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Evaluation of human papillomavirus DNA detection-guided aminolaevulinic acid-mediated photodynamic therapy for the treatment of condyloma acuminata

Jingying Wang, Songshan Li, Junpeng Li, Zhijia Li, Qi Wang, Xiaoming Peng, Minglan Shi, Qilei Che, Hui Liu, Lifen Jiang, Kang Zeng*

Department of Dermatology and Venereology, Nanfang Hospital, Southern Medical University, Guangzhou, 510515, China

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ABSTRACT

Background: Aminolaevulinic acid-mediated photodynamic therapy (ALA-PDT) is used to treat condyloma acuminata (CA), yielding a high clearance rate and low recurrence rate. Consecutive human papillomavirus (HPV) DNA detection can be used to dynamically monitor the therapeutic efficiency of PDT. Here, we evaluated the efficacy of ALA-PDT in the context of different HPV infection states.

Methods: One hundred thirty-eight patients with HPV infection and visible anogenital warts were enrolled. Microwave or radiofrequency was used to remove visible lesions before PDT. HPV DNA detection was performed using real-time polymerase chain reaction before each PDT session and at follow-up. Treatment was halted after the patient showed two negative results for HPV DNA detection in a row.

Results: Of the 138 patients enrolled in the study, 72 completed treatment. Multisite HPV-infected patients required more sessions of PDT than did single-site infected patients to reach the endpoint of treatment. Compared with patients with only external CA, individuals with internal CA required more sessions to eliminate HPV infection. The total number of PDT sessions performed in the multitype HPV-infected group was significantly higher than that in the single-type infected group. Patients with non-high-risk (HR)-HPV infection required fewer PDT sessions than did those with HR-HPV infection by the end of treatment. Sixty-nine patients were followed-up for at least 6 months, only 2.9% of whom showed recurrence.

Conclusions: Combined ALA-PDT and HPV DNA detection was an effective strategy for the treatment of CA. Patients with multisite and multitype HPV infection required more PDT sessions to eliminate the virus.

1. Introduction

Condyloma acuminata (CA), commonly known as genital warts, is one of the most frequently encountered sexually transmitted diseases in the sexually active population and is associated with several types of human papillomavirus (HPV) infection [1]. More than 200 HPVs have been identified and sequenced, among which, low-risk HPV (LR-HPV), mainly HPV-6 and -11, are predominantly responsible for CA. High-risk types (HR-HPV), such as HPV-16, -18, -31, and -35, have also been occasionally detected (usually as coinfections with HPV-6 or HPV-11) [2,3]. Persistent or latent HPV infection, without symptoms or visible warts, often leads to refractory lesions after treatment [4], which is the most significant challenge of conventional strategies for treating CA.

Conventional treatments for CA aim to clear visible warts and often include repeated local drug application or surgical methods, such as CO_2 laser treatment, cryotherapy, or electric cauterization. All of these treatments have not been definitively demonstrated to be effective in eliminating HPV infection and preventing CA from recurring [1]. Because of the high efficiency for eliminating HPV and preventing recurrence, 5-aminolaevulinic acid-mediated photodynamic therapy (ALA-PDT) has been increasingly used for CA treatment [5]. As a noninvasive therapy, the simplicity of topical use of photosensitizers and the easy accessibility of light exposure make this approach more suitable for inaccessible sites.

The type and viral load of HPV are associated with disease progression. High viral loads resulting from active viral replication may

* Corresponding author.
E-mail addresses: 180166333@qq.com (J. Wang), 2082511791@qq.com (S. Li), 641798777@qq.com (J. Li), 1160101491@qq.com (Z. Li), wangqi1987@smu.edu.cn (Q. Wang), 448064200@qq.com (X. Peng), shi_minglan@163.com (M. Shi), che_qilei@163.com (Q. Che), 1625216105@qq.com (H. Liu), 857721495@qq.com (L. Jiang), nfpfkzk@126.com (K. Zeng).

