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Original Research

Development of high-risk HPV associated cervical dysplasia despite HPV-vaccination: a regional dysplasia center cohort study

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Summary

The present study was conducted to examine the frequency of CIN/squamous intraepithelial lesions in patients after vaccination and respective HPV types observed at their referral to a regional center for screening of cervical dysplasia.

Key words: HPV; Vaccination; Cervical intraepithelial neoplasia.

Introduction

HPV has been shown to be primarily responsible for the development of cervical dysplasia and neoplasia [1]. It is the main causal agent in cervical cancer, as well as cervical dysplasia and cancerous lesions of the vulva, the penis, and the anus [2].

Up to now more than 200 different HPV related types have been described of which about 40 can be sexually transmitted and are associated with infection of the female genital tract. HPV subtypes fall into two categories, low-risk HPVs causing skin warts and benign condyloma acuminata (e.g. HPV types 6 and 11) and high-risk HPVs (e.g., types 16 and 18) that are responsible most HPV induced carcinoms [3].

Cervical cancer is still the second most common malignancy in women worldwide and over 80% of HPV-associated diseases are reported from low income countries. Risk factors for high-risk HPV infection are early onset of sexual activity, high numbers of sexual partners, and tobacco smoking [4-6].

Currently, the FDA has approved three vaccines that are effective at preventing HPV infection covering 2, 4, or 9 HPV serotypes, respectively. The Committee on Adolescent Health Care and Immunization Expert Work Group of the American College of Obstetricians and Gynecologists updated their vaccination recommendations accordingly and suggest routine HPV vaccination for girls and boys at the target age of 11–12 years (but it may be given from the age of 9 years) [7]. It can be given to females and males up to the age of 26 or 21 years as part of the adolescent immunization platform in order to help reduce the incidence of anogenital cancer and genital warts associated with HPV infection [8]. Obstetrician–gynecologists and other healthcare providers should stress to parents and patients the benefits and safety of HPV vaccination and offer HPV vaccines in their offices [7, 9, 10].

The quadrivalent vaccine Gardasil (against HPV type 6,11,16 and 18) was introduced in 2006 followed in 2007 by the introduction of the bivalent vaccine Cervarix (against HPV type 16 and 18) [9-12].

The present study was conducted to examine the frequency of CIN/squamous intraepithelial lesions in patients after vaccination and respective HPV types observed at their referral to a regional center for screening of cervical dysplasia. Another interesting factor was the period from vaccination to detection of abnormal cytological smears and finally CIN lesions in vaccinated patients and if this differs between HPV subtypes.

Materials and Methods

The study comprises a group of 19 females, HPV vaccinated patients with HSIL (25 to 33 years of age) out of a total of 1,276 (19 vaccinated and 1,257 non-vaccinated) subjects clinically observed at a regional cervical dysplasia referral center within late 2011 until early 2017 suspicious for dysplastic cervical lesions at first appointment (Table 1). All patients were first seen within routine gyneacologic check-up and sent for referral center becau-

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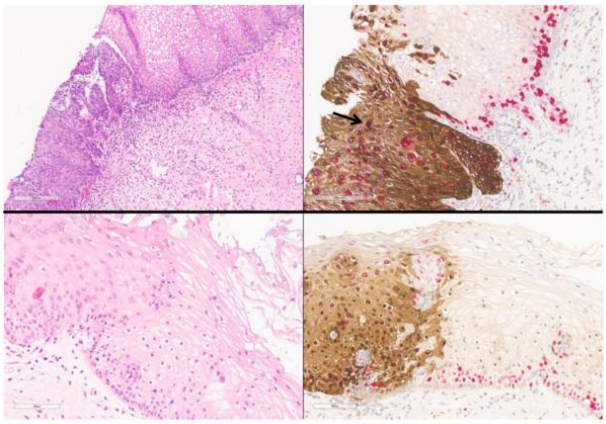

Figure 1. — Cervical intraepithelial neoplasia/HSIL. A) CIN 3 (high grade)/HSIL in a patient with HPV type 16 infection showing abnormal maturation and dysplastic cells with pleomorphic nuclei occupying the full thickness of the epithelium (left lower half), normal epithelium (right upper half). (HE, ×5). B) Immunohistological dual staining reveals specific cytoplasmic and nuclear p16 reaction (brown). Ki67 (red nuclei) marks proliferating cells where co-expression with p16 indicates a positive test result (see arrowhead) (×20). C) CIN 2 (intermediate grade)/HSIL in a patient with HPV type 59 infection with moderately dysplastic cells occupying the middle third of the epithelium. (HE, ×20). D) These cells show positive p16 immunostaining and the Ki67 proliferation zone is shifted towards the middle third (left half) which in normal epithelium is restricted to the basal cell layer (right half) (×20),

