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Efficacy of 2LPAPI®, a Micro-Immunotherapy Drug, in Patients with High-Risk Papillomavirus Genital Infection

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Abstract

Human papillomaviruses (HPVs) are well known for being linked to the development of cervical cancers, most of them being caused by the high-risk (HR) oncogenic genotypes, mainly 16 and 18. The efficacy of 2LPAPI® (Labo'Life), a micro-immunotherapy homeopathic drug, has been evaluated in HR-HPV infected women (n = 18), in a private gynecology practice, by comparing them to an untreated control group (n = 18). Patients were 20 to 45 years old and had cytology with Atypical Squamous Cells of Undetermined Significance (ASCUS) or Low grade Superficial Intra Lesions/Cervical Intraepithelial Neoplasia Grade I (LSIL/CINI). Patients freely chose to be treated with the drug or not. Those deciding not to take the drug remained untreated and were followed as a control group. The drug was taken at the regimen of one capsule per day during 6 months. HR-HPV and cytology were evaluated at 6 and 12 months. After 12 months, HR-HPV was cleared in 78% of the patients taking the drug versus 44% in those not taking it (p = 0.086). In patients over 25 years, HR-HPV clearance in the treated group was significantly higher (81.3%) than in the control group (20%) (p = 0.004). The difference in the regression of the lesion grades almost reached statistical significance (p = 0.053). This follow-up confirms that the micro-immunotherapy drug 2LPAPI® is a safe and effective therapeutic approach to treat HR-HPV cervical lesions in women over 25 years.

Keywords

High-Risk Human Papillomavirus, Micro-Immunotherapy, Genital Infection, Homeopathy, 2LPAPI®

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1. Introduction

Human papillomaviruses (HPVs) are well known for being linked to the development of cervical cancers [1], with nearly all of them being caused by persistent infection of high-risk (HR) oncogenic genotypes, mainly 16 and 18 [2]. HPV has also been implicated in several other conditions such as anal, vulvar, vaginal but also penile cancers [3], as well as oropharyngeal carcinoma [4]. According to the World Health Organization, cervical cancer was the fourth most common cancer in women in 2012, accounting for a total of 266,000 deaths worldwide. The European population was not impacted as much as the population of the less developed countries (where cervical cancer is the second most common cancer), with 34,000 women being diagnosed in the European Union during the same year [5].

HPVs are transmitted through genital contact. The prevalence of HR-HPV is higher in women in their twenties, reaching 53.6%. This prevalence tends to decrease with age, with about 20% in women between 30 and 50 years of age, and about 16% in older women [6].

Immunity, either innate or adaptive, plays a capital role in HPV infection. HPV expresses several early (E) proteins during the first part of the viral cell cycle, some being needed for the others to be expressed. Two of these proteins, E6 and E7 [7], are crucial for the development of cervical cancer because they inactivate the oncosuppressor proteins p53 (via binding of E6, p53 is degraded, preventing cell apoptosis and promoting viral DNA replication) and pRb (through E7). E6 and E7 also play a role in the immune tolerance due to the down regulation of type-1 interferons expression in keratinocytes. Another E protein, E5, is responsible for the down regulation of the class I human leukocyte antigen (HLA-I) expression [8].

No allopathic treatment is currently available to eradicate HPV, even if promising advances have been made in the immunological field [9] [10].

Most of the infected patients will clear the virus naturally within 2 years and more than 80% of the low grade intraepithelial lesion will regress spontaneously, especially in young patients.

Health authorities have established specific monitoring recommendations. For Atypical Squamous Cells of Undetermined Significance (ASCUS) or Cervical Intraepithelial Neoplasia Grade I (CINI), regular 6-month follow-up examinations are recommended via pap smear test, while for CINII, cervical conization is performed.