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promote viral persistence [6]. LR-HPVs are mainly responsible for CA, whereas persistent HR-HPV infection may lead to anogenital cancers. Compared with single-type HPV infection, multitype HPV co-infection often results in more and larger warts, a longer disease duration, and a greater frequency of recurrence [7], necessitating more sessions of PDT to achieve infection clearance [8]. HPV DNA detection, including genotype identification and viral load measurement, can not only determine the infection state of patients with CA but also accurately guide ALA-PDT, identify unnoticed lesions, and improve and evaluate efficiency [9]. From our clinical experience, patients who ended treatment after obtaining two negative HPV DNA detection results in a row appeared to have a lower risk of recurrence during follow-up, suggesting this parameter could be used as an endpoint for ALA-PDT.

Accordingly, in this study, we aimed to evaluate the efficacy of ALA-PDT in the context of different HPV infection states using HPV DNA detection as an indicator of the treatment endpoint in patients with CA.

2. Materials and methods

2.1. Patients

Data and samples were collected from patients attending the Department of Dermatology and Venereology, Nanfang Hospital in Guangzhou, Guangdong, China from January 2016 to March 2019. Patients were diagnosed as having CA based on medical history, clinical examination, aceto-whitening test, HPV DNA detection, or pathology. Patients underwent a systemic examination before treatment to confirm the presence of any other sexually transmitted diseases or systemic diseases. All patients were free of syphilis, human immunodeficiency virus infection, or autoimmune diseases. No patient had received systemic corticosteroid therapy or immunosuppressive therapy in the previous month.

All patients signed informed consent before treatment according to the recommendations of our institutional review board, and this study was approved by the Ethics Committee of Nanfang Hospital.

2.2. PDT

ALA-PDT was administered after removing visible lesions by microwave or radiofrequency. A 20% (w/v) 5-ALA (Fudan Zhangjiang Bio-Pharm Co. Ltd., Shanghai, China) gel was prepared (dissolving ALA powder into sterile 0.9% NaCl) immediately prior to application. The 5-ALA gel was placed on the aceto-whitening-positive sites and the adjacent normal skin within a 1 cm radius and then covered by a layer of opaque film and surgical gauze to avoid loss of solution and to facilitate absorption. After 3 h incubation, light irradiation at 100 J/cm^2 and 100 mW/cm^2 was applied to the treatment field using a cylindrical semiconductor laser fibre (LED-IB PDT instrument; Wuhan Yage Optic and Electronic Technique Co. Ltd., Wuhan, China) emitting 635-nm laser. Patients were required to receive ALA-PDT once every week after HPV DNA detection.

2.3. HPV DNA detection

HPV DNA detection, including genotyping and viral load measurement, was used to dynamically monitor the virus infection state throughout treatment and follow-up. Before patients underwent HPV DNA detection during their first visit, photographs were acquired to record the lesion distribution and location. After the lesions were removed or cured, samples were collected from the same locations according to the obtained photographs. Samples were obtained by rubbing separate dry Dacron swabs over the entire surface of the lesion at different anatomical sites before being placed in a tube containing 2 mL phosphate-buffered saline. DNA extraction and real-time polymerase chain reaction (PCR) were performed using an HPV genotyping and quantification kit (BioPerfectus Technologies, Jiangsu, China) according to the manufacturer's instructions. The genotyping and quantification processes were based on the BioPerfectus Multiplex Real Time (BMRT) HPV assay, which was designed to detect each of the 21 most prevalent HPV types, including 18 HR-HPV genotypes (HPV-16, -18, -26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68, -73, and -82) and three LR-HPV genotypes (HPV-6, -11, and -81). PCR amplification was conducted in a final reaction volume of 20 μL with 2 μL samples DNA. The reactions were pre-incubated at 50 °C for 5 min, followed by an initial denaturation at 95 °C for 10 min and 45 cycles at 95 °C for 10 s and 58 °C for 40 s. In each PCR assay, human *TOP3*, a single-copy gene encoding DNA topoisomerase III, was amplified as a control for determining the relative number of viral copies in a given sample. Perfectus Software v1.0 (Bioperfectus Limited Corp., China) was used for genotyping and quantitative analysis. A previous study reported the sensitivity, specificity, and concordance rate (accuracy) of the BMRT HPV assay to be 98.4%, 99.6%, and 99.6%, respectively [10].

