A randomized controlled trial analyzing nonthermal atmospheric plasma for the treatment of verruca vulgaris in pediatric patients

Courtney L. Walker, MD, MSCR,[a] Chelsea N. Shope, MD, MSCR,[b] Laura A. Andrews, MD, MSCR,[b] Kelly M. Atherton, MD, MSCR,[a] Tyler Beck, MD, PhD,[a] Gabriella Santa Lucia, MD, MSCR,[b] Gregory Fridman, PhD,[c] Peter C. Friedman, MD, PhD,[d] Colleen H. Cotton, MD,[e,f] and Lara Wine Lee, MD, PhD[b]

Background: Verruca vulgaris (VV) is a common viral disease in children. Treatment options are often not well tolerated in children due to pain or adverse effect risk. Nonthermal atmospheric plasma (NTAP), which generates reactive oxygen/nitrogen species, is well tolerated and without adverse effects.

Objective: Determine efficacy of NTAP as compared to standard of care (SOC) therapy for VV in children.

Methods: This prospective open-label study randomized lesions 1:1 to receive NTAP or SOC (cryotherapy). Patients were treated at 4-week intervals for a maximum of 3 treatments. They were evaluated 4 weeks postfinal treatment for sustained response. Primary outcome was lesion response.

Results: One hundred twelve VV lesions in 14 patients were enrolled. Patients were mostly White (92.9%) males (71.4%) with mean age of 9.5 [±2.5] years. Responses of SOC- and NTAP-treated lesions, respectively, included no response (5.4%, 7.1%); partial response (33.9%, 41.1%); and complete resolution (60.7%, 51.8%; *P* value = .679). Patients were more likely to report pain in SOC lesions post-treatment (*P* value <.001). No significant adverse events (AEs) occurred.

Limitations: Limitations include single-site, maximum of 3 treatments, and short post-treatment follow-up.

Conclusion: NTAP is an efficacious, safe intervention for treatment of VV in children. (J Am Acad Dermatol 2025;92:46-50.)

Key words: cryotherapy; pediatric dermatology; plasma; randomized controlled study; RCT; treatment; warts.

INTRODUCTION

Verruca vulgaris (VV) is a common viral skin disease in children that results from infection with a double-stranded DNA virus. Diagnosis of these lesions is clinical, with VV presenting as irregularly surfaced domed lesions.[1] Although benign and generally self-limited, VV is contagious and can lead to complications such as inflammation, pruritus,

From the College of Medicine, Medical University of South Carolina, Charleston, South Carolina[a]; Department of Dermatology and Dermatologic Surgery, Medical University of South Carolina, Charleston, South Carolina[b]; AAPlasma LLC, Philadelphia, Pennsylvania[c]; The Skin Center Dermatology Group, New City, New York[d]; Division of Dermatology, Children's National Hospital, Washington, District of Columbia[e]; and Department of Dermatology, George Washington School of Medicine and Health Sciences, Washington, District of Columbia.[f]

Funding sources: None.

Patient consent: Consent for the publication of recognizable patient photographs or other identifiable material was obtained by the authors and included at the time of article submission to the journal stating that all patients gave consent with the understanding that this information may be publicly available.

IRB approval status: Reviewed and approved by Western IRB; approval #106689.

Accepted for publication July 23, 2024.

Correspondence to: Courtney L. Walker, MD, MSCR, 135 Rutledge Ave, 11th floor, Charleston, SC 29425. E-mail: linkousc@musc.edu.

Published online August 27, 2024.

0190-9622/$36.00

© 2024 by the American Academy of Dermatology, Inc.

https://doi.org/10.1016/j.jaad.2024.07.1522

dermatitis, discomfort, scarring, and superinfection.[2,3] There is little consensus on the ideal management of this condition, but treatment options include mechanical removal (ie, curettage), destruction with cryosurgery or heat, topical irritants, topical vesicants, and topical acids.[1] These strategies are often irritating and painful, particularly in children requiring treatment of multiple lesions. Furthermore, they have well-documented risk of dyspigmentation and scarring. The lack of tolerability and variable efficacy of these therapies warrants exploration of more effective and less painful treatment options for the pediatric population.

CAPSULE SUMMARY

- This randomized study confirms efficacy and safety of nonthermal atmospheric plasma for treatment of verruca vulgaris in pediatrics, as previously reported in case series.
- This article suggests emerging utility of this technology as a well-tolerated treatment for verruca vulgaris in children, for which standard therapies are poorly tolerated.

