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GYNECOLOGIC ONCOLOGY

Indication of prophylactic vaccines as a tool for secondary prevention in HPV-linked disease

Annalisa Pieralli[1] · Claudia Bianchi[1] · Noemi Auzzi[1] · Maria Grazia Fallani[1] · Cecilia Bussani[1] · Massimiliano Fambrini[1] · Giuseppe Cariti[1] · Gianfranco Scarselli[1] · Felice Petraglia[1] · Alessandro Ghelardi[2]

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Abstract

Purpose To determine whether quadrivalent HPV vaccination is effective in reducing recurrent disease in women with a previous history of HPV disease.

Methods All women under 45 years of age treated for HPV-linked disease and with negative HPV test, cytology and colposcopy 3 months after treatment were enrolled. Women were randomly assigned into two groups: a group that received HPV vaccine post treatment and a group that was only submitted to follow-up. Follow-up was performed every 6 months for a duration of at least 3 years. Kaplan–Meier curve was used to estimate the overall disease-free survival during the follow-up period. Statistical analysis was performed by Fisher's exact test.

Results From November 2013 to October 2014, we enrolled a total of 178 women at Careggi University Hospital in Florence and at Azienda USL in Massa Carrara. 12 out of 89 patients in the non-vaccination group recurred (13.5%), while 3 out of 89 patients in the vaccination group recurred (3.4%). The Kaplan–Meier curves showed a statistically difference in the log rank test ($p = 0.0147$) for the overall disease-free survival in the study groups during follow-up. The rate of recurrence was significantly higher in the non-vaccination group, with a $p = 0.0279$ by Fisher exact test.

Conclusion The introduction of anti-HPV vaccination during the follow-up post treatment for HPV-linked disease is recommended to reduce the risk of recurrence. The clinical implication of this could be very important to influence post-treatment management of HPV disease.

Keywords HPV vaccination · Squamous intraepithelial lesion · HPV · Secondary prevention

Introduction

Human papillomavirus is considered the most important oncogenic virus affecting humans and the primary cause of cancer of the cervix.

Persistent viral infection with high-risk HPV genotypes can cause most cervical cancer. The high-risk HPV genotypes 16 and 18 cause approximately 70% of all cervical cancers worldwide, and types 31, 33, 45, 52 and 58 cause an additional 20%. While, HPV types 6 and 11 cause approximately 90% of anogenital warts [1, 2].

HPV vaccines have been developed to protect against the acquisition of HPV infection and the development of subsequent HPV-associated disease.

Three different vaccines, which vary according to the number of HPV types they contain and target, have been developed: bivalent vaccine (Cervarix) targets HPV types 16 and 18; quadrivalent HPV vaccine (Gardasil) targets HPV types 6, 11, 16 and 18. The latest 9-valent vaccine (Gardasil 9) targets the same HPV types as the quadrivalent vaccine as well as types 31, 33, 45, 52, and 58. HPV vaccines are prophylactic vaccines that protect against infection by HPV and subsequent HPV-associated lesions.

HPV vaccines are licensed for safe administration from 9 years of age up to 45. The optimal time for HPV immunization is prior to the individual's sexual debut. In fact, the advisory committee on immunization practices (ACIP)

✉ Claudia Bianchi
clabianchi85@gmail.com

[1] Division of Obstetrics and Gynecology, Department of Biomedical, Experimental and Clinical Sciences, University of Florence, Largo Brambilla 3, 50134 Florence, Italy

[2] Azienda USL-1 Massa e Carrara, Via Minzoni Don Giovanni 3, 54033 Carrara, MS, Italy

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recommended the HPV vaccination to be given from 11 to 12 years for females. At this age, patients have the highest probability to not be so far exposed to the HPV virus and have a higher immunologic response to the vaccination [3–6].

