective cohort study. J Pain Sympt Manage 2019;58:92–99.e1. https://doi.org/10.1016/j.jpainsymman.2019.03.017.

[7] Rzepecki A, Birnbaum M, Ohri N, Daily J, Fox J, Bodner W, et al. Characterizing the effects of radiation dermatitis on quality of life: A prospective survey-based study. J Am Acad Dermatol 2022;86:161–3. https://doi.org/10.1016/j.jaad.2019.03.011.

[8] Finkelstein S, Kanee L, Behroozian T, Wolf JR, van den Hurk C, Chow E, et al. Comparison of clinical practice guidelines on radiation dermatitis: a narrative review. Support Care Cancer 2022. https://doi.org/10.1007/s00520-022-06829-6.

[9] Layer K, Layer JP, Glasmacher AR, Sarria GR, Böhner AMC, Layer YL, et al. Risk assessment, surveillance, and nonpharmaceutical prevention of acute radiation dermatitis: results of a multicentric survey among the German-speaking radiation oncology community. Strahlenther Onkol 2023. https://doi.org/10.1007/s00066-023-02074-w.

[10] Layer JP, Layer K, Glasmacher AR, Sarria GR, Böhner AMC, Layer YL, et al. Pharmaceutical management of acute radiation dermatitis in the German-speaking radiation oncology community. J Der Dtsch Dermatologischen Gesellschaft 2023. https://doi.org/10.1111/ddg.15279.

[11] Behroozian T, Bonomo P, Patel P, Kanee L, Finkelstein S, van den Hurk C, et al. Multinational Association of Supportive Care in Cancer (MASCC) clinical practice guidelines for the prevention and management of acute radiation dermatitis: international Delphi consensus-based recommendations. Lancet Oncol 2023;24:e172–85. https://doi.org/10.1016/S1470-2045(23)00067-0.

[12] Gan L, Zhang S, Poorun D, Liu D, Lu X, He M, et al. Medical applications of nonthermal atmospheric pressure plasma in dermatology. J Der Dtsch Dermatologischen Gesellschaft 2018;16:7–13. https://doi.org/10.1111/ddg.13373.

[13] Stratmann B, Costea T-C, Nolte C, Hiller J, Schmidt J, Reindel J, et al. Effect of cold atmospheric plasma therapy vs. standard therapy placebo on wound healing in patients with diabetic foot ulcers: a randomized clinical trial. JAMA Netw Open 2020;3:e2010411. 10.1001/jamanetworkopen.2020.10411.

[14] Kletschkus K, Haralambiev L, Mustea A, Bekeschus S, Stope MB. Review of innovative physical therapy methods: introduction to the principles of cold physical plasma. In Vivo 2020;34:3103–7. 10.21873/invivo.12143.

[15] Brandenburg R. Dielectric barrier discharges: progress on plasma sources and on the understanding of regimes and single filaments. Plasma Sources Sci Technol 2018;27:79501. https://doi.org/10.1088/1361-6595/aaced9.

[16] Gelbrich N, Stope MB, Burchardt M. Cold atmospheric plasma for the treatment of urological tumors. Urologe A 2019;58:673–9. https://doi.org/10.1007/s00120-018-0754-8.

[17] Bernhardt T, Semmler ML, Schäfer M, Bekeschus S, Emmert S, Boeckmann L. Plasma medicine: applications of cold atmospheric pressure plasma in dermatology. Oxid Med Cell Longev 2019;2019:3873928. https://doi.org/10.1155/2019/3873928.

[18] Assadian O, Ousey KJ, Daeschlein G, Kramer A, Parker C, Tanner J, et al. Effects and safety of atmospheric low-temperature plasma on bacterial reduction in chronic wounds and wound size reduction: a systematic review and meta-analysis. Int Wound J 2019;16:103–11. https://doi.org/10.1111/iwj.12999.

