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Efficacy of a *Coriolus versicolor*–Based Vaginal Gel in Women With Human Papillomavirus–Dependent Cervical Lesions: The PALOMA Study

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Objective: The aim of the study was to evaluate the efficacy of Papilocare, a *Coriolus versicolor*–based vaginal gel, in repairing human papillomavirus (HPV)-related low-grade cervical lesions.

Methods: The study is a multicenter, open-label, randomized, parallel-group, watchful waiting approach-controlled trial involving 91 HPV-positive women with low-grade Pap smear alterations and consistent colposcopy.

Results: The percentage of patients with normal Pap smear and concordant colposcopy 3 and 6 months after receiving treatment (78.0% and 84.9%) was significantly higher than without treatment (54.8% and 64.5%), especially in high-risk HPV patients (79.5% and 87.8% vs 52.0% and 56.0%). At 6-month visit, overall HPV clearance was achieved by a greater number of patients receiving treatment (59.6%) compared with those without treatment (41.9%), especially high-risk HPV ones (62.5% vs 40.0%). The cervical re-epithelization score was significantly higher with treatment (mean = 4.5) than without (mean = 4.1). Compared with baseline, perceived stress decreased in the treatment group (from 21.1 to 19.0) and increased in the control group (from 17.7 to 20.7). A total of 7 possible or probable treatment-related adverse events were reported, most of them (n = 6) being mild or moderate in severity.

Conclusions: Treatment with Papilocare has demonstrated a better clinical benefit than the conventional watchful waiting approach in clinical practice for total and high-risk HPV patients in terms of its efficacy to treat HPV-related cervical lesions and to clear all HPV strains after a single 6-month period. It has demonstrated an adequate safety and tolerability and confers additional benefits such as higher re-epithelization, stress reduction, and high treatment adherence.

Key Words: efficacy, Papilocare, *Coriolus versicolor*, human papillomavirus, cervical lesions, epithelization, vaginal health, clearance

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The human papillomavirus (HPV) is one of the most common sexually transmitted infections and the most important etiological agent in cervical cancer.[1] Most HPV infections are asymptomatic and clear within 2 years without treatment.[2] Despite its low risk of progression to cervical cancer, many women experience negative emotional responses and even long-term psychological distress associated with an HPV-positive diagnosis and an abnormal cytology.[3,4] When added to the fact that the conventional management strategy for this condition, especially in cases of low-grade lesion, is watchful waiting, the approach may become long and challenging for both the women and their physicians. Thus, a safe and nonsurgical treatment that can help repair the low-grade lesions and enhance viral clearance might be highly useful under these circumstances.[5] Papilocare (Procare Health, Valencia, Spain) is a *Coriolus versicolor*–based vaginal gel that combines ingredients boasting known properties, such as moisturizing, tissue regeneration, and balancing of the vaginal microbiota (hyaluronic acid, *Asian centella, Aloe vera,* and α-glucan oligosaccharide),[6,7] with other ingredients with demonstrated positive effects on both HPV-dependent cervical lesions and HPV clearance (*C. versicolor, Azadirachta indica*, and carboxymethyl-β-glucan).[8–10] It has been hypothesized that HPV integration could be minimized by reducing the potential mitotic surface by re-epithelization.[11] On the other hand, the vaginal microbiota status

plays a decisive role in the persistence or clearance of local infections.[12–14] Improved re-epithelization and vaginal microbiota restoration have been observed in previous pilot studies involving the use of Papilocare in both asymptomatic, healthy women and HPV-positive patients with no cervical lesions.[15,16] Moreover, a combination of *C. versicolor* and neem extract has shown to induce a local immune response in both in vitro and animal model studies[17–19] hindering the oncogenic action of the HPV.

The purpose of the present study was to evaluate the efficacy of Papilocare in repairing cervical mucosa lesions in HPV-positive women with low-grade Pap smear alterations and consistent colposcopy observations.