Micro-immunotherapy is a non-conventional therapeutic approach using ultra-low doses of endogenous molecules produced by recombinant DNA technique and Specific Nucleic Acids (SNA®). The oromucosal administration of the drug allows direct contact with the immunocompetent cells of the sublingual membrane. Several studies have demonstrated the efficacy of micro-immunotherapy drugs against viral infection [11] and allergy [12]. Grauwet [13] has previously published a follow-up on 34 HR-HPV positive patients treated with the antiviral micro-immunotherapy treatment 2LPAPI® for 6 months at a posology of one capsule/day, which showed promising results with 80% eradication within the HR-HPV population. However no control group was available for comparison. The aim of this prospective controlled follow-up was to study a women population between 20 and 45 years, HR-HPV positive, with an ASCUS or CINI cytology, and to observe its evolution with or without the immune support of the micro-immunotherapy drug 2LPAPI®.

2. Patients and Methods

2.1. Patients

Thirty-six (N = 36) female patients aged 20 to 45 years, with a normal body mass index (BMI) and non-smokers, were followed from July 2011 to January 2014. Patients were all HR-HPV positive with ASCUS or CINI cytology (normal pap smear of less than 12 months).

Patients undergoing immune or chemical treatment, whether oral or topical, or nutritional supplementation, were not included in the follow-up. The patients presenting recurrences or having more than one sexual partner during the last year were not included either.

2.2. Methods

This prospective follow-up was conducted in a gynecology practice.

At first visit (T0), if the pap smear test revealed an ASCUS or low grade lesion, HR-HPV research was done by using the h2c high-risk HPV DNA Test® of Quiagen. This test is based on nucleic acid hybridization with signal amplification detected by chemoluminescence. It allows the qualitative detection of the thirteen following

HR-HPV in cervical specimens: HPV16/18/31/33/35/39/45/51/52/56/58/59/68. If the patient was HR-HPV and had a cytology showing ASCUS or CINI, she was asked if she was willing to be treated with 2LPAPI® or not, and was therefore considered in the medication group (2LPAPI® for 6 months) or in the control group (conventional monitoring without any medication). Overall, there were 18 patients both in the control and treated group.

When included in the treated group, the patient would take the medicine at the posology of 1 capsule per day, to be opened and its content poured under the tongue, in the morning, fasting, during 6 months. Patients choosing not to take the medicine were followed in the control group and did not receive any medication.

At the second visit, scheduled at the end of the 6-month treatment period, or after six months without treatment for the control group, patients had a pap smear and HR-HPV testing. Treatment compliance and tolerance were evaluated.

The final visit took place 6 months later, at Month 12, one year after the beginning of the follow-up. Final pap smear and HR-HPV testing were performed.

The objectives were to assess the therapeutic efficacy of the micro-immunotherapy treatment on both clearance of HR-HPV and cytology (decrease from CINI to ASCUS, or to normal cells) compared to an untreated control group.

The evaluation was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki.

2.3. Investigational Product

The micro-immunotherapy medicine 2LPAPI®ismanufactured by Labo'Life, and notified to the Belgian Federal Agency for Medicines and Health Products (FAMHP) under notification number 1507CH41F1. It is an oromucosally administered sequential medicine. Ultra-low doses of immunological substances (Interleukin-1 [IL-1], IL-2, Interferon alpha [IFNα], Ribonucleic Acid [RNA]), cyclosporine A and Specific Nucleic Acid (SNA®) targeting HPV proteins or human HLAII gene, obtained by dilution-sucussion process, are impregnated on lactose saccharose globules according to the European Pharmacopoeia homeopathic manufacturing process (**Table 1**). The globules are presented in capsules, and have to be taken daily by placing them under the tongue, fasting, during 6 months.

2.4. Statistical Analyses

All statistical analyses have been performed by an independent statistician, using IBM-SPSS Statistics (Version 21.0). It should be mentioned that statistical analyses had not been planned by the investigators and therefore the power had not been calculated a priori. Differences in clinical outcomes between treated and control group at 12 months prompted the investigators to question the statistical significance of the data a posteriori.