[19] Boekema B, Stoop M, Vlig M, van Liempt J, Sobota A, Ulrich M, et al. Antibacterial and safety tests of a flexible cold atmospheric plasma device for the stimulation of wound healing. Appl Microbiol Biotechnol 2021;105:2057–70. https://doi.org/10.1007/s00253-021-11166-5.

[20] Riley PA. Free radicals in biology: oxidative stress and the effects of ionizing radiation. Int J Radiat Biol 1994;65:27–33. https://doi.org/10.1080/09553009414550041.

[21] Dejonckheere CS, Torres-Crigna A, Layer JP, Layer K, Wiegreffe S, Sarria GR, et al. Non-invasive physical plasma for preventing radiation dermatitis in breast cancer: A first-in-human feasibility study. Pharmaceutics 2022;14. https://doi.org/10.3390/pharmaceutics14091767.

[22] Offersen B, Boersma LJ, Kirkove C, Hol S, Aznar MC, Biete Sola A, et al. ESTRO consensus guideline on target volume delineation for elective radiation therapy of early stage breast cancer. Radiother Oncol 2015;114:3–10. https://doi.org/10.1016/j.radonc.2014.11.030.

[23] Böhner AMC, Koch D, Schmeel FC, Röhner F, Schoroth F, Sarria GR, et al. Objective evaluation of risk factors for radiation dermatitis in whole-breast irradiation using the spectrophotometric L*a*b* colour-space. Cancers (Basel) 2020;12. 10.3390/cancers12092444.

[24] Sakiyama Y, Graves DB, Chang H-W, Shimizu T, Morfill GE. Plasma chemistry model of surface microdischarge in humid air and dynamics of reactive neutral species. J Phys D Appl Phys 2012;45:425201. https://doi.org/10.1088/0022-3727/45/42/425201.

[25] Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 n.d. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_5x7.pdf.

[26] Noble-Adams R. Radiation-induced skin reactions. 3: evaluating the RISRAS. Br J Nurs 1999;8:1305–12. 10.12968/bjon.1999.8.19.1305.

[27] Russell NS, Knaken H, Bruinvis IA, Hart AA, Begg AC, Lebesque JV. Quantification of patient to patient variation of skin erythema developing as a response to radiotherapy. Radiother Oncol J Eur Soc Ther Radiol Oncol 1994;30:213–21. https://doi.org/10.1016/0167-8140(94)90460-x.

[28] Momm F, Bartelt S, Haigis K, Grosse-Sender A, Witucki G. Spectrophotometric skin measurements correlate with EORTC/RTOG-common toxicity criteria. Strahlenther Onkol 2005;181:392–5. https://doi.org/10.1007/s00066-005-1345-3.

[29] Dejonckheere CS, Abramian A, Lindner K, Bachmann A, Layer K, Anzböck T, et al. Objective, clinician- and patient-reported evaluation of late toxicity following adjuvant radiation for early breast cancer: long-term follow-up results of a randomised series. J Clin Med 2023;12:4212.

[30] Barnes L, Kaya G, Rollason V. Topical corticosteroid-induced skin atrophy: a comprehensive review. Drug Saf 2015;38:493–509. https://doi.org/10.1007/s40264-015-0287-7.

[31] Haruna F, Lipsett A, Marignol L. Topical management of acute radiation dermatitis in breast cancer patients: a systematic review and meta-analysis. Anticancer Res 2017;37:5343–53. 10.21873/anticanres.11960.

[32] Tam S, Zhou G, Trombetta M, Caini S, Ryan Wolf J, van den Hurk C, et al. Topical corticosteroids for the prevention of severe radiation dermatitis: a systematic review and meta-analysis. Support Care Cancer 2023;31:382. https://doi.org/10.1007/s00520-023-07820-5.

[33] Yee C, Wang K, Asthana R, Drost L, Lam H, Lee J, et al. Radiation-induced skin toxicity in breast cancer patients: a systematic review of randomized trials. Clin Breast Cancer 2018;18:e825–40. https://doi.org/10.1016/j.clbc.2018.06.015.