METHODS

Study Design and Patients

This was a multicenter, open-label, parallel-group, randomized, controlled clinical trial involving women with HPV-related low-grade cytological alterations and concordant colposcopy observations who attended the gynecologist office between July 2016 and January 2018 (PALOMA trial, #NCT04002154). Nine Spanish hospitals (4 public and 5 private) participated in the study. The study was carried out in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practices guidelines. The protocol was evaluated and approved by the following institutional review boards: the Comité Ético de Investigación Clínica del Hospital Universitario de la Princesa, the Comité Ético de Investigación Clínica de Clínica Tres Torres-Centre Cardiovascular Sant Jordi, the Comité de Ética de la Investigación Provincial de Málaga, the Comité Ético de Investigación Clínica del Grupo Hospitalario Quirón, the Comité Ético de Investigación Clínica del Hospital General Universitario Gregorio Marañón, the Comité Ético de Investigación Clínica de la Fundació Sanitària del Hospital de la Santa Creu i Sant Pau, the Comité Ético de Investigación Clínica del Hospital General de Alicante, and the Comité Ético Investigación Clínica GAE HCSC Area 7. All patients provided signed informed consent to participate in the study. The inclusion criteria were as follows: age between 30 and 65 years; positivity for low-risk or high-risk (HR) cervical HPV subtypes (tested within the 3 months before the inclusion); and a cytology of atypical squamous cells of undetermined significance, low-grade squamous intraepithelial lesion (LSIL), or atypical glandular cells of undetermined significance together with a concordant colposcopic observation. The exclusion criteria included a history of an HPV vaccination, the existence of a symptomatic vulvovaginal infection, a history of surgical cervical excision (within the last year) or total hysterectomy, a history of any clinically relevant immune alterations or autoimmune diseases, ongoing treatment with immunosuppressant drugs, a history of a gynecologic malignancy or abnormal genital bleeding (within the 6 months before the inclusion), the use of vaginal contraceptives, a contraindication for Papilocare, pregnancy and lactation, or participation in another clinical trial (within the 4 weeks before the inclusion).

Treatment Groups and Procedures

Women were randomized (1:1:1) into 3 treatment groups: scheme A, scheme B, or control (watchful waiting approach). Scheme A comprised using Papilocare once daily, preferably at bedtime, for 21 consecutive days followed by 7 days with no treatment throughout the first month, then an alternate-day therapy (except during the menstrual cycle) lasting up to 6 months. Scheme B was similar to scheme A except for the fact that the 21-day treatment/7-day rest period lasted for 3 months (instead of one) and that the alternate-day therapy lasted up to 3 months. Randomization was carried out using a centralized, computer-generated randomization list. Although sexual relations were not forbidden, the use of condoms was strongly recommended. The use of douches and vaginal deodorants was not allowed.

Analyzed Variables

The primary end point of this study included the repair of the lesions after 6 months of treatment, defined as a normalized cytology and concordant colposcopy observations. An atypical squamous cell of undetermined significance, LSIL, or atypical glandular cells of undetermined significance cytology was diagnosed using a Pap smear test and following the Bethesda System 2001 classification.[20] The colposcopic concordance was evaluated based on the definitions established by the Nomenclature Committee of the International Federation for Cervical Pathology and Colposcopy.[21] The secondary end points included HPV clearance, cervical re-epithelization, vaginal health, perceived stress, satisfaction with the product, adherence to the treatment, and incidence and severity of adverse events (AEs). Overall HPV clearance was determined by the sum of the total and partial clearances. Total clearance was defined as a negative HPV test or the disappearance of all species detected at baseline, whereas partial clearance was defined as the disappearance of at least 1 species together with normal Pap smear findings and concordant colposcopic observations. Clinically relevant types of HPV were identified by polymerase chain reaction technology (Clart HPV4; Genomica, Madrid, Spain). Both the Pap smear and HPV screening tests were blinded and centrally conducted by an independent researcher at the *Instituto de Estudios Celulares y Moleculares* laboratory (Lugo, Spain). The colposcopy, in contrast, was not blinded and was locally performed on site by the respective local investigator. The degree of re-epithelization of the cervical mucosa was evaluated by colposcopy and quantitatively rated with a 5-point Likert scale (where a score of 1 equated to severe ectopy and bleeding and a score of 5 equated to no ectopy).[15] Vaginal health was evaluated using the Bachmann Vaginal Health Index,[22] whereby lower scores indicate the existence of greater atrophy in the genitourinary tract. Perceived stress was evaluated using the Spanish version of the 14-item Perceived Stress Scale,[23] whereby higher scores represent a greater degree of perceived stress. The patients' degree of satisfaction with Papilocare was assessed with a 7-point Likert scale, and the satisfaction data were grouped as follows: satisfied (scores 1–3), neither satisfied nor dissatisfied (score 4), and dissatisfied (scores 5–7). Complete data collection was performed at baseline and after 3 and 6 months (except the overall HPV clearance data, which were only collected at 6 months).