Physical plasma refers to ionized gas and is considered the fourth state of matter. Nonthermal, cold, or nonequilibrium plasma are all synonymous for plasma that is not in thermodynamic equilibrium because the electron temperature is significantly higher than that of the surrounding ions. This leads to differing velocity distributions between electrons and ions, which can result in temporary cell membrane permeabilization and modification of DNA, proteins, and membranes.[2,4] Most nonthermal atmospheric plasmas (NTAPs) are generated in helium or argon mixed with other reactive gases, which create reactive nitrogen and oxygen species that induce a targeted and transient antiviral or antibacterial milieu.[2,5,6] NTAP has not been shown to cause damage to skin, and thermal injury does not occur due to low temperature.[2,7] Thus, this technology has exhibited a promising safety profile for medical applications.

Given its safety, NTAP, also known as cold atmospheric plasma, has been introduced to the field of dermatology for the treatment of various cutaneous conditions. Multiple clinical studies have established its success in wound healing and in the treatment of onychomycosis, actinic keratosis, and viral warts.[2,8] A 2020 case series using NTAP for the treatment of warts in 5 children demonstrated safety and efficacy of this technology in the pediatric population, with no reports of pain or adverse events such as blistering, scarring, significant pigmentary alteration, or persistent nail changes.[9] NTAP has demonstrated efficacy in treatment of other cutaneous viral processes as well. In a single case report, NTAP was shown to be an effective treatment for molluscum in a 12-year-old boy, as the lesions cleared after 4 treatments without significant inflammation.[10] While several patient cases have supported the safety and treatment success of NTAP for VV in children, randomized controlled studies exploring the use of NTAP as compared to standard of care (SOC) therapies are lacking. In this study, we aimed to investigate the efficacy and safety of NTAP as compared to the current SOC therapies for VV in the pediatric population.

METHODS

Trial design

This investigator-initiated study was an open-label, randomized controlled trial exploring the efficacy of NTAP for the treatment of VV in pediatric patients. Institutional review board approval (Pro00106689), registration on ClinicalTrials.gov (NCT05070754), and written informed consent from patients' parents or legal guardians were obtained before any protocol-related procedures were performed. Patients were approached and enrolled in the study within our institution's pediatric dermatology clinic and completed study follow-up between December 2021 and November 2022.

Participant lesions were randomized 1:1 through alternation to receive treatment with either NTAP or SOC therapy, which was liquid nitrogen cryotherapy. Lesions were randomized by study enrollment team members (C.N.S., L.A.A., K.M.A., and T.B.), and patients could have up to 20 lesions enrolled in the study, with each individual lesion randomized to be treated with either NTAP or SOC. Therefore, participants were permitted to be treated with both NTAP and SOC therapy so long as the treatment modality for each lesion was consistent for the duration of the study. Lesions randomized to receive SOC treatment with cryotherapy were pretreated with topical 4% lidocaine cream, according to our SOC procedure. Cryotherapy was applied with either cotton tip applicator or cryospray unit, and SOC lesions were treated with a minimum of 2 10-second freeze cycles with thawing in between, dependent on lesion characteristics. At enrollment visits, baseline demographics, medical history, and photos were captured; participants received appropriate treatment of their lesion(s); and pain levels were recorded using the validated visual analog scale. Patients received a phone call 7 days following their first visit to assess for lesion improvement and adverse events (AEs) such as pain, numbness, erythema, and other color changes. Following enrollment visits,

Abbreviations used:

AE: adverse event
NTAP: nonthermal atmospheric plasma
SOC: standard of care
VV: verruca vulgaris

patients followed up approximately every 4 weeks to assess degree of lesion resolution and to identify any AEs. Need for retreatment of individual lesions was determined by the patient's dermatologist (L.W.L. or C.H.C.), and lesions were retreated according to the initial randomization scheme for a maximum of 3 treatments. Additionally, patients followed up 4 weeks following their final treatment to assess for sustained treatment response. During the course of the study, use of home topical or systemic treatments for VV was not permitted. The primary outcome was lesion resolution, and secondary outcomes included AEs and tolerability as measured by degree of pain using the visual analog scale.

Study population

The study population consisted of patients 4 to 21 years of age with at least one lesion of VV who were seen in our institution's outpatient pediatric dermatology clinic. Warts included were periungual, palmoplantar, and common warts. Exclusion criteria included treatment of the target lesion(s) within the past month with any modality, diagnosis of immunodeficiency, adverse response to prior treatments for target lesion(s), signs of self-resolution of the target lesion(s), facial or genital lesions, and conditions that lead to excessive scarring.