[34] Behroozian T, Milton L, Zhang L, Lou J, Karam I, Lam E, et al. How do patient-reported outcomes compare with clinician assessments? A prospective study of radiation dermatitis in breast cancer. Radiother Oncol J Eur Soc Ther Radiol Oncol 2021;159:98–105. https://doi.org/10.1016/j.radonc.2021.03.020.

[35] Jagsi R, Griffith KA, Vicini F, Boike T, Dominello M, Gustafson G, et al. Identifying patients whose symptoms are underrecognized during treatment with breast radiotherapy. JAMA Oncol 2022;8:887–94. https://doi.org/10.1001/jamaoncol.2022.0114.

[36] Kluge S, Bekeschus S, Bender C, Benkhai H, Sckell A, Below H, et al. Investigating the mutagenicity of a cold argon-plasma jet in an HET-MN model. PLoS One 2016;11:e0160667. https://doi.org/10.1371/journal.pone.0160667.

[37] Schmidt A, von Woedtke T, Stenzel J, Lindner T, Polei S, Vollmar B, et al. One year follow-up risk assessment in SKH-1 mice and wounds treated with an argon plasma jet. Int J Mol Sci 2017:18. https://doi.org/10.3390/ijms18040868.

[38] Bekeschus S, Schmidt A, Kramer A, Metelmann H-R, Adler F, von Woedtke T, et al. High throughput image cytometry micronucleus assay to investigate the presence or absence of mutagenic effects of cold physical plasma. Environ Mol Mutagen 2018;59:268–77. https://doi.org/10.1002/em.22172.

[39] Jablonowski L, Kocher T, Schindler A, Müller K, Dombrowski F, von Woedtke T, et al. Side effects by oral application of atmospheric pressure plasma on the mucosa in mice. PLoS One 2019;14:e0215099. https://doi.org/10.1371/journal.pone.0215099.

[40] Bernhardt T, Manda K, Hildebrandt G, Stachs O, Bekeschus S, Vollmar B, Emmert S BL. Kaltes Atmosphärendruckplasma zur Behandlung einer Radiodermatitis und Untersuchung objektiver Verlaufsparameter im Mausmodell, 2021.

[41] Peña-Eguiluz R, López-Callejas R, González-Mendoza B, Mercado-Cabrera A, Rodríguez-Méndez BG, Valencia-Alvarado R. Acute neck radiodermatitis treated by nonthermal plasma therapy: case report. IEEE Trans Radiat Plasma Med Sci 2022;6:503–6. https://doi.org/10.1109/TRPMS.2021.3070784.

[42] Behroozian T, Milton L, Li N, Zhang L, Lou J, Karam I, et al. Predictive factors associated with radiation dermatitis in breast cancer. Cancer Treat Res Commun 2021;28:100403. https://doi.org/10.1016/j.ctarc.2021.100403.

[43] Schmeel LC, Koch D, Stumpf S, Leitzen C, Simon B, Schüller H, et al. Prophylactically applied Hydrofilm polyurethane film dressings reduce radiation dermatitis in adjuvant radiation therapy of breast cancer patients. Acta Oncol (Madr) 2018;57:908–15. https://doi.org/10.1080/0284186X.2018.1441542.

[44] Schmeel LC, Koch D, Schmeel FC, Bücheler B, Leitzen C, Mahlmann B, et al. Hydrofilm polyurethane films reduce radiation dermatitis severity in hypofractionated whole-breast irradiation: an objective, intrapatient randomized dual-center assessment. Polymers (Basel) 2019:11. https://doi.org/10.3390/polym11122112.