2.4. Treatment procedure

Before each ALA-PDT session, HPV DNA detection was performed. Patients received the treatment once a week until the result of HPV DNA detection was negative for two consecutive tests; this point was considered the endpoint of PDT. As a follow-up, HPV DNA detection was required once a month for 3 months, followed by once at 6 months after ending treatment.

2.5. Statistical analyses

All statistical analyses were performed using SPSS Statistics 22.0 (SPSS, Chicago, IL, USA). Chi-square tests were performed to compare differences in infection sites among single- and multitype HPV-infected groups. Mann-Whitney U tests were used to assess differences in the total number of PDT sessions performed until the endpoint of therapy between different groups. Pearson's correlation analysis was used to analyse the correlations of HPV infection status (co-infection or single infection), lesion location (internal or not), and site distribution (single or multiple). Multiple linear regression was performed in multivariate analysis. All statistical tests were two-sided; P values of less than 0.05 were considered statistically significant.

3. Results

3.1. Characteristics of HPV-infected individuals

In total, 138 patients (101 men and 37 women; aged 18–69 years) who visited our clinic from January 2016 to March 2019 with positive results of HPV DNA detection at one or more anatomical sites were enrolled in this study. HPV DNA detections were performed on one or more of 13 different anatomical sites, including the urethra, urethral orifice, glans, coronal sulcus, frenulum, prepuce, penis, scrotum, vulva, vagina, cervix, perianal area, and anal canal, depending on the distribution of warts. Overall, 257 samples were included in this study. The clinical characteristics of patients are presented in Table 1. Sixty-one (61/138, 44.2%) patients suffered from single-site infection, whereas the remaining patients (77/138, 55.8%) were infected at multiple sites. Fig. 1 shows the distribution of all 257 samples. The perianal area was the most susceptible infection site (48/257, 18.7%) followed by the penis (31/257, 12.1%), vulva (28/257, 10.9%), and cervix (26/257, 10.1%).

Fig. 2 shows the prevalence of HPV types in all cases (n = 138). HPV-6 was the most prevalent type of LR-HPV infection (76/138, 55.1%), followed by HPV-11 (49/138, 35.5%). HPV-52 (21/138, 15.2%), HPV-16 (12/138, 8.7%), and HPV-58 (12/138, 8.7%) were three of the most common types of HR-HPV infection. LR-HPV predominated, with a prevalence of 96.4%. Among all patients, 70 (70/138, 50.7%) were infected with a single type of HPV, whereas 68 (68/

Table 1
Clinical characteristics of HPV-infected individuals (n = 138).

Patients (Total No./% of total)		No.		No.
Patients in roll (138)	Female	37	Male	101
	Age	33.5(y)	(18–69y)	
Single-site infection (61/44.2%)	Female	12	Male	49
Multisite infection (77/55.8%)	Female	25	Male	52
Single-type infection (70/50.7%)	Female	19	Male	51
	LR-HPV	60	HR-HPV	10
Multitype infection (68/49.3%)	Female	18	Male	69
	LR-HPV	8	HR-HPV	7
	LR/HR-HPV	53		

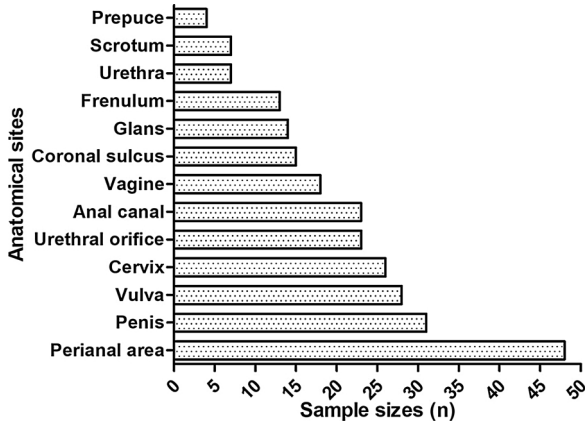

Fig. 1. Distribution of 257 samples.