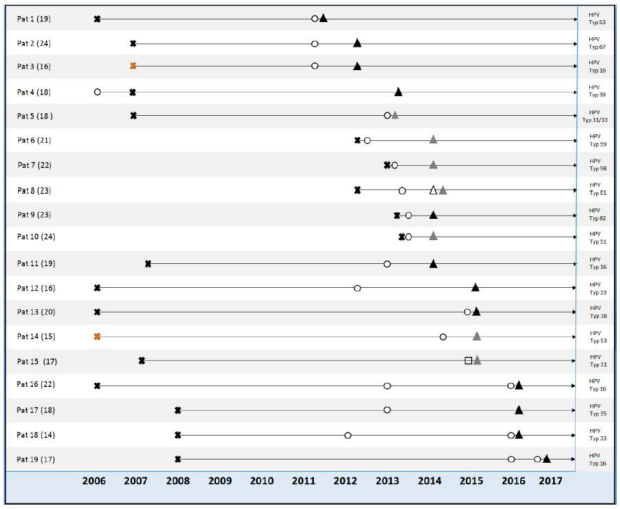

Figure 2. — Clinical course of patients.
Date of vaccination with Gardasil (HPV 6, 11, 16, 18): (**x**) and with Cervarix (HPV 16,18) (**x**) in all patients after inconspicuous prior cytology. Findings at referral center: cytological findings NILM (□) and HSIL (○). Histological and immunohistological findings CIN III/HSIL (▲), CIN II/HSIL (▲) and CIN I/LSIL (△). PCR based HPV subtype (on the right). Patients' year of age at vaccination in brackets , e.g. "Pat 1 (19)". Overall observation time period after vaccination ten years, after first cytological control five years.

-se of recurrent suspicious cervical smears. Most of those patients seen at the referral center had not received vaccination or had only reactive atypic smears during controls, meaning cytologically suspicious findings because of persistent infection, cervical or uterine polyps a.s.o. Our cohort of 19 vaccinated patients were 1.4% of in total 1,276 patients investigated.

All patients were sent to the certified regional cervical dysplasia referral center (led by JW). Clinical work-up comprised thorough recall regarding vaccination status, prior suspicious cytology smears, and sexual activity followed by colposcopy. Fractioned curretage and/or excision biopsy was done and if the suspicious cytologic findings were verified by histology, the patient was sent for conization, i.e. surgical removal of the dysplastic tissue.

Specimens were routinely stained by H&E and p16/Ki67 immunohistochemistry (IHC) was performed on formalin-fixed paraffin embedded tissues using an automated immunostainer system. In all conization specimens step-wise sectioned slides were produced for histological diagnosis. Cases were claimed "positive" via histology if CIN2 or CIN3 (HSIL) was diagnosed, either on cervical samples or conization specimens.

Immunostaining was done by p16 [p16 with monoclonal mouse anti-p16^{INK4a} (E6H4, ready to use primary antibody)] and Ki67 (rabbit anti-human Ki-67 polyclonal antibody, dilution 1:200) using a special p16/Ki67-double-staining procedure. Evaluation of p16/Ki67 IHC double staining was focused on dysplastic cells with nuclear co-expression.

For HPV subtyping, Processing was done to identify specific subtypes, with confirmation by two external laboratories [University Pathology Institute of Cologne (n=4) and Goettingen (n=15)]. HPV subtyping at the Institute of Pathology, University of Goettingen was performed using two analysis systems. Six cases were analyzed by F-HPV typing using a multiplex PCR with fragmentation length analysis on an genetic analyzer. For further nine cases the VisionArray HPV Chip was used. Both methods were applied according to the manufacturer's protocol. At the Institute for Pathology at the University Hospital Cologne, HPV detection and subtyping was achieved by polymerase chain reaction and enzyme-linked immunosorbent assay (PCR-ELISA) as previously described. Statistical calculations were done using Mann-Whitney U Test and significance was set at $p < 0.05$.

The study is in agreement with the guidelines of the responsible local ethics committee (Landesärtzekammer Hessen, Wiesbaden, Germany). Research Review Board approval was not necessary.