HPV vaccination is now possible up to 45 years as a personal preventive tool, as indicated in the datasheet of HPV vaccine. The vaccine is also safe at this age, is well-tolerated and is able to determine an antibody response [7, 8]. Therefore, HPV vaccine can also induce protection in older women, and most of all, the administration of the vaccine to patients previously treated for HPV-related disease can reduce the recurrence rate of the disease [9–11].

This study was conducted to evaluate whether vaccination with the quadrivalent HPV vaccine in patients under 45 years of age with a previous history of HPV disease is effective in reducing recurrent disease and abnormal cytology.

Materials and methods

A prospective randomized controlled trial was approved by The Institutional Ethic Committee and Review Board of University teaching Hospital of Careggi and was conducted enrolling patients from Colposcopic Laser Surgery Unit of Careggi in Florence and of Azienda USL in Massa Carrara.

All women under 45 years of age, treated for cervical squamous intraepithelial lesion and with negative HPV test, cytology and colposcopy 3 months after treatment were enrolled. Written informed consent was obtained from all participants. In the informed consent, it was clearly expressed that the study was not blind to the placebo, thus the patients who provided the consent were aware of being selected for either of the two different groups.

Therefore, the selection criteria for the inclusion of the patients in the study group were: women, under 45 years of age, with previous history of treatment for HPV-related disease.

The exclusion criteria, evaluated at a follow-up visit 3 months after treatment, were: positive HPV test, abnormal cervical cytology or HPV-related disease evident at enrollment colposcopy; women positive for HIV, HBV, HCV and other conditions influencing immune system response, pregnancy state at enrollment.

At the first follow-up visit, 3 months after the treatment, patients who did not meet the exclusion criteria were randomly assigned (1:1) according to the lesion treated in two groups: a group was submitted to only follow-up and the other group received the HPV quadrivalent vaccine at 0, 2 and 6 months. The randomized numbers were assigned in an unreadable computer file by clinicians and biologists. The investigator could open the file only after the enrolled patients were entered and accepted.

Follow-up visits were done every 6 months for a duration of at least 3 years. Each visit consisted of a Pap smear, HPV test and a colposcopy. Colposcopic-directed punch biopsies were taken in the case of any suspected HPV lesion.

Positive histologic results during the follow-up were considered as recurrent disease. The recurrent diseases were treated with ablative or excisional treatments according to the grade of the lesion.

The primary endpoint was to evaluate whether the vaccine was effective in reducing recurrent disease by the comparison of the overall disease-free survival.

Secondary objectives were to check whether the vaccination was able to reduce the rate of abnormal cytology and persistent abnormal cytology during the follow-up period.

Persistent abnormal cervical cytology was defined as the detection of abnormal Pap smear in two consecutive cervical samples collected at a distance of 6 months.

When the recurrent disease occurred, HPV genotyping was performed to verify the HPV types involved.

In all women during the entire study, reports of serious adverse events were collected. The end of the study analysis was conducted once all patients had completed the follow-up visits over the 3 years.

Kaplan–Meier curve was used to estimate the fraction of patients living without recurrent disease or positive cervical cytology during the follow-up period.

The comparison between the two groups' Kaplan–Meier curves was done by the log rank test. Additionally, p values were calculated using the Fisher's exact test to compare the proportions of events (recurrent disease or abnormal cytology) between vaccine and control groups. p values < 0.05 were considered statistically significant.

The time interval between the date of enrollment and the date of the first recurrence or abnormal cytology was compared in the two groups using t test ($p < 0.05$).

The planning of the study protocol pointed out a prior calculated intention to treat population of 73 women for each group with observation time of at least 3 years.

Results

From November 2013 to October 2014, we enrolled a total of 178 women who had been treated for cervical disease at Careggi University Hospital in Florence and at Azienda USL in Massa Carrara and did not meet the exclusion criteria at the 3 months follow-up visit after the treatment.