Treated and control patients were compared at baseline and at each follow-up time point using: Independent Student's t tests for continuous variables, Mann-Whitney's tests for discrete ordinal variables, Chi-square or Fisher's exact tests (as appropriate) for discrete nominal variables.

Many studies have demonstrated that the viral clearance is much higher in young women aged 25 years or less. There were more young women in the control group than in the treated group, therefore creating a potential

Table 1. Composition of 2LPAPI® (Labo'Life, Les Isnes, Belgium).

Ingredient	Concentration
Interleukin-1	10-17CH
Interleukin-2	10-17CH
Interferon-α	10-17CH
Cyclosporine A	7-10-17CH
Ribonucleic acid	10-18CH
Specific Nucleic Acid (SNA®) HLAII	10-18CH
Specific Nucleic Acid (SNA®) PAPI	10-18CH

CH = Homeopathic Centesimal Hahnemannian dilution.

bias in favor of the control group. Exploratory analyses were performed to determine if the age of the population had an impact on the HR-HPV clearance and on the cytology in both groups.

Therefore, the primary endpoint and the three secondary endpoints were tested in two subpopulations of patients: women aged 25 years or less and women aged over 25 years. In the two age category subsets, all endpoints were compared between the two treatment groups using Mann-Whitney's tests.

By general convention, p values lower than 0.05 were considered statistically significant. However, no correction being applied for multiplicity of endpoints and analyses, the statistical significance of p values for secondary endpoints should be considered with caution. The p value was considered conclusive for the primary endpoint only.

3. Results

The demographics and baseline characteristics of the patients can be found in **Table 2**. Although treated patients appeared older than control patients, the difference between the two groups was not statistically significant (p = 0.127): 29.0 ± 5.8 (mean ± SD) in the control group and 32.2 ± 6.3 in the treated group. The distribution of patients in the two age groups (≤25 years and >25 years) was not statistically different between the two treatment groups (p = 0.060) but there was a clear tendency for a predominance of young women in the control group (44.4% versus 11.1% in the treated group). Cervix cells abnormalities were uniformly distributed among the two groups: 38.9% ASCUS and 61.1% low grade dysplasia in both groups (p = 1.000). As already mentioned, all patients were HR-HPV positive.

Considering that, as previously mentioned, the women's age influences the HR-HPV clearance rate, the ages of the patients (>25 years) have been compared between treated and control group and are presented in **Table 3**. Age distribution was found to be similar between the two groups.

The primary endpoint was the HR-HPV clearance occurring one year after the beginning of the treatment (Month 12). HPV clearance occurred in 8 patients (44.4%) of the control group and 14 patients (77.8%) of the treated group. This difference was not statistically significant (p = 0.086). At Month 6 (end of the treatment), HR-HPV clearance occurred in 2 patients (11.1%) in the control group and 5 patients (27.8%) in the treated group. This difference was again not statistically significant (p = 0.402) (**Figure 1** and **Table 4**).

Table 2. Demographics and baseline characteristics of the patients.

Parameter	Category	Treated group					Control group				
		N	Mean or %	SD	Min	Max	N	Mean or %	SD	Min	Max
Age (year)	All patients	18	32.2	6.3	24	43	18	29.0	5.8	21	42
	≤25 years old	2	24.5	0.7	24	25	8	24.3	1.4	21	25
	>25 years old	16	33.1	6.0	26	43	10	32.8	5.2	26	42
HR-HPV	Positive	18	100.0				18	100.0			
	Negative	0	0.0				0	0.0			
Cytology	ASCUS	7	38.9				7	38.9			
	CINI	11	61.1				11	61.1			

SD = standard deviation; Min = minimum; Max = maximum; HR-HPV = high-risk human papilloma virus; ASCUS = atypical squamous cells of undetermined significance; CINI = cervical intraepithelial neoplasia grade I.

Table 3. Age distribution of the patients >25 years in the treated and control groups.