Sample Size and Statistical Analyses

Given the exploratory nature of the study and the lack of published data, the sample size was determined by considering the results expected by the board of scientific experts and the trials' investigators, that is, repair of lesions in approximately 80% and 30% of treated and untreated women, respectively. Therefore, it was determined that a sample size of 28 patients in each group (84 in total) would be able to detect a 50% difference in the proportion of patients achieving a repair in their lesions after 6 months of treatment with a 95% statistical power. Thus, the estimated total sample size was 96 considering the differentiation of the treatment group into 2 schemes and the missing information (approximately 10% of the participants). Categorical variables were expressed as absolute and relative frequencies, and continuous variables were expressed as a mean with its SD. Comparisons between groups were analyzed using the χ^2 or Fisher exact test for categorical variables or the Kruskal-Wallis test for continuous ones, when appropriate. Statistical significance was defined as a p value of .05 or less. All statistical procedures were performed using the SAS 9.4 software. Because evaluating the efficacy of Papilocare versus the control approach was the primary objective of the trial, data

from treatment schemes A and B were pooled into a single-treatment group for the main analysis. A predefined subgroup analysis with HR HPV–infected women, including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68,[9] was performed only to examine the repair of the cervical lesions and the HPV clearance. This analysis was conducted with the modified intention-to-treat population ("full-analysis set" described by the International Conference on Harmonization E9 guideline),[24] i.e., women randomized who received at least 1 dose of Papilocare and had, at least, baseline data and 1 posttreatment value available for the primary end point analysis.

Role of the Funding Source

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Results

Of the total of 101 patients who were randomized in the study, 91 were evaluable for efficacy (see Figure 1): 59 patients receiving treatment (44 considered as HR HPV patients) and 32 control patients (26 considered as untreated HR HPV patients). The characteristics of the patients of the treatment and control groups were fully comparable at baseline: predominantly White women (93.4% of the total), with a mean age of 40.5 years (SD = 7.9 years), and a mean body mass index of 22.8 kg/m^2 (SD = 3.4 years; see Table 1).

TABLE 1. Demographic and Clinic Characteristics of the Patients

	Treatment group	Control group (no treatment)	*p*
Age			.249
N available	59	32	
Mean (SD) age, y	41.4 (8.2)	38.8 (7.0)	
Race			.679
N available	59	32	
White, *n* (%)	56 (94.9)	29 (90.6)	
Hispanic (Latin American), *n* (%)	3 (5.1)	3 (9.4)	
Body mass index			.179
N available	56	31	
Mean (SD), kg/m^2	22.8 (3.6)	22.8 (3.3)	
Previous pregnancies			.312
N available	20	10	
Mean (SD)	2.2 (1.2)	1.6 (1.0)	
Previous miscarriages			.921
N available	20	10	
Mean (SD)	0.5 (0.7)	0.4 (0.5)	
Vaginal deliveries			.776
N available	20	8	
Mean (SD)	1.7 (0.9)	1.1 (0.6)	
Cesarean deliveries			.778
N available	19	8	
Mean (SD)	0.4 (0.7)	0.4 (0.5)	
Sexual relations within the last month			.295
N available	59	32	
Mean (SD)	4.9 (5.8)	5.0 (5.5)	

Repair of Cervical HPV-Induced Lesions

The percentage of patients presenting with normal Pap smear findings and concordant colposcopy observations after 6 months was significantly higher in the treatment group than in the control group (84.9% vs 64.5%, $p = .031$; see Figure 2), with this difference being even more noticeable in the HR HPV subgroup (87.8% vs 56.0% for the treatment and control groups, respectively, $p = .003$). Significant differences were also observed at 3 months in the total sample (78.0% vs 54.8% for the treatment and control groups, respectively, $p = .023$) and the HR HPV subgroup (79.5% vs 52.0%, $p = .017$).