Device

This study utilized a floating electrode-dielectric barrier, or cold atmospheric plasma device, loaned to our institution by P.C.F. (Supplementary Fig 1, available via Mendeley at https://data.mendeley.com/datasets/dm2y8ztgvm/1). To create plasma, a pulse generator, which supplied 17 kHz, 6 μs width pulse at a power density of approximately 2 W/cm2 (AAPlasma, Philadelphia, PA) was connected to a 10 mm diameter quartz-covered copper electrode of 50 mm length and 1 mm quartz thickness with one end fused. These pulse parameters were selected to provide sufficient treatment dose at the high level of plasma uniformity required to avoid any tissue damage. The device is used by hovering either the tip or side of the electrode directly over the target lesion, and plasma forms only where the gap between the electrode and the target surface does not exceed 1 mm. We treated the lesions randomized to NTAP by circling the electrode gently over each treatment area for a duration of 2 minutes per lesion. The treatment protocol and device settings were developed by P.C.F., as previously reported.[9-11]

Statistical analysis

Initial power analysis calculated that, with an effect size of 0.4384062, a power of 80%, and a type I error of 5%, 67 total lesions were required. Effect size was determined based on a prior NTAP study conducted; of 17 lesions treated, 9 (53%) had full resolution, 3 (18%) had some improvement, and 5 (29%) showed no improvement.[12] Our study received IRB approval for an increased enrollment goal and ultimately achieved 96% power using the same effect size and type I error, with 112 total VV lesions enrolled.

After study follow-up was complete, study team members blindly compared baseline and final photos for each lesion to assess overall improvement and degree of post-treatment sequelae such as pigmentary changes, erythema, and scarring. Lesion improvement was graded using the following scale: 0 = unchanged, 1 = mild improvement (<25%), 2 = moderate improvement (~50%), 3 = marked improvement (~75%), 4 = near resolution (>90%), and 5 = complete resolution (Fig 1, *A*). Degree of hyperpigmentation and hypopigmentation were graded using this scale: 0 = no difference in pigment, 1 = mild difference, 2 = moderate difference, 3 = high difference. Final post-treatment lesion resolution scores and residual sequelae were determined by the study's principal investigator (L.W.L.).

Study results were analyzed using an intention-to-treat analysis. Descriptive statistics were used to characterize patient demographics, degree of lesion resolution, and frequency of adverse events. Categorical data was analyzed using Pearson chi-square testing. Numerical data such as age and pain ratings were assessed with two-sample t-tests for normally distributed data and nonparametric tests for data without normal distribution. A two-sided *P* value of ≤.05 was considered significant. Statistical analysis was performed using SPSS software (IBM Corp).

RESULTS

From a total of 14 patients, 112 VV lesions were enrolled in this study. Patients were most often White (92.9%), male (71.4%), and a mean of 9.5 [±2.5] years old (Supplementary Table I, available via Mendeley at https://data.mendeley.com/datasets/dm2y8ztgvm/1). Responses of SOC- and NTAP-treated lesions, respectively, included: no response (5.4%, 7.1%); partial response (33.9%, 41.1%); and complete resolution (60.7%, 51.8%; *P* value = .679). These responses were defined based on the scale shown in Fig 1, where

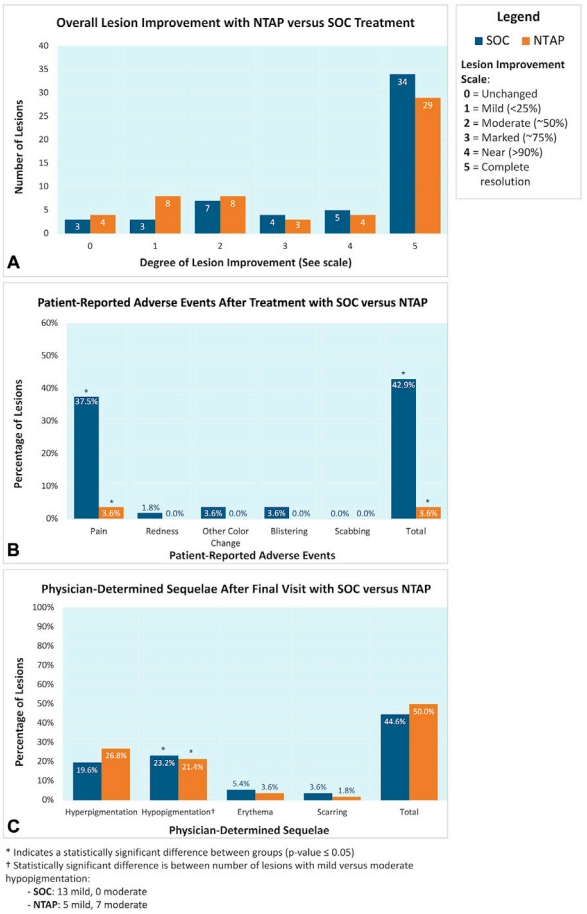


Fig 1. Efficacy and adverse events of standard of care (SOC) therapy versus nonthermal atmospheric plasma (NTAP) for VV. **A,** Final improvement after treatment of VV lesions with SOC therapy versus NTAP. **B,** Patient-reported adverse events after treatment with SOC versus NTAP. **C,** Physician-determined sequelae after final visit with SOC versus NTAP therapy. *VV*, Verruca vulgaris.