Results

In total the study comprised of 19 patients who developed cervical high-grade squamous intraepithelial lesions (HSIL) although they had been vaccinated in previous years. HPV screening (cytology based) and p16/Ki67 double-staining as well as HPV subtyping (histology based) revealed hrHPV type infection. No patient was hrHPV negative. No glandular dysplasia or microinvasive carcinoma of the cervix was detected. Accordingly, p16/Ki67 immunostaining revealed strong double-positivity in all investigated cases due to atypic distracting morphology to rule out immature metaplasia and confirm CIN2/HSIL (Fig-

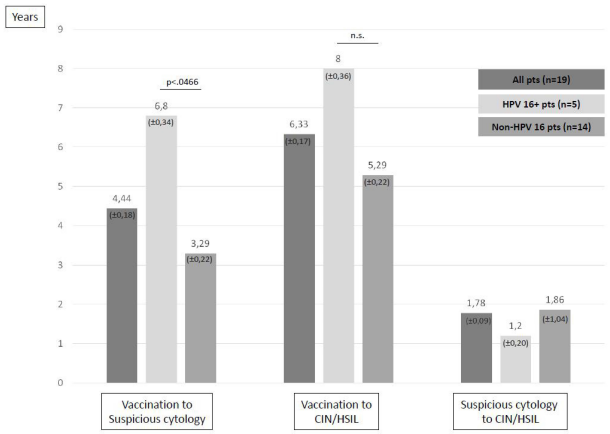

Figure 3. — Time to suspicious cytology and CIN (HSIL) diagnosis after vaccination.

ure 1).

Of the two patients who had received vaccination with Cervarix one was positive for HPV 16 and the other for HPV 53. All other 17 patients were vaccinated with Gardasil and were positive for HPV type 16 (4x), 31,33(2x),35,39,51(2x),53,58,59,67 or the potential hrHPV type 82. One patient had a HPV 31/33 double-infection (Figure 2).

Within the referral center, each patient had another cytology performed first, followed by conization if cytology was positive for HSIL. These 19 women of this cohort were vaccinated during the years 2006 to 2013 and mostly by quadrivalent vaccine Gardasil (covering HPV subtype 6, 11, 16, and 18). Data about prior cytology status were not available. Only two patients received the bivalent vaccine Cervarix (HPV subtype 16 and 18). Two dose vaccination at the time should have been standard but could not be proved. Nowadays the standard of three vaccination could not be confirmed. Median age at vaccination was 19.3 (ranging from 14 to 24) years. No immunsuppressive therapy or risk factors have been documented.

Within this study, 11 excision biopsies, nine curettage materials, as well as nine conization samples were sent to the Institute of Pathology Nordhessen for histopathological diagnostics.

Cytologically, 18 patients presented cervical smears with recurrent HSIL-results and one patient was sent because of NILM and highly suspicious anamnesis with CIN II (HSIL) or higher on a prior cervical biopsy (Pat #15). Further patients' examination on histological samples revealed CIN III (HSIL) in 12 (HPV type 16(5x), 33(2x), 35, 39, 53, 67 and 82) and CIN II (HSIL) in seven patients (HPV type 31, 51(2x), 53, 58 and 59 as well as 31/33 dual-infection) (Figure 2).

Most cases were observed within the years 2011 to 2017. In median, it took 4.4 years from vaccination to suspicious cervical smear and again 1.7 years to detect manifest intraepithelial neoplasia (histologically proven). Thus, "protocolled progress" in daily routine from normal findings and vaccination to CIN (SIL) occurred with a median of 6.3 years (Figure 3). The present authors underline that no standardized research protocol or artificially changed surveillance besides daily routine which was implemented.

Table 1. — *Patients with suspicious cytology sent for dysplasia referral clinic grouped by age range. Approximately 1,276 patients per year (p.p.y.)*

Year of investigation	Patients < 25 years of age (%)	25–35 years of age (%)	Patients > 35 years of age (%)
2011	11.9	40.48	47.62
2012	7.86	36.43	55.71
2013	9.16	43.66	47.18
2014	12.33	54.79	32.87
2015	10.10	40.40	49.49
2016	11.71	40.97	47.32
Average / 1,276 p.p.y	**10.5 % /** n = 133	**42.8 % /** n = 546	**46.7 % /** n = 597

Focussed on herein presented five HPV 16 associated CIN lesions (HSILs), it took 6.8 years from vaccination to detection within routine clinical setting of suspicious smear, and again 1.2 years to detect cervical intraepithelial neoplasia (HSIL). Time from vaccination to CIN lesion (HSIL) detection was 8.0 years. In comparison, within the non-HPV 16 cohort, the mean time from vaccination to suspicious cervical smear detection was significantly shorter (3.3 years), and it took another 1.9 years from suspicious smear to detection of CIN lesion (HSIL). General detection of CIN lesion (HSIL) from vaccination took 5.3 years (Figure 3). All data showed no statistic significance.