Overall HPV Clearance

A nonstatistically significant trend toward a higher HPV clearance was observed at 6 months in the treatment group (31/52 [59.6%] of the patients, with missing data for 1 patient) compared with the control group (41.9% [13/31], $p = .118$). Scheme B with Papilocare showed significant differences in clearance compared with the control group (75.9% vs 41.9%, $p = .008$), whereas no significant differences were observed with scheme A (39.1% vs 41.9%, $p = .836$). In the HR HPV group, a trend toward greater clearance was observed among the treated patients compared with the control group (62.5% vs 40.0%, $p = .076$). In this patient cohort, scheme B also resulted in significant differences in clearance with respect to the control group (81.8% vs 40.0%, $p = .004$), whereas scheme A did not (38.9% vs 40.0%, nonsignificant).

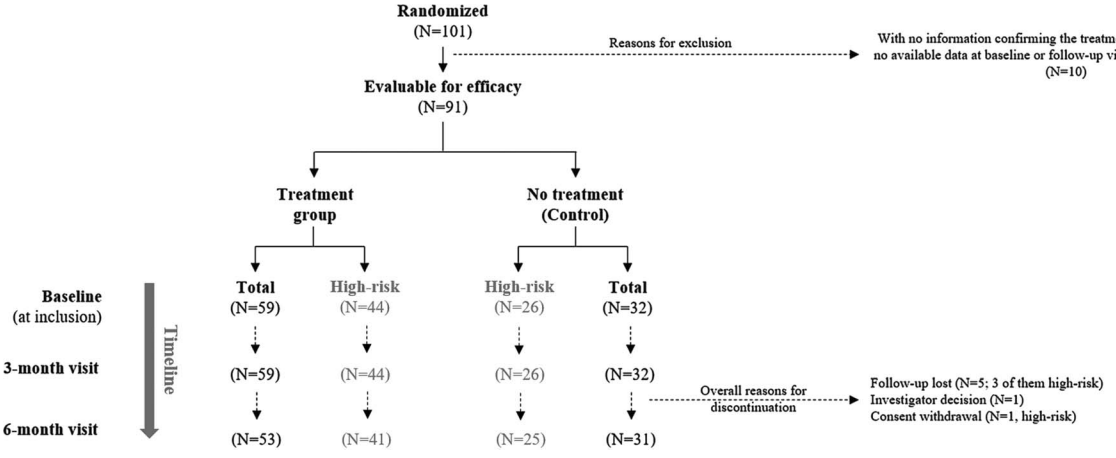

FIGURE 1. Patient flowcharts showing the study groups, the total and HR HPV patient pools, and the timeline.

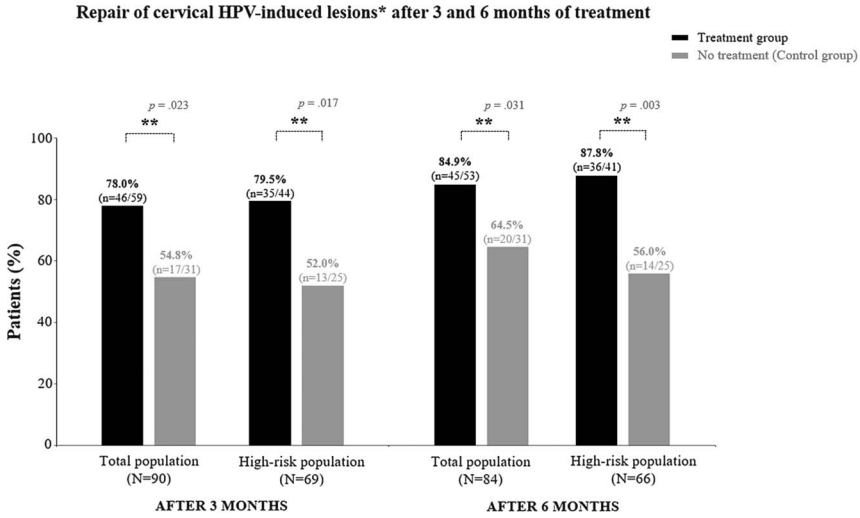

FIGURE 2. Repair of HPV-dependent cervical lesions after 3 and 6 months of treatment in the total and HR HPV patient pools. *Normalized cytology together with concordant colposcopic observations. **χ^2 test. One high-risk HPV patient of the control group had missing data.

