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Effectiveness of HPV vaccination in women reaching screening age in Italy

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ABSTRACT

Background and objectives: A randomized trial was conducted in Tuscany, Italy, to evaluate the effectiveness of HPV vaccination for 25 year old (yo) women who attend at the first time cervical cancer screening. The trial also evaluated immune response after vaccination, reductions of cytological abnormalities and the impact of vaccination on screening activity.

Study design: During 2010–2011, all 25 yo women who were invited to the Florence cervical cancer screening programme were also asked to participate in the trial. Enrolled women were randomized into study and control groups. Those in the study group were offered HPV vaccination after the usual Pap test. The cytology distribution and prevalence for any high risk (hr) HPV type were compared at the subsequent screening round in an intention-to-treat analysis. The impact of HPV vaccination was evaluated per protocol comparing vaccinated women with the control group.

Results: Our results showed a reduction in HPV prevalence at recall for any hr-HPV type but it was not statistically significant, being 17.1% vs 21.4%, $p = 0.20$ in the study and control groups, respectively. If we restricted the analysis to vaccinated women, strong reductions of the HPV 16,18,31,33,45 and HPV 31,33,45 infections were observed, being 5.3% vs 12.8%, $p < 0.01$ and 2.1% vs 6.5%, $p = 0.02$, respectively. Significant reductions for any hr-HPV infection and for HPV 16 infection were also observed in women HPV 16/18 negative at enrolment, being 12% vs 21.4%, $p < 0.01$ and 0.6% vs 6.7%, p-value < 0.01, respectively. In women hr-HPV negative at enrolment no infections due to HPV 16 or HPV 18 were observed and there was a big reduction for any hr-HPV infection (7.1% vs 21.4% $p < 0.01$). A strong antibody response was observed not only for HPV 16 & 18 but also for their related types.

Conclusions: Our findings suggest that HPV vaccination at the age 25 is beneficial if it is offered to hr-HPV negative women. Our data will assist in developing a cost effectiveness model for choosing the best strategy to integrate screening and vaccination for the coming years.

Clinical trial registration number is NCT02296255.

1. Background

Prophylactic human papillomavirus (HPV) vaccination against HPV 16/18 infections has been introduced for adolescent girls in Italy since 2007, as in most countries of Western Europe [1,2]. In Tuscany, an administrative region of Italy, the HPV vaccination programme offers vaccination with bivalent Cervarix® (Glaxo-SmithKline) to 12- to 17-year-old (yo) girls free of charge. In addition, there is a strong interest for the vaccination among adult women [3,4], even if vaccination effectiveness on CIN2+ among

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vaccinated women decreases with increasing age at vaccination [5].

One important aspect in this context is to assess the potential impact of HPV vaccination on cervical cancer screening and to identify the best strategy for screening in a vaccinated population in order to minimize unnecessary procedures and costs. HPV vaccination and cervical cancer screening should work in an integrated manner. Two strategies have been proposed, but need validation. The first one is a 'vaccinate and screen' strategy [6] in which women would be vaccinated and, one year later, screened for the presence of high risk (hr) HPV in the cervix. The second one is a 'screen and vaccinate' strategy [7] in which women would first be screened for hr-HPV.

1.1. Objectives

The objective of this study was to evaluate the impact of vaccination among 25yo women during their first cervical cancer screening appointment. We aimed to evaluate the consequences of vaccination on screening activity, to monitor the immune response following vaccination, to study the dynamics of infection after vaccination and, finally, to evaluate reductions of cytological abnormalities among vaccinated women.

2. Materials and methods

The study was conducted by the Cancer and Prevention Institute (ISPO) in Florence and was approved by the local ethics committee and funded by Istituto Toscano Tumori. It was a randomized controlled trial offering vaccination integrated into the first two screening rounds of the cervical cancer screening and was offered for women who were new participants to the screening in the year 2010/2011. All 25yo women (birth cohorts 1985/1986), resident in the province of Florence and targeted by the cervical cancer screening programme, were invited by letter to participate. Women who accepted invitation and gave their informed consent were randomized into study and control groups in a 1 to 2 ratio. Women were enrolled regardless of being sexually active or not. The results of assignment were communicated to consenting women by the person taking the cervical smear.