Out of them, 30 were treated for low-grade squamous intraepithelial lesions and 148 received a treatment of conization for a diagnosis of high-grade squamous intraepithelial lesion according to the 2014 World Health Organization

(WHO) classification of cervical tumors. 89 patients (mean age 31.8 years; range 23–44) were followed with follow-up alone and 89 patients (mean age 32.1 years; range 23–44) received HPV quadrivalent vaccine.

Of the 89 patients in the vaccination group, 3 (3.4%) developed recurrence during the follow-up period. All recurrences were low-grade cervical squamous intraepithelial lesions. In the non-vaccination group, 12 (13.5%) developed recurrence: eight low-grade squamous intraepithelial lesions, three affecting vulva and vagina and five affecting cervix, and four developed high-grade cervical squamous intraepithelial lesions.

The mean time between the date of enrollment and the date of relapse was 14.5 months (range 6–24) in the non-vaccination group and 18 months (range 12–24) in the vaccination group. The difference between two groups, analyzed with *t* test, did not show a statistical significance ($p=0.29$).

In Fig. 1, the Kaplan–Meier curves showed a statistically significant difference in the log rank tests, $p=0.0147$ for the overall disease-free survival in the two study groups during the 3 years follow-up.

From the statistical analysis carried out with Fisher's exact test, the vaccination with quadrivalent HPV vaccine was a tool to reduce the incidence of recurrence of HPV-related disease in women previously treated for cervical squamous intraepithelial lesion (Table 1).

HPV genotyping performed in case of recurrence in the vaccination group highlighted the high-risk (HR) HPV types in all cases: one positive to HPV type 16, one positive to HPV types 18 and 33 and one positive to HPV type 31.

In the non-vaccination group, of the three cases of vulva and vaginal recurrences, two were related to low-risk (LR)

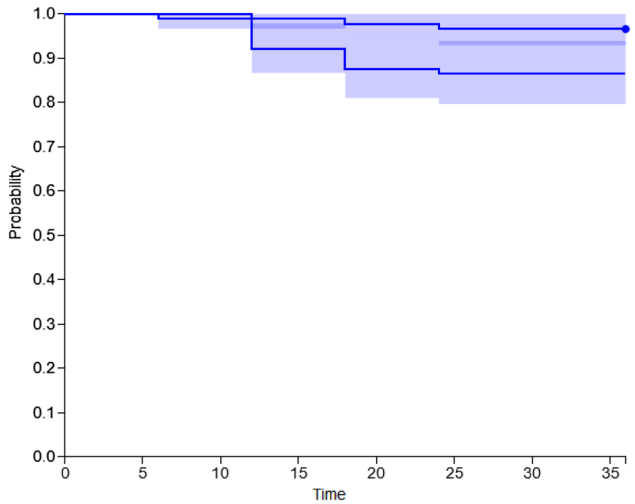

Fig. 1 Kaplan–Meier's curves show the difference during the follow-up period for the overall disease-free survival in two groups. The comparison between the two groups, done with the log rank test, was statistically significant ($z=2.44$, $p=0.0147$)

Table 1 Statistical analysis using the Fisher's exact test showed that vaccination was a useful tool for reducing the incidence of post-treatment recurrence ($p=0.0279$)

	Relapse	No relapse	Marginal row total
Vaccination	3	86	89
Non-vaccination	12	77	89
Marginal column total	15	163	178 (grand total)

The Fisher's exact test statistic value is 0.0279. The result is significant at $p<0.05$

HPV (one to HPV type six and one HPV type 53) and one to HR HPV type 52 and LR HPV type 55.

All five low-grade cervical squamous lesions were related to HR HPV: two to HPV type 16 and two to HPV type 18 and one to HPV type 31. The HPV type 16 was detected in four cases of high-grade disease recurrences associated with HR HPV types 35 and 31 and LR HPV type 53.

During the follow-up period, 23 (25.8%) patients had an abnormal Pap test in the non-vaccination group and 7 (7.9%) patients had an abnormal Pap test in the vaccination group.