Cervical Re-epithelization

The cervical re-epithelization score of the treated patients improved significantly between the baseline (mean = 4.2 [SD = 0.9]) and month 6 (mean = 4.5 [SD = 0.7], $p = .001$; see Figure 3). At the 6-month visit, the score was significantly higher among the patients of the treatment group compared with those of the control group (mean = 4.1 [SD = 0.9], $p = .017$). A trend toward a higher percentage of patients with no ectopy was observed with Papilocare (from 45.8% at baseline to 62.3% at 6 months) in comparison with the control group (from 37.5% to 35.5%). At the 3-month visit, significant differences were observed between the percentage of patients with no ectopy in the treatment group versus the control group (52.5% vs 40.6%, $p = .013$).

Vaginal Health

Compared with the baseline, the percentual change in the vaginal health index after 3 and 6 months of follow-up was numerically higher in the treatment group (mean percentual change of 2.5% [SD = 13.1] at the 3-month visit and of 3.7% [SD = 15.2] at the 6-month visit) compared with the control group (−0.1% [SD = 10.8], $p = .249$ and −1.3% [SD = 12.3], $p = .067$, respectively). A trend toward an improvement in the vaginal health from baseline was observed in the treatment group (41.4% and 42.3% of women achieved an improvement at the 3- and 6-month visit, respectively) in comparison with the control group (31.3% and 29.0%, respectively).

Perceived Stress

A differential but nonsignificant trend in this parameter was observed between both groups, in such a way that it decreased in the treatment group (from a mean of 21.1 [SD = 8.8] at baseline, to a mean of 19.7 [SD = 9.0] at 3 months, and, finally, a mean of 19.0 [SD = 9.1] at 6 months) and increased in the control group (from a mean of 17.7 [SD = 7.2] at baseline, to a mean of 17.4 [SD = 6.3], and, finally, a mean of 20.7 [SD = 9.8] at 3 and 6 months, respectively). The number of patients reporting an improvement in perceived stress was higher at the 3-month (35 of 56 women [62.5%]) and 6-month visits (29 of 50 women [58.0%]) in the treatment group compared with the control group (15 of 31 women [48.4%] and 11 of 28 women [39.3%], respectively).

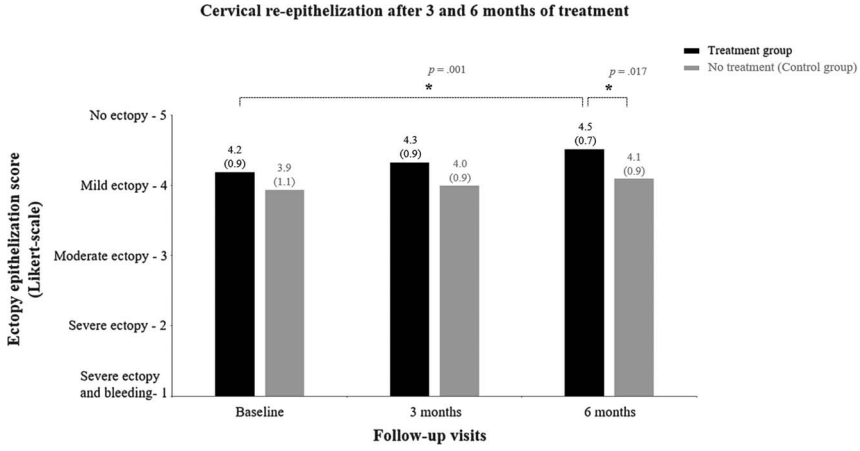

FIGURE 3. Changes in the cervical re-epithelization score (Likert scale) between baseline and the 3- and 6-month visits. Values are expressed as the mean together with the SD (between parentheses). *χ^2 test.

Satisfaction With the Product and Therapeutic Adherence

At the 3- and 6-month visits, 49 (83.1%) of 59 patients and 45 (86.5%) of 52 patients treated with Papilocare, respectively, were satisfied with the treatment. None of the patients were dissatisfied with the product at 6 months. Treatment adherence was 93.2% during the first 3 months (55 of 59 patients) and 94.3% after 6 months (50 of 53 patients). Causes of nonadherence during the entire study included omissions (4 patients), discomfort (1 patient), influenza infection (1 patient), and menstruation (1 patient).