0 = no response, 1-4 = partial response, and 5 = complete response. When comparing individual lesion resolution on the 0−5 resolution scale, no significant difference existed between the 2 interventions (Fig 1, *A*, *P* value = .690). However, when degree of lesion resolution was averaged for NTAP and SOC lesions within each patient enrolled, SOC lesions were found to have slightly superior median degree of lesion resolution (4.7; see scale in Fig 1) as compared to NTAP lesions (4.0, *P* value = .028). Fig 2 demonstrates an enrolled VV lesion achieving complete resolution with NTAP therapy during this study, with 2A showing the lesion pretreatment and 2B post-treatment.

Overall, patients reported more AEs in SOC lesions (42.9%) than in NTAP lesions (3.6%, *P* value <.001). Median pain levels (rated 0-10) for individual patients during active treatment were 5.63 for SOC lesions and 0.815 for NTAP lesions, and patients were

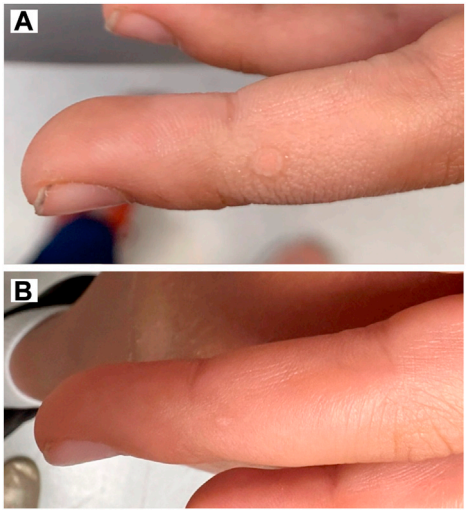


Fig 2. Nonthermal atmospheric plasma (NTAP) treatment response of verruca vulgaris (VV) lesion. **A,** An untreated wart on the lateral third finger, and (**B**) complete resolution of the lesion after 3 NTAP treatments with mild residual hypopigmentation.

more likely to report a presence of pain, stinging, or burning within 1 week of first treatment in SOC lesions (37.5%) than in NTAP lesions (3.6%, *P* value <.001). Additionally, topical lidocaine was applied prior to cryotherapy, which may have confounded pain results in acute peritreatment time. There was no difference in the patient-reported rates of redness, blistering, scabbing, or other color change between NTAP and SOC treatments, as rates were low in both treatment groups (Fig 1, *B*, Supplementary Table II, available via Mendeley at https://data.mendeley.com/datasets/dm2y8ztgvm/1). Of physician-determined sequelae at the end of the study, more SOC lesions (13) were found to have mild hypopigmentation as compared to NTAP lesions (5), but more NTAP lesions (7) were found to have moderate hypopigmentation as compared to SOC lesions (0), *P* value = .003). However, there was no difference in degree of hyperpigmentation, erythema, or scarring (Fig 1, *C*, Supplementary Table II, available via Mendeley at https://data.mendeley.com/datasets/dm2y8ztgvm/1). No significant AEs occurred throughout the duration of this study.

DISCUSSION

NTAP is a promising new therapy in dermatology due to its painless nature and promising safety profile. Our results demonstrate that NTAP has an efficacy equivalent to that of current SOC treatments for VV with regard to lesion resolution, and it exhibited a significantly lower rate of patient-reported AEs, such as pain. Although comparison

of mean SOC and NTAP lesion resolution within each patient showed a higher median improvement for SOC lesions, both NTAP and SOC resolution medians were between 90% and 100%. Thus, the difference is not clinically significant. Additionally, a lower number of patient-reported AEs with NTAP treatment provides justification for its use, particularly in young children with a lower pain threshold. Though satisfaction level of treatment modalities was not included as a secondary study outcome, anecdotally, patients and parents consistently expressed high satisfaction with the NTAP device as compared to SOC cryotherapy.