Of the abnormal Pap tests in the vaccination group, five were LSIL (low-grade squamous intraepithelial lesion) related to HR HPV and two were ASCUS (atypical squamous cell of undetermined significance), two were related to HR HPV and two to LR HPV.

Of the 23 abnormal Pap tests in the non-vaccination group, one was ASCH (atypical squamous cell, but cannot exclude high-grade squamous intraepithelial lesion), one was AGC (atypical glandular cells not otherwise specified), eight were LSIL and 13 were ASCUS. 18 (1 ASCH, 1 AGC, 6 LSIL and 10 ASCUS) of the abnormal cytologies were related to HR HPV, four (2 LSIL and 2 ASCUS) to LR HPV and one (ASCUS) to a negative HPV test.

The mean time during which the Pap tests resulted abnormal in the follow-up period was 11.20 months (range 6–24) in the non-vaccination group and 16.29 months (range 12–24) in the vaccination group, not showing a statistical significance difference with *t* tests between the two study groups ($p=0.07$).

In the vaccination group, the abnormality of the seven Pap tests was not confirmed in any case at the following 6 months follow-up visit. While in the non-vaccination group, the abnormality of the Pap smear was confirmed in nine cases (39.1%) in the 6-month follow-up visit.

So, the vaccination was able to reduce both the rate of abnormality to Pap test and the rate of persistent abnormal cervical cytology ($p<0.05$) (Tables 2, 3).

In Figures 2, 3, the Kaplan–Meier's curves showed the difference during the follow-up period in free interval between abnormal cytology and persistent abnormal cytology in the two groups. The difference was statistically

Table 2 Statistical analysis using the Fisher's exact test showed that the quadrivalent vaccine reduced the rate of positive cytology results during follow-up ($p=0.0023$)

	Pap test +	Pap test −	Marginal row total
Vaccination	7	82	89
Non-vaccination	23	66	89
Marginal column total	30	148	178 (grand total)

The Fisher's exact test statistic value is 0.0023. The result is significant at $p<0.05$

Table 3 Statistical analysis using the Fisher's exact test showed that the vaccine reduced the rate of persistent positive cervical cytology results during follow-up ($p=0.0023$)

	Pap test +	Pap test −	Marginal row total
Vaccination	0	89	89
Non-vaccination	9	80	89
Marginal column total	9	169	178 (grand total)

The Fisher's exact test statistic value is 0.0032. The result is significant at $p<0.05$

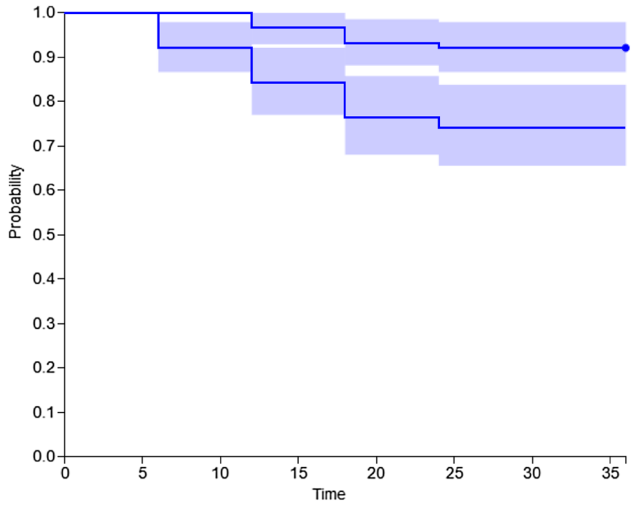

Fig. 2 Kaplan–Meier's curves show the difference in the intervals free from positive Pap smears in the two groups during the 3 years of follow-up. The comparison between the two groups, done with the log rank test, was statistically significant ($z=3.26$, $p=0.00113$)

significant for both secondary endpoints ($p<0.001$ and $p<0.002$, respectively).