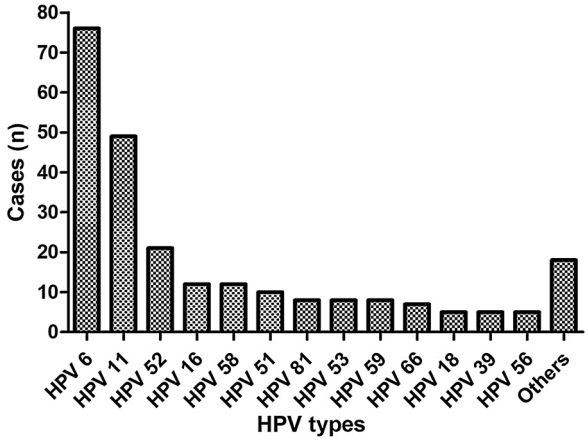

Fig. 2. Prevalence of HPV types in 138 cases.

138, 49.3%) were infected with multiple types of HPV. In single-type infection cases, 85.0% (60/70) were LR-HPV, and 15.0% (10/70) were HR-HPV. Among the multitype infection cases, LR- and HR-HPV combined infection was most frequent (n = 53), followed by only LR-HPV (n = 8) and only HR-HPV infection (n = 7).

Table 2 shows the infection characteristics of all patients. In single-type HPV-infected patients, 34 suffered single-site infections, and 36 had multisite infections. In contrast, in multitype infection cases, there were 27 and 41 with single-site and multisite infections, respectively. These results indicated that multitype HPV infection may be not one of the factors causing multisite lesions ($P = 0.309$).

Table 2
Infection characteristics of all patients (n = 138).

Patient (No.)	Single-site	Multisite	Total	P value
Single-type	34	36	70	0.309
Multitype	27	41	68	
Total	61	77	138	

Table 3
Characteristics of patients who completed the treatment (n = 72).

Characteristics	n (%)	Sessions of PDT (Mean ± SD)	P value
Gender			
Female	20 (27.8)		
Male	62 (72.2)		
Sites distribution			
Single-site	38 (52.8)	4.29 ± 0.31	P < 0.001
Multisite	34 (47.2)	6.21 ± 0.46	
Internal lesions			
Yes	25 (34.7)	5.68 ± 0.38	P = 0.032
No	47 (65.3)	4.96 ± 0.39	
HPV prevalence			
Single-type	45 (62.5)	4.29 ± 0.21	P < 0.001
Multitype	27 (37.5)	6.70 ± 0.59	
In single-type infection	45		
LR-HPV	39 (86.7)	4.33 ± 0.24	P = 0.757
HR-HPV	6 (13.3)	4.00 ± 0.45	
In multitype infection	27		
LR-HPV	4 (14.8)	4.25 ± 1.03	P = 0.082
LR/HR or HR/HR-HPV	23 (85.2)	7.13 ± 0.64	
HR-HPV infection			
Yes	29 (40.3)	6.48 ± 0.57	P = 0.001
No	43 (59.7)	4.33 ± 0.23	

3.2. Patients with multisite or internal HPV infections required more PDT sessions

Among all cases, 72 patients completed the treatment, and HPV DNA detection was negative after PDT (66 of 138 patients were excluded because they were lost to follow up or did not undergo the entire treatment protocol). In these 72 cases, 38 had single-site HPV infections, and 34 had multisite infections (Table 3). Compared with the single-site infected group (4.29 ± 0.31 sessions), the multisite infected group required significantly more sessions of PDT (6.21 ± 0.46 sessions, $P < 0.001$) to reach the endpoint. Next, the completed cases were divided into two groups according to the presence of internal lesions in the urethra, vagina, cervix, and anal canal. The results indicated the cases with internal HPV infection (25 cases, 5.68 ± 0.38 sessions) required more PDT sessions than cases with external lesions only (47 cases, 4.96 ± 0.39 sessions, $P = 0.032$). Correlation analysis showed that patients with internal HPV infection may have a higher risk of multisite infection ($P = 0.009$, correlation coefficient = 0.307).

3.3. Patients with multitype HPV infection required more PDT sessions

To determine whether there were differences in the total number of PDT sessions between patients with single-type and multitype HPV infections, the 72 patients were grouped according to their infection state (Table 3). There were 27 cases infected with more than one HPV type, and this necessitated significantly more sessions of PDT to end the treatment (6.70 ± 0.59 sessions) than that in patients with single-type HPV infections (45 cases, 4.29 ± 0.21 sessions, $P < 0.001$). In addition, among cases of single-type HPV infection, there were no significant differences in PDT sessions between LR- or HR-HPV-infected groups ($P = 0.757$). In cases of multitype HPV infection, there were no significant differences among HR-HPV and non-HR-HPV-infected groups ($P = 0.082$).