[45] Dejonckheere CS, Dejonckheere E, Layer JP, Layer K, Sarria GR, Koch D, et al. Barrier films for the prevention of acute radiation dermatitis in breast cancer: A systematic review and meta-analysis of randomised controlled trials. Breast 2023. https://doi.org/10.1016/j.breast.2023.07.001.
[46] Dejonckheere CS, Stope MB, Schmeel LC. Concise review of non-invasive physical plasma as a promising treatment option for radiation injuries of the skin 2023:3–5. 10.1111/wrr.13077.
[47] Hill A, Hanson M, Bogle MA, Duvic M. Severe radiation dermatitis is related to Staphylococcus aureus. Am J Clin Oncol 2004;27:361–3. https://doi.org/10.1097/01.coc.0000071418.12121.c2.
[48] Kost Y, Rzepecki AK, Deutsch A, Birnbaum MR, Ohri N, Hosgood HD, et al. Association of staphylococcus aureus colonization with severity of acute radiation dermatitis in patients with breast or head and neck cancer. JAMA Oncol 2023. https://doi.org/10.1001/jamaoncol.2023.0454.
[49] Kost Y, Deutsch A, Mieczkowska K, Nazarian R, Muskat A, Hosgood HD, et al. Bacterial decolonization for prevention of radiation dermatitis: A randomized clinical trial. JAMA Oncol 2023. https://doi.org/10.1001/jamaoncol.2023.0444.
[50] Puligundla P, Mok C. Potential applications of nonthermal plasmas against biofilm-associated micro-organisms in vitro. J Appl Microbiol 2017;122:1134–48. https://doi.org/10.1111/jam.13404.
[51] Daeschlein G, Napp M, Lutze S, Arnold A, von Podewils S, Guembel D, et al. Skin and wound decontamination of multidrug-resistant bacteria by cold atmospheric plasma coagulation. J Der Dtsch Dermatologischen Gesellschaft 2015;13:143–50. https://doi.org/10.1111/ddg.12559.
[52] Duchesne C, Frescaline N, Blaise O, Lataillade J-J, Banzet S, Dussurget O, et al. Cold atmospheric plasma promotes killing of staphylococcus aureus by macrophages. MSphere 2021;6:e0021721. https://doi.org/10.1128/mSphere.00217-21.
[53] Mirpour S, Fathollah S, Mansouri P, Larijani B, Ghoranneviss M, Mohajeri Tehrani M, et al. Cold atmospheric plasma as an effective method to treat diabetic foot ulcers: A randomized clinical trial. Sci Rep 2020;10:10440. https://doi.org/10.1038/s41598-020-67232-x.
[54] Haertel B, von Woedtke T, Weltmann K-D, Lindequist U. Non-thermal atmospheric-pressure plasma possible application in wound healing. Biomol Ther (Seoul) 2014;22:477–90. https://doi.org/10.4062/biomolther.2014.105.
[55] Kisch T, Schleusser S, Helmke A, Mauss KL, Wenzel ET, Hasemann B, et al. The repetitive use of non-thermal dielectric barrier discharge plasma boosts cutaneous microcirculatory effects. Microvasc Res 2016;106:8–13. https://doi.org/10.1016/j.mvr.2016.02.008.
[56] Duchesne C, Banzet S, Lataillade J-J, Rousseau A, Frescaline N. Cold atmospheric plasma modulates endothelial nitric oxide synthase signalling and enhances burn wound neovascularisation. J Pathol 2019;249:368–80. https://doi.org/10.1002/path.5323.
[57] Arndt S, Unger P, Wacker E, Shimizu T, Heinlin J, Li Y-F, et al. Cold atmospheric plasma (CAP) changes gene expression of key molecules of the wound healing machinery and improves wound healing in vitro and in vivo. PLoS One 2013;8:e79325. https://doi.org/10.1371/journal.pone.0079325.
[58] Marschewski M, Hirschberg J, Omairi T, Höfft O, Viöl W, Emmert S, et al. Electron spectroscopic analysis of the human lipid skin barrier: cold atmospheric plasma-induced changes in lipid composition. Exp Dermatol 2012;21:921–5. https://doi.org/10.1111/exd.12043.
[59] Eggers B, Stope MB, Marciniak J, Götz W, Mustea A, Deschner J, et al. Non-invasive physical plasma generated by a medical argon plasma device induces the expression of regenerative factors in human gingival keratinocytes, fibroblasts, and tissue biopsies. Biomedicines 2022:10. https://doi.org/10.3390/biomedicines10040889.