Discussion

HPV vaccine is higly effective in women when given prior to their sexual debut namely before initial exposure to the virus. However, it is still unclear whether the vaccine has

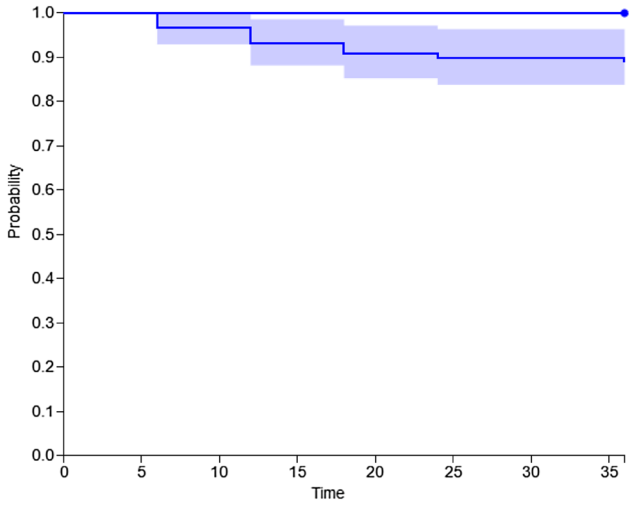

Fig. 3 Kaplan–Meier's curves show the difference in the intervals free from persistent positive Pap smears in the two groups, during the 3 years of follow-up. The comparison between the two groups, done with the log rank test, was statistically significant ($z=3.07$, $p=0.00214$)

any benefit in women who have previously been treated for the HPV-related disease.

Recent studies have shown that the vaccination would be able to reduce the risk of developing subsequent HPV-linked disease in women already treated for HPV-related disease.

In 2012, Joura et al. demonstrated that previous HPV vaccination with quadrivalent HPV vaccine was associated with an important reduction in the recurrence of high-grade cervical disease in women who were treated for HPV-linked disease [9]. Subsequently, Kang et al. showed a lower rate of recurrent cervical disease among those who were vaccinated post-excisional treatment with LEEP for high-grade squamous intraepithelial lesion [9].

The latest study of Garland et al. confirmed that women who undergo surgery for cervical lesion after receiving HPV vaccine may continue to benefit from vaccination, in fact the bivalent vaccine was effective in reducing the risk of recurrence for high-grade and low-grade cervical disease [11].

None of these studies could directly evaluate the impact of vaccination on women who had undergone treatment before vaccination, because the studies of Joura et al. and Garland et al. enrolled women who had been vaccinated before the treatment [9, 11]. Meanwhile, in the non-randomized trial of Kang et al., patients were vaccinated one week after excisional treatment with LEEP, therefore it was not possible to evaluate the possible residual disease, recurrent disease, abnormal cytology or positive HPV test at enrollment [10].

To our knowledge, this is the first study evaluating the direct impact of HPV vaccination on women with previous history of HPV disease without recurrent/residual disease,

negative cytology and HPV test at enrollment. Our results confirm the benefits of HPV vaccination after treatment for HPV-related disease; in fact, the rate of recurrence was higher in the non-vaccination group than in the vaccination group during the follow-up period (13.5% vs 3.4%; $p < 0.05$).

Our study, together with the other studies in the literature, suggests that these women, who had already manifested an inability to clear persistent oncogenic HPV infection and are actually the women who would have benefited from primary HPV vaccination, have a significant capacity to benefit from secondary vaccination. In fact, the high antibody levels following the vaccination seem to prevent new areas of epithelial infection, whether due to dissemination from existing sites of HPV infection or from new HPV exposure, and thus disease [12].

Results from Phase III vaccine trials [13, 14] promised that natural immune response may be boosted by vaccinating previously infected HPV women when they naturally would develop low anticorpal level and clear up from DNA detection. This hypothesis could represent one of the supportive mechanisms to validate prophylactic vaccines as protective for women who underwent surgery for HPV-linked lesions, as well as giving them a tool to protect themselves against newly acquired or cross-dependant infections, within the safety range of age of the vaccine itself.