Tolerability

Information on the tolerability was available for 96 patients, whereby a total of 11 patients (11.5% of the total) experienced an AE, 9 of which belonged to the treatment group (14.1%). Of 17 reported AEs, 7 (31.8%) were considered as possibly or probably related to the treatment and most of them (n = 6) were mild or moderate in severity (see Table 2). Temporary or permanent discontinuation was required in 3 cases.

DISCUSSION

Approximately 30% of Spanish women younger than 30 years are HPV carriers.[25] The likelihood of HPV infection decreases over time, from 47% between the ages of 15 and 19 years to 12% in women older than 45 years.[26] The HPV is a fundamental factor for the development of cervical cancer.[27] The World Health Organization has recently established the elimination of cervical cancer as a priority medium-term objective.[28] This trial aimed to explore whether treatment with Papilocare provides better results than the conventional watchful waiting strategy in improving low-grade cervical lesions, which are the first step in the natural history of cervical cancer.[29] Clearance of LSILs after a conservative approach is of approximately 59% within 2 years of the diagnosis. Nevertheless, the likelihood of progression of these lesions to a high-grade squamous intraepithelial lesion within 5 years is 12.7%.[30] In addition, treatments for cervical cancer severely compromise the reproductive health of women. In a systematic review of Cochrane, Kyrgiou et al.[31] demonstrated a higher baseline risk for prematurity in women with cervical intraepithelial neoplasia, which increased with excisional and ablative treatments. Therefore, the scenario is conflicting, as choosing an excisional or ablative approach for the treatment of LSILs can result in negative reproductive outcomes, but avoiding treatment and just monitoring the disease results in its progression to high-grade lesions in 12 of 100 women. The current recommendation in Spain also includes monitoring the disease.[32,33] However, based on the results of this present study, a novel strategy involving Papilocare has become available. The results of this treatment in the repair of low-grade lesions have demonstrated to significantly exceed those obtained with the watchful waiting approach (8 in 10 women achieved a normalization in their lesions).

Spontaneous clearance of the HR HPV occurs in approximately 29% and 41% of cases at 6 and 18 months, respectively.[34] The HPV clearance rates seen in our study were higher, i.e., more than twice (63%) the established value at 6 months. This finding has important clinical implications considering the fact that the only guidelines that a physician can provide to their female patients to clear HPV is to maintain their vaginal health or, in the case or active smokers, to quit smoking. This absence of solutions may increase stress levels in many women. Indeed, the results of our study revealed a differential trend with respect to perceived stress, with increased reported levels in the control group and reduced ones in the treatment group. Thus, based on the results of our study, we believe that Papilocare should be included among the advices for the treatment of HPV considering its ability to double the clearance rate of HR HPV.

The results of our study have been replicated in several independent, observational, noncontrolled studies performed in Spanish public university hospitals.[35–37] The 6-month effectiveness of Papilocare was evaluated and confirmed in all of the studies (between 53% and 72.5% of cases achieved a negative cytology and/or HPV clearance/reduced viral load). An update of the study by Riera et al.,[38] which included a watchful waiting control group, revealed a significantly higher percentage of responders in the group treated with Papilocare compared with the controls (80.0% vs 51.4%). Recent interim results from the observational, multicenter, prospective, single-cohort PAPILOBS study (#NTC04199260) revealed a notable effect of Papilocare at 6 months in repairing HPV-dependent low-grade cervical lesions and clearing HPV (66% and 63% of patients, respectively) under real-life conditions.[39] Data obtained in the present study in relation to the HPV clearance, especially in HR HPV patients, are consistent with real-world data obtained in other studies.[40] Moreover, in this trial, the degree of cervical re-epithelization was significantly higher with Papilocare compared with the control group. This beneficial effect on re-epithelization was also observed by Palacios et al.[15] in a pilot study performed with 21 asymptomatic non–HPV-infected women, in which women treated Papilocare achieved improved cervical re-epithelization (from 3.1 to 4.4 according to the same