Parameter	Category	Treated group	Control group
Age (year)	26 - 30 years old	44%	40%
	31 - 35 years old	31%	40%
	36 - 40 years old	0%	0%
	>40 years old	25%	20%

In patients over 25 years, HR-HPV clearance at Month 12 occurred in 2 patients (20.0%) of the control group and 13 patients (81.3%) of the treated group. This difference was statistically significant (p = 0.004) (**Figure 1** and **Table 4**).

Changes in cytology were considered as a secondary endpoint. The changes in the cervix cells abnormalities between baseline and Month 6 did not differ statistically significantly between the two groups (p = 0.111). There was an improvement of 2 grades in no patient (0%) of the control group and 2 patients (11.1%) of the treated group, an improvement of 1 grade in 4 patients (22.2%) of the control group and 7 patients (38.9%) of the treated group, a status quo in 9 patients (50.0%) of the control group and 6 patients (33.3%) of the treated group, and a deterioration of 1 grade in 5 patients (27.8%) of the control group and 3 patients (16.7%) of the treated group (**Figure 2** and **Table 4**).

The change in the dysplasia grade between baseline and Month 12 did not differ statistically significantly between the two groups (p = 0.192). There was an improvement of 2 grades in 3 patients (16.7%) of the control group and 4 patients (22.2%) of the treated group, an improvement of 1 grade in 5 patients (27.8%) of the control group and 9 patients (50.0%) of the treated group, a status quo in 8 patients (44.4%) of the control group and 4 patients (22.2%) of the treated group, and a deterioration of 1 grade in 2 patients (11.1%) of the control group and 1 patient (5.6%) of the treated group (**Figure 2** and **Table 4**).

If we compare the population of patients over 25 years in the two groups, the change in dysplasia grade was borderline but not statistically significant at Month 6 (p = 0.060) and close to significance at Month 12 (p = 0.053) (**Table 4**).

The patients taking 2LPAPI® tolerated it very well and no adverse events were reported by the patients.

4. Discussion

As previously mentioned, there is no allopathic treatment available on the market to treat patients infected by HR-HPV. The aim of this private follow-up was to determine the efficacy of the medicine 2LPAPI® on HR-HPV clearance, compared to the natural clearance occurring in untreated patients, along with its impact on bringing the cells back to a normal cytology.

The design of the current follow-up is relatively similar to that of Grauwet [13], except that an untreated control group has been used for comparison with 2LPAPI®. Grauwet reported an 80% mean eradication of HR-HPV in HR-HPV positive patients treated with 2LPAPI® during 6 months, at the posology of one capsule daily. Therefore these recommendations have been followed and a 6-month treatment period has been prescribed.