Moreover, HPV infection status was not correlated with site distribution (multisite infection or not, $P = 0.116$) or lesion location

(internal lesion or not, $P = 0.183$). The multivariate analysis indicated that multitype HPV infection was independently associated with longer treatment period ($P < 0.001$). Additionally, patients with multisite HPV infection may require more sessions of PDT to be cured ($P = 0.003$).

3.4. HR-HPV-infected patients required more PDT sessions

There were 29 cases with only HR-HPV or combined HR-HPV infection among the 72 patients who achieved two consecutive negative HPV DNA detection results (Table 3). Compared with the 43 cases only infected with LR-HPV (4.33 ± 0.23 sessions), patients with HR-HPV infection were more likely to require long-term PDT to achieve virus elimination (6.48 ± 0.57 sessions, $P = 0.001$). This result indicated that HR-HPV-infected patients may require more sessions of PDT to reach the endpoint than non-HR-HPV-infected patients.

3.5. Follow-up and recurrence rates after treatment

Sixty-nine patients attended follow-up after they reached the endpoint of PDT. Thirty-three patients were free of HPV infection and visible warts at the 6-month follow-up, as verified by negative results of HPV DNA detection, whereas 16 patients showed negative results for HPV DNA detection and were free of visible warts at the 3-month follow-up (also claiming that there were no visible warts at the 6-month telephone follow-up). Eighteen patients refused to undergo HPV DNA detection but stated they did not find any new lesions during telephone follow-up. At the end of the 6-month follow-up period, only two patients (2.9%) showed recurrence of HPV infection, with positive HPV DNA detection results but no visible warts.

4. Discussion

HPV infection is one of the most common sexually transmitted infections worldwide and can cause various urogenital tract diseases. CA is the most common clinical manifestation of HPV infections. Although not as life-threatening as cervical cancer, which is related to persistent HR-HPV infection, genital warts carry a huge psychological and economic burden. To date, several vaccines have been developed to provide excellent protection against certain types of HPV infection. Evaluation of HPV prevalence in patients with CA may facilitate the implementation of vaccination programs. In our study, 138 patients underwent HPV DNA detection, and LR-HPV was found to predominate, with a prevalence of 96.4%. The three most common infection types were HPV-6, HPV-11, and HPV-52. These results were slightly different from results observed in other countries [11], but similar to previous studies carried out in Guangdong [12] and Xi'an [13], suggesting the occurrence of regional differences. We also found that multiple types of HPV co-infection were common in our study, although the rate of co-infection was higher than in several previous studies [14–16] but consistent with a recent report [17]. Although multitype HPV infections are associated with a poorer prognosis [7], we did not find a significant relationship between multitype infection and multisite infection, which could be explained by the small sample size of this study.

Conventional treatments for CA mainly aim to remove visible warts but not eliminate HPV infection entirely [18]. Previous studies have shown that the clinical clearance rates of traditional therapies vary from 50% to 75% [19,20]. Because of the efficacy of ALA-PDT for the treatment of CA, this approach has become quite popular, yielding clearance rates of 95.27% and 95.93% in two previous studies [21,22]. The mechanism through which the viral load was decreased may involve the higher capacity of virus-infected cells to synthesize PpIX from ALA and to be effectively killed by PDT [23]. Our previous study also indicated that ALA-PDT reduces HPV viral load via autophagy and apoptosis by upregulation of Ras/Raf/MEK/ERK and downregulation of PI3K/AKT pathways [24]. Nevertheless, there is currently no widely accepted laboratory indicator to evaluate the efficacy of PDT in virus elimination. Our previous studies have shown that the dynamics of HPV viral loads can reflect the treatment effects of PDT and that patients finishing PDT with a negative HPV DNA detection result may have a lower possibility of recurrence [8,9]. In this study, we used two consecutive negative results of HPV DNA detection as a treatment endpoint and compared sessions of ALA-PDT throughout the process to evaluate the efficacy of this approach.