There are several limitations to our study. Though the study was adequately powered with the number of lesions enrolled, the study was conducted at a single center with a small number of patients ultimately enrolled and low enrollment of patients with skin color. A lack of matching cohorts by wart size and location may also introduce bias into study results and conclusions. Patients were limited to only 3 treatments with the NTAP device, and many patients showed continued improvement without complete clearance by the end of the study treatment period. We hypothesize that more patients could have achieved full lesion clearance with one or several more NTAP treatments. Additionally, follow-up assessment of sustained treatment response was limited to 4 weeks and may not represent long-term treatment response. Similarly, lack of post-treatment follow-up after physician-determined sequelae were measured at the end of the study period limits our ability to determine whether these sequelae (such as pigmentary changes) persisted or self-resolved. Additionally, the risk of hypopigmentation with SOC treatments is higher in patients with skin color, which was underrepresented in our cohort. NTAP may be even more advantageous in this patient population. Prolonged treatment time with NTAP may be a limiting factor for patients with numerous lesions. Finally, though all study team members were trained to use the NTAP device, potential user errors cannot be fully excluded. It is important to note that this device is not currently commercially available.

Results of this study demonstrate that NTAP is as effective as current SOC therapy with lower rates of AEs. Given that NTAP is an efficacious and risk-free intervention for the treatment of VV, we propose that NTAP deserves further development as an up-and-coming treatment modality for VV in pediatric patients. Given its effectiveness and that it is relatively painless, use of NTAP in other pediatric dermatologic conditions should be considered. Larger-scale, multicenter clinical studies employing NTAP in the pediatric population are warranted to confirm safety and efficacy.

The authors would like to acknowledge Dr Mathew Gregoski, PhD, MS, for his assistance with statistical analysis. This project was supported, in part, by the National Center for Advancing Translational Sciences of the National Institutes of Health under Grant Number UL1 TR001450. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Additionally, Tyler Beck was supported by an NIH training grant (F31 HL158243).

Conflicts of interest

None disclosed.

REFERENCES

1. Stulberg DL, Hutchinson AG. Molluscum contagiosum and warts. *Am Fam Physician*. 2003;67(6):1233-1240.
2. Shope C, Beck T, Andrews L, Friedman P, Wine Lee L. Nonthermal atmospheric pressure plasma technology in dermatology. *SKIN J Cutan Med*. 2022;6(5):365-373. https://doi.org/10.25251/skin.6.5.2
3. Silverberg N. Pediatric molluscum contagiosum: optimal treatment strategies. *Paediatr Drugs*. 2003;5(8):505-512. https://doi.org/10.2165/00148581-200305080-00001
4. Kong M, Kroesen GMW, Morfill G, et al. Plasma medicine: an introductory review. *New J Phys*. 2009;11:12-26. https://doi.org/10.1088/1367-2630/11/11/115012
5. Dobrynin D, Fridman G, Friedman G, Fridman A. Physical and biological mechanisms of plasma interaction with living tissue. *New J Phys*. 2009;11:115020. https://doi.org/10.1088/1367-2630/11/11/115020
6. Morfill G, Kong M, Zimmermann J. Focus on plasma medicine. *New J Phys*. 2009;11:115011. https://doi.org/10.1088/1367-2630/11/11/115011
7. Fluhr JW, Sassning S, Lademann O, et al. In vivo skin treatment with tissue-tolerable plasma influences skin physiology and antioxidant profile in human stratum corneum. *Exp Dermatol*. 2012;21(2):130-134. https://doi.org/10.1111/j.1600-0625.2011.01411.x
8. Friedman PC. Cold atmospheric pressure (physical) plasma in dermatology: where are we today? *Int J Dermatol*. 2020;59(10):1171-1184. https://doi.org/10.1111/ijd.15110
9. Friedman PC, Fridman G, Fridman A. Using cold plasma to treat warts in children: a case series. *Pediatr Dermatol*. 2020;37(4):706-709. https://doi.org/10.1111/pde.14180
10. Friedman PC, Fridman G, Fridman A. Cold atmospheric pressure plasma clears molluscum contagiosum. *Exp Dermatol*. 2023;32:562-563. https://doi.org/10.1111/exd.14695
11. Friedman PC, Miller V, Fridman G, Fridman A. Use of cold atmospheric pressure plasma to treat warts: a potential therapeutic option. *Clin Exp Dermatol*. 2019;44(4):459-461. https://doi.org/10.1111/ced.13790
12. Friedman PC, Miller V, Fridman G, Lin A, Fridman A. Successful treatment of actinic keratoses using nonthermal atmospheric pressure plasma: a case series. *J Am Acad Dermatol*. 2017;76(2):349-350. https://doi.org/10.1016/j.jaad.2016.09.004