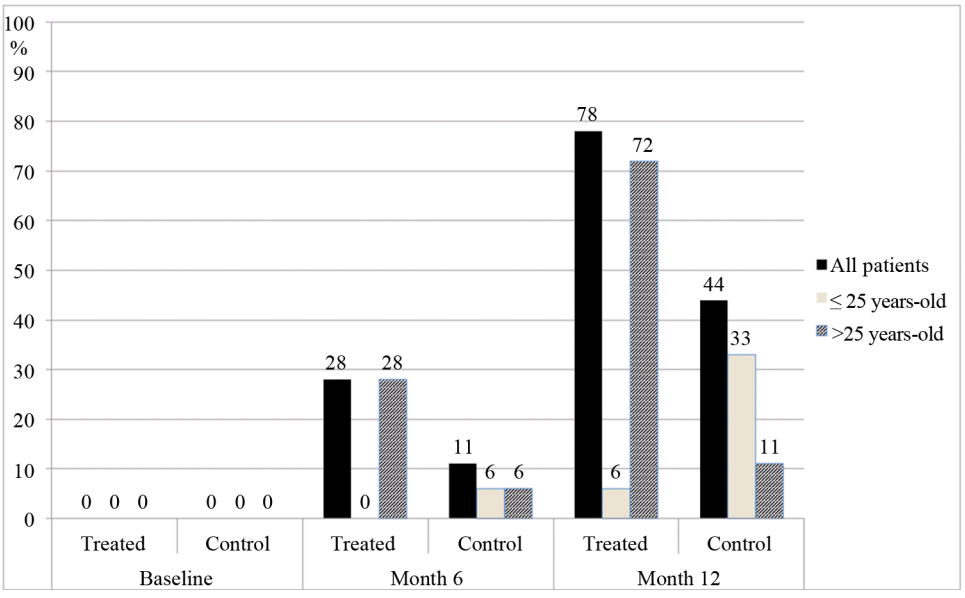

Figure 1. Clearance of HR-HPV (%) in the 2LPAPI® treated group (N = 18) and in the untreated control group (N = 18). At Month 12, the difference between treated women (72%) and control women (11%) aged over 25 years is statistically significant (p = 0.004).

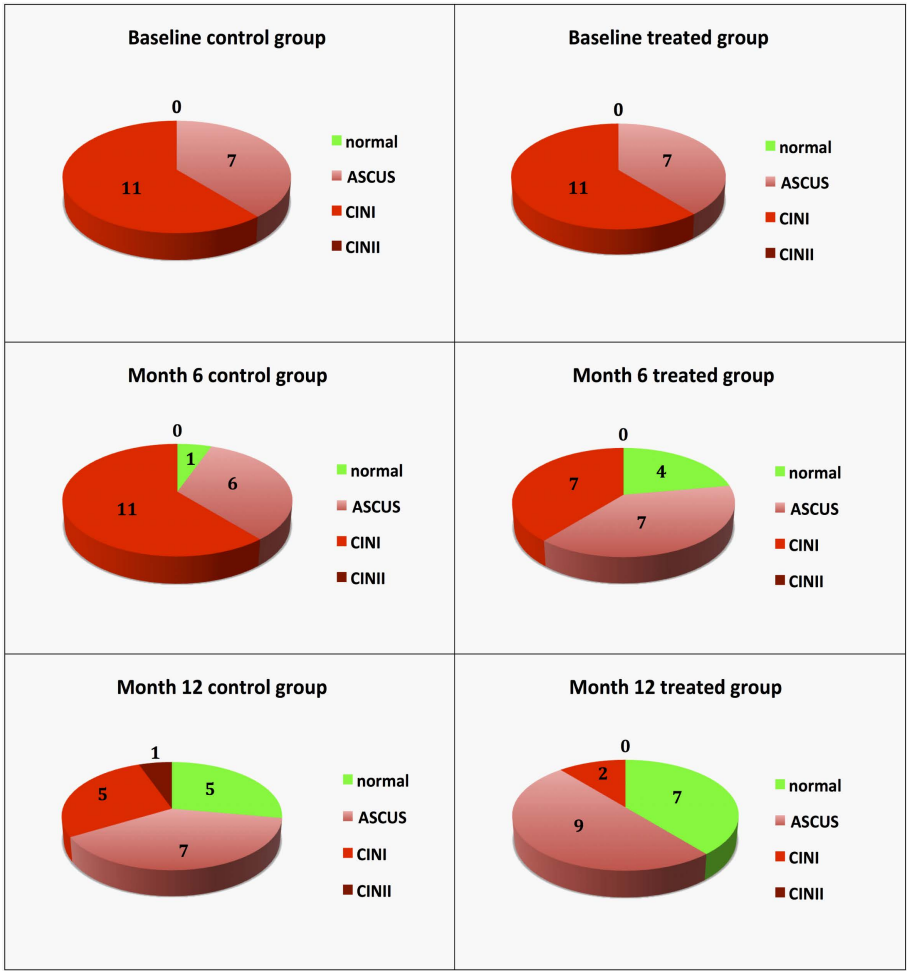

Figure 2. Cytology of HR-HPV patients in the 2LPAPI® treated group (N = 18) and in the untreated control group (N = 18) at baseline, after 6 months and after 12 months (ASCUS = Atypical Squamous Cells of Undetermined Significance; CINI or CINII = Cervical Intraepithelial Neoplasia Grade I or Grade II).