In our study, we collected samples from all anatomical sites covered by genital warts and performed HPV DNA detection. Thus, the infection field could be detected fully. From our clinical observations, patients with more than one HPV-infected site seemed to require more sessions of PDT, and this speculation was confirmed by our data. Moreover, patients with multisite HPV infections required longer treatments than patients with single-site infections. These findings were reasonable considering the larger infected field.

Managing internal CA is still an intractable problem. A barrier to successfully treating internal CA is that there are no therapies with few side effects or widely approved topical therapies available. Many studies have shown that PDT exhibits superior efficacy for the treatment of urethral [25] and intra-anal [9,26,27] CA and is safe to administered during pregnancy [28]. Our data indicated that patients with internal CA required significantly more sessions of PDT than patients suffering from external genital warts. These findings are consistent with the results of a retrospective study, which concluded that having both intra-anal and perianal CA was a significant predictor requiring multiple surgical treatments for clearance [29]. The treatment field for some internal anatomical sites, such as the vagina, cervix, and anal canal, is commonly folded and hard to expose sufficiently, and CA in the urethra is difficult to access completely, which could lead to insufficient treatment and increase the total sessions of PDT.

Multitype HPV infections are not rare in CA. Patients with multiple HPV types have a slightly elevated median time to clearance [30] and are more likely to have not only persistent infection but also cytologic abnormalities [31]. Another study also showed that the median volume of warts in multitype infections was larger than that of single-type infections. Additionally, co-infection with multiple types of HPV is usually associated with an extended disease course and a greater frequency of recurrence [7]. The average virus load of multitype HPV infection is significantly higher than that of single-type infection after six sessions of PDT in CA [8]. Our results were similar, demonstrating that, compared with single-type infection, patients with multitype HPV infection required more sessions of PDT to reach the endpoint of therapy. These findings also emphasized the important role of HPV DNA detection throughout the disease process as an indicator of the severity of HPV infection and prognosis and for prediction of the length of treatment course in single- and multitype HPV infections.

HR-HPV types are etiological agents of cervical and anal cancers as well as a subset of other genital tract cancers, such as vulvar, vaginal, and penile carcinomas [32]. Patients with both LR- and HR-HPV types have increased risk of developing CA and persistent infection [33]. Once a persistent HR-HPV infection is established, malignancy is promoted in the absence of any known additional risk factors [34]. A cross-sectional study has shown that HR-HPV types are prevalent in warts of longer duration than in warts with a duration of less than 1 month, potentially because HR-HPV genotypes take longer to clear than other genotypes [35]. To determine whether HR-HPV infection influenced the treatment efficiency of PDT, we compared the total session numbers in the HR-HPV- and non-HR-HPV-infected groups. The results showed that the former required more sessions of PDT than the latter, indicating that treatment of CA with HR-HPV infection may require more time.

Early research has shown that PDT can effectively eliminate HPV infection [36]. A meta-analysis including 20 randomized controlled trials with 1903 patients concluded that local application of ALA-PDT significantly reduced the recurrence rate compared with CO_2 laser treatment, cryotherapy, and microwave therapy. Moreover, a

combination of therapies destroying the visible part of warts as well as peripheral latent HPV-infected cells, similar to our current approach, may increase the efficacy of PDT [37]. In contrast to these previous studies, we set the endpoint of treatment as two consecutive negative results of HPV DNA detection for the first time. After follow-up for at least 6 months, only 2.9% of 69 patients suffered from recurrence in our study; this recurrence rate was lower than that in patients who only accepted three or six sessions of PDT, regardless of the results of HPV DNA detection [8,38].

In summary, in this study, we showed the predominance of ALA-PDT in patients with CA, combined with HPV DNA detection. We also demonstrated the superiority of ending treatment after obtaining two consecutive negative results of HPV DNA detection. We concluded that patients with multisite and multitype HPV infection required more sessions of PDT to eliminate the virus, particularly those suffering from internal CA or HR-HPV infection. Although the study was limited by the small sample size, our findings provide some insights into appropriate approaches for the treatment of CA. Further studies with larger sample size from multiple centres will be carried out to elucidate the importance of HPV DNA detection-guided ALA-PDT in treating CA.

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Declaration of Competing interest

The authors have no conflict of interest to declare.

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