It is therefore reasonable to discuss the potential benefits of vaccination for women previously treated for HPV-related diseases. However, women must be informed that vaccination will not treat existing areas of infection or disease nor prevent all future HPV infections and that therefore attendance at follow-up and future screening remain important.

In our study, HPV quadrivalent vaccine is effective in reducing abnormalities in Pap tests. In fact, during the follow-up period, 7.9% of the Pap smears were abnormal in the vaccination group compared to 25.8% of the Pap smears resulting abnormal in the non-vaccination group. The interesting result is that the abnormality to Pap test was not confirmed in the next follow-up visit in the vaccination group compared to the non-vaccination group in which the abnormality of the Pap test was confirmed in 39.1% of the cases.

Our data suggest that the implications of post-treatment vaccination would be very important as it influences the post-treatment management of HPV diseases. The follow-up after treatment could be changed in the patients who undergo vaccination after treatment for HPV-related disease.

Numerous studies have suggested that HPV testing is more sensitive than cytology and has a negative predictive value of almost 100% for detection of CIN2 + [15–17].

It is suggested that "double-negative" HPV DNA and cervical cytology testing indicate high prognostic assurance against risk of future high-grade squamous intraepithelial lesion and may safely allow longer follow-up intervals for such women [18].Therefore, in cases of negative post-treatment HPV testing and cervical cytology, the frequency of follow-up could be reduced and an early return to screening could be planned. In our study, among vaccinated patients in 92.1% (82/89) of cases, HPV test and cervical cytology were both negative during the follow-up period, therefore we could already plan an early return to screening 2 years after the treatment. The choice of return to screening 2 years after treatment is strengthened by the fact that the mean time between the date of enrollment and the date of relapse and abnormal Pap test occurred within 2 years after treatment.

Viral genotyping, performed in case of relapse, showed in most cases the presence of HPV types contained in the latest 9-valent vaccine. Therefore in our study, vaccination with 9-valent vaccine could have reduced the relapse rate in both groups. Taking into account the contribution of HPV genotypes, the vaccination with 9-valent vaccine expands the potential to prevent HPV disease and it will be therefore an important tool not only for primary prevention but also for secondary prevention in HPV-linked disease. In two cases of recurrences in the vaccination group, viral genotyping detected the presence of HPVHR 16 and 18, therefore vaccination has not led to protection. The cause is probably related to the individual's immune system. These are the rare cases of not responding to vaccination or women responding to vaccination without sufficient antibody production.

In conclusion, the HPV vaccine can be recommended in women already treated for HPV-related disease given the significant reduction in the incidence of recurrence of HPV diseases and abnormality of the Pap test in the vaccination group. The HPV vaccination has not led to a statistically significant change compared to non-vaccination group at the time of occurrence of disease recurrence of abnormally to the Pap test.

Our study shows that the introduction of HPV vaccination during the follow-up post treatment for HPV-linked disease is recommended to reduce the risk of recurrence. The introduction of the vaccination as a routine procedure after treatment is also supported by a previous study that demonstrated that the HPV vaccination is feasible in 82.4% of cases considering a cohort of Italian women under the age of 45 previously treated for HPV-related diseases [19]. In addition, no adverse events or adverse reactions were reported in the vaccination group, demonstrating the safety of the vaccine [20–22]. Moreover, these women, sensitized by their personal history, accept willingly the opportunity to have a vaccination, if properly informed.

Author contribution Protocol/project development: AP, AG, MF. Conceptualization: AP. Funding acquisition: CB, MF. Data collection and management: CB, NA. Blind colposcopists and physicians: AP, AG, MGF. Blind biologists: CB. Data Analysis: CB, NZ. Manuscript writing/editing: CB, NA, GC, FP. Supervision: GC, FP.

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Compliance with ethical standards

Conflict of interest We declare that we have no conflict of interest.

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