TABLE 2. Incidence and Severity of the AEs Reported During the Study Period in the Group Receiving Treatment With Papilocare

	Total
Reported AEs, n (%)	17 (100.0)
Infections/infestations	8 (47.1)
Herpes zoster	2 (11.8)
Candidiasis	2 (11.8)[a]
Influenza	2 (11.8)
Bacterial vaginosis	1 (5.9)
Urinary tract infection	1 (5.9)
Skin/subcutaneous disorders	1 (5.9)
Rash	1 (5.9)
General disorders and alterations in the administration site	6 (35.3)
Vulvovaginal stinging/burning	4 (23.5)[a]
Intolerance to the product	1 (5.9)[a]
Vulvovaginal pruritus	1 (5.9)[a]
Benign, malignant, or unspecified neoplasm	1 (5.9)
Breast cancer	1 (5.9)
Psychiatric disorders	1 (5.9)
Anxiety	1 (5.9)
Severity of the AEs, n (%)	
Mild	11 (64.7)
Moderate	4 (23.5)
Severe	2 (11.8)
Serious AEs, n (%)	1 (5.9)[b]
Association of the AE with Papilocare	
Probable	1 (5.9)
Possible	6 (35.3)
Not related	10 (58.8)

[a]Probably or possibly related to the product under study (only 1 of the 2 candidiasis was related to Papilocare).

[b]Not related to the study product.

cervical epithelization scoring system used in the present study). Similarly, Gálvez et al.[16] reported a significant 20% improvement in cervical re-epithelialization in HPV-positive lesion-free women treated with Papilocare (3.8 vs 4.5) compared with baseline.

In addition, treatment with Papilocare is safe, as most AEs reported in this study were mild or moderate in severity. Moreover, most of the participants were satisfied with the treatment, and therapeutic adherence exceeded 90%. Nevertheless, our study has certain limitations: (1) the lack of information about cofactors for the progression of cervical HPV infection to cancer, such as smoking[41] (lacking in the present study), or adjusting all analyses by covariables in patients, which could have avoided potential interferences in the results, and (2) it should be noted that we used a cytology plus concordant colposcopic observations to evaluate the normalization of lesions. Biopsies were not performed in the study because all women presented low-grade cytological alterations with normal colposcopy or grade 1 changes, decision in agreement with guidelines recommendations,[32,42] and also because biopsy can modify the natural history of the lesion. Thus, the present article does not provide evidence on the treatment and prevention of dysplasia; (3) colposcopic concordance and epithelization assessments were also performed in an unblinded manner; thus, subjectivity associated with these outcomes could have resulted in an assessment bias; (4) the "partial clearance" was included as part of the overall HPV clearance secondary end point (see method section) according to the concept that 1 HPV-related lesion is very frequently caused by just 1 viral subtype, despite the presence of multiple HPV types.[43] With regression of the lesion as well as clearance of one of the oncogenic HPV subtypes, it is likely that the causative virus has been eliminated. Nevertheless, the risk of future lesions and dysplasia remains if the residual HPV subtype is a HR HPV, (5) Given the exploratory nature of the study and the concomitant determination of the sample size, the number of patients was insufficient for identifying statistical differences in certain secondary variables for which tendencies were observed. (6) Moreover, for our initial exploration, we aimed to analyze all women, not only those with HR HPV, and to obtain results that could be extrapolated for the general population. Additional subanalyses that already were planned in the protocol will determine the efficacy and safety of Papilocare on HR HPV patients. Further ongoing clinical trials have already included only HR HPV patients (the PAPILOCAN clinical trial #NCT04210336 and the PALOMA II clinical trial #NTC04199078). Besides these limitations, comparisons made with the conventional approach applied in standard clinical practice are very useful and provide evidence that new interventions for the management of HPV are required.

CONCLUSIONS

Treatment with Papilocare has demonstrated a better clinical benefit than the conventional watchful waiting approach in clinical practice for HPV-positive patients, especially for those with HR HPV. Papilocare has shown significant efficacy in the treatment of low-grade cervical lesions associated with HPV and a positive trend in increasing HPV clearance after a 6-month period. Moreover, it shows good safety and tolerability and confers additional benefits, such as a significant improvement in cervical re-epithelization, a positive trend in perceived stress reduction, and high therapeutic adherence.

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