Table 4. Clinical efficacy of 2LPAPI® in treated versus control high-risk human papilloma virus (HR-HPV) women.

Time point	Parameter	Status	Population	Treated group N = 18		Control group N = 18	
				n	%	n	%
Baseline (Month 0)	Cytology	HR-HPV negative	All patients	0	0	0	0
			≤25 years old	0	0	0	0
			>25 years old	0	0	0	0
		ASCUS	All patients	7	39	7	39
			≤25 years old	1	6	3	17
			>25 years old	6	33	4	22
		CINI	All patients	11	61	11	61
			≤25 years old	1	6	5	28
			>25 years old	10	55	16	33

Continued

Month 6	Cytology	HR-HPV negative	All patients	5	28	2	11
			≤25 years old	0	0	1	6
			>25 years old	5	28	1	6
		Normal	All patients	4	22	1	6
			≤25 years old	0	0	1	6
			>25 years old	4	22	0	0
		ASCUS	All patients	7	39	6	33
			≤25 years old	1	6	4	22
			>25 years old	6	33	2	11
		CINI	All patients	7	39	11	61
			≤25 years old	1	6	3	17
			>25 years old	6	33	8	44
Month 12	Cytology	HR-HPV negative	All patients	14	78	8	44
			≤25 years old	1	6	6	33
			>25 years old	13	72	2	11
		Normal	All patients	7	39	5	28
			≤25 years old	0	0	4	22
			>25 years old	7	39	1	6
		ASCUS	All patients	9	50	7	39
			≤25 years old	2	11	2	11
			>25 years old	7	39	5	28
		CINI	All patients	2	11	5	28
			≤25 years old	0	0	2	11
			>25 years old	2	11	3	17
		CINII	All patients	0	0	1	6
			≤25 years old	0	0	0	0
			>25 years old	0	0	1	6

HR-HPV = High-Risk Human Papilloma Virus; ASCUS = Atypical Squamous Cells of Undetermined Significance; CINI = Cervical Intraepithelial Neoplasia Grade I; CINII = Cervical Intraepithelial Neoplasia Grade II

A similar clearance of 78% in all patients, and 72% in patients aged over 25 years, has been found.

The overall results show a clear reduction of the HR-HPV infection in the treated patients, without reaching statistical significance ($p = 0.086$). It is important to note that HR-HPV clearance measured after 12 months was higher than clearance measured right after the end of the treatment. This could corroborate a long-term therapeutic action of the medicine on the immune system, still impacting it after stopping the treatment.

The clearance of HR-HPV tends to decrease with age. The results corroborate the literature: a higher natural clearance in younger patients (33% in patients aged less than 25 years, versus 11% in patients over 25 years). 2LPAPI® significantly increases the clearance ($p = 0.004$) of the virus in the population above 25 years, which is at the highest risk of developing cervical cancer. In this category of women, the drug has the strongest effect on cell normalization (grade regression from CINI to ASCUS and from ASCUS to normal), almost reaching statistical significance versus control group ($p = 0.053$).

Despite methodological limitations (limited sample size which had not been calculated a priori, lack of placebo control group, no randomization nor blinding), this follow-up highlights the efficacy of the medicine

2LPAPI® on the clearance of the HR-HPV in the population at highest risk of later developing cervical cancer, and confirms the medicine safety (medicine was well-tolerated and no adverse events were reported by the patients in the treated group). These results remain to be confirmed in a double-blind randomized placebo-controlled well-powered study, in HR-HPV women aged over 25 years.

In the absence of allopathic treatment, this drug constitutes a therapeutic option for patients infected by HR-HPV, which may be safely prescribed by gynecologists in their daily practice.

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