Women in the control group received usual care, i.e. the collection of a cervical sample for the Pap test. Women in the study group received care comprised of the collection of two cervical samples (for Pap test and HPV test collected in HC2 DNA Collection Device for cervical specimen collection in Specimen Transport medium), a blood sample (for HPV antibody testing on serum before vaccination), urine sample (for HPV testing, data already published) [8], and free vaccination with Cervarix® [9]. The care for women in both groups at the second screening round comprised of the collection of cervical samples (for Pap test and HPV test) and, for the study group only, a second blood sample for HPV antibody testing.

At enrolment, women with abnormal Pap test results (atypical squamous cells of undetermined significance or more severe, ASC-US+) were referred to colposcopy. If colposcopy revealed a final histological diagnosis of CIN2+ lesions, women were recommended for an excisional treatment, but if colposcopy was negative, they were recalled at 30 months from enrolment.

Women in study group with normal cytology but with HPV-positive results at enrolment, were recalled at 12 months for HPV and Pap retests and if either test was positive, they were referred to colposcopy. If colposcopy revealed final histological diagnosis of CIN2+ lesion, they were recommended for treatment, but if colposcopy was negative they were recalled at 30 months from enrolment.

Finally, women with normal cytology at enrolment in the control group and HPV-negative women with normal cytology in the study group were recalled by letter to attend a second round of screening after 30 months (the screening interval was reduced to 30 months from the usual 3 years for their first screening round only).

As previously described, for women in the study group, in addition to the Pap test, an HPV test was also made at enrolment. Those women with normal cytology but with HPV-positive results at enrolment, were recalled at 12 months for HPV and Pap retests and if either test was positive, they were referred to colposcopy. Thus doing an HPV test at enrolment could generate a possible diagnostics anticipation for women in the study group. Hence, in order to compare the study group with the control group, we considered the HPV positive women with normal cytology at baseline in the study group who were diagnosed with a CIN2+ lesion at 12-months as a result at the 30-month follow-up.

2.1. HPV testing

The presence of hr-HPV was evaluated by Hybrid Capture 2 (HC2) (Qiagen, Gaithersburg, USA), which contains only the 'high-risk' mixture of probes, which is designed to detect HPV types 16,18,31,33,35,39,45,51,52,56,58,59, (Group 1) and 68 (probably carcinogenic to humans, Group 2) [10]. Samples were considered hr-HPV positive if the relative light units/cut-off (RLU/CO) ratio was $\geq$1, as recommended by the manufacturer. Genotyping was performed in all HPV positive samples by INNO-LiPA HPV Genotyping *Extra* [8]. All HPVX samples (HPV-control line positive but no specific probe in the strip) were amplified and genotyped by "Linear Array HPV Genotyping Test" (Roche, Molecular Diagnostics, Pleasanton, USA). The system can identify 37 HPV types: 6,11,16,18,26,31,33,35,39,40,42,45,51,52,53,54,55,56,58,59,61,62, 64,66,67,68,69,70,71,72,73,81,82,83,84,IS39, and CP6108.

2.2. Serological HPV testing

Serum samples were tested for HPV antibodies using Luminex technology [11,12], heparin coating of the beads and pseudovirions (PsVs) of 17 HPV types belonging to the alpha species: 3,6,11,16,18,31,32,33,35,39,45,52,56,58,59,68,73, and of four HPV types belonging to the beta species: 5,15,38,76.

The cut-off values to define seropositivity were calculated independently for each HPV type by analysing the mean fluorescence intensity unit (MFI) values obtained from the sera of 107 children. The cut-off algorithm recommended by the global HPV LabNet 20 (mean MFI value of a negative control serum panel plus three standard deviations) was used [13]. However, if the calculated cut-off value was less than 400 MFI, we used 400 MFI as the cut-off.

2.3. Statistical analysis

We calculated the cytological distribution and the prevalence for any hr-HPV type at recall for both groups with the relative 95% confidence interval (intention-to-treat analysis). Difference in proportions was evaluated by Pearson's chi-square ($\chi 2$) test.

Given alpha = 0.05 and power = 0.80 (one tail test), the study would have been able to detect as statistically significant a reduction in referral rate to colposcopy of 55% or more, in the vaccinated group compared to unvaccinated group with a sample size of 2100 women. Regarding HPV infections, expecting an 8% HPV no −16 nor −18 prevalence in the control group, such a sample size would have been able to detect as statistically significant (alpha = 0.05, power = 0.80, two tails) a difference of about 3% of infections for the same types (i.e. lower than 5.2% or higher than 11.4%).