Discussion

Decrease of high-risk HPV associated cervical lesions has been stated in various studies in different countries [8, 13, 14]. Herein the authors show that after vaccination against HPV strains 16 and 18, cervical dysplasias may develop. In 75% of the cases (14 out of 19) infections by HPV types were not covered by the vaccines (bivalent and quadrivalent). All cases showed high-grade intraepithelial neoplasia (HSIL/ CIN II and CIN III). The protocolled time to high-grade CIN (HSIL) diagnosis after vaccination was 6.3 years and time to suspicious cervical smear was 4.4 years (in median).

About 90% of cervical cancers world-wide develop due to HPV infections. In most cases (60-80%) intermitting HPV infections get eradicated by the individual's immune system within approximately 12 months to two years. Only 20-40% show persistent infections usually by hrHPV.

Since 2006 the HPV vaccine Gardasil and since 2007 Cervarix have been available in Germany which raises the question of the usefulness of screening in this subgroup of patients. The German Standing Vaccination Committee (STIKO) has advised young girls at the age of 9 to 14 to

receive HPV vaccination, best before the first sexual activity. Vaccination follows a two-dose regimen. Girls or youngwomen older than 15 years receive three doses [10].

Since April 2016, a new vaccine was approved in Germany named Gardasil 9 which is designed to protect against nine HPV types (6,11,16,18,31,33,45,52,58). A cross-sectional study on the relative contribution to cervical cancer and precancerous lesions of HPV types found that these nine types were responsible for 95.5% of all cervical lesions in North America [15]. HPV types 31, 33, 45, 52, and 58 accounted for 16.9% of total lesions [12]. Remaining risk of less oncogenic and potentially oncogenic HPV types exists leading to cervical dysplasia if the more powerful HPV types are reduced succesfully.

In the present series the new nonavalent vaccine would potentially have protected nearly half of cases who developed CIN lesions (SIL) after correctly applied vaccination (n=9/19; 47%; Patient #3,5,11,12,13,15,16,18,19). Focussing only on non-HPV16 positive patients, the nonavalent vaccine would have potentially protected five patients, so practically nine would have been infected anyway (n=9/14; 64%).

Published data report eight to 18 months for HPV infection to clear or turn into persistant state. Time from initial HPV infection to CIN lesion (HSIL) ranges from one to ten years [7]. The mean time from suspicious cytology (ASCUS) to CIN II/ CIN III (HSIL) or cancer diagnosis by cytology in oncogenic HPV infected patients was reported as 73.4 months (6.1 yearss) in women aged 31-65 as well as 80.4 months (6.7 years) in women aged 16-30. From LSIL to HSIL it took 68.4 months (5.7 years) *vs*. 75.6 months (6.3 years) in older and younger women [16].

Significant differences in comparison of the two applied vaccines have not been detected. Although the authors present a small cohort, without detailed data about demographic information, vaccination schedules and sexual intercourse, they found HPV 16 positive patients in both vaccine groups.

The present cohort, more precisely the 19 vaccinated women aged 25 to 33 years out of 546 patients within this age group out of in total 1,276 represent, 3.5% of patients were positive for high-risk HPV. Compared to other data showing HPV positivity in vaccinated women ranging from 3.3% to 6.4%, these findings match [17]. Detailed data about high-risk HPV prevalence in vaccinated women in Germany, other European contries or the U.S. are not yet available.

Prospective screening guidelines for cervical dysplasia, e.g. HPV testing alone or along with cytology, should be also be considered carefully in the subgroup of vaccinated patients. Currently, new screening guidelines are being discussed. Thus, the Joint Federal Committee has recently published new screening guidelines for cervical dysplasia. Special guidelines for vaccinated women have not been approved. It might be reasonable to have a regular screening, e.g. by one-, three-, and five-year control using RT-PCR and genotype analysis as proposed in the USA [18].

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