Primary cervical cancer truly negative for high-risk human papillomavirus is a rare but distinct entity that can affect virgins and young adolescents

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Summary

Cancer of the uterine cervix is almost exclusively associated with human papillomavirus (HPV). Carcinogenesis is slow, the minimal time from initial HPV infection to invasive carcinoma seems to be less than ten years. In order to identify rapid onset cervical cancer, we carried out a retrospective re-analysis of an extended cohort of patients with invasive cervical cancer, and reviewed cases identified within the cancer registry of Lower Saxony or using Medline or ISI data. No instances of a rapid-onset cancer or true HPV-DNA negative cancer were found among our hospital cohort of 178 women with primary cancer of the uterine cervix. Registry data identified four out of 5,878 patients who were diagnosed with primary cervical cancer at 14 to 20 years of age. They were classified as clear-cell and endometriod adenocarcinoma and tested persistently negative for high-risk HPV-DNA. Fourteen more cases of cervical cancer in virgins and very young women were identified by a Medline search, mostly with unknown histologic type or rare subtypes of adenocarcinoma. In conclusion, rare adenocarcinoma of the uterine cervix may represent an entity unrelated to HPV, thus explaining instances of rapid onset cervical cancer.

Key words: HPV-negative; Cervical cancer rapid onset.

Introduction

Persistent infection with a genital high-risk human papillomavirus (HPV) is essential for the development of almost all invasive cervical cancers. On average, the complete cycle from initial HPV infection to invasive disease seems to require more than 20 years [1]. HPV-prevalence rates show a peak in women younger than 25 years, while the peak of cervical cancer prevalence is found in 50-55 year-old women [2-5]. Based on the natural history of HPV-associated cervical intraepithelial neoplasia the minimum period of carcinogenesis seems to be in the range of eight to ten years after infection with HPV. As a sexually transmitted infection with HPV is always the first step of carcinogenesis, and the minimal latency between infection and invasive cancer is seven years, true rapid-onset cervical cancers should not occur within seven years of sexual initiation, nor should invasive cancers be seen in virgins. Furthermore, before the age of 20 years, invasive cervical cancer should occur only as a result of sexual child abuse.

However, a number of studies reported cases of cervical cancers with a shorter onset as well as HPV-negative primary cervical cancers [6]. Such a subset of invasive cervical carcinoma would escape any screening strategy based on HPV-testing and/or HPV vaccination protocols.

In order to clarify the existence and to determine the frequency of rapid onset as well as HPV-negative primary cervical cancer we analysed different cohorts of cervical cancer patients prospectively and retrospectively, and did a review of the literature by a Medline research.

Materials and Methods

We reanalysed an extended cohort of 178 cervical cancer patients who were recruited prospectively at the Medizinische Hochschule Hannover (MHH) Department of Gynaecologic Oncology in Hannover 1999-2003 and in Wolfsburg 2003-2007 [7]. All women underwent HPV-testing using the Hybrid Capture 2 assay (HC-2, Digene TM). Data on age at first intercourse and lifetime screening results were collected from all participants. HPV-negative tumours as well as cases with an interval of less than ten years between first sexual intercourse and invasive disease underwent further evaluation.

The MHH cancer registry 1980-2004 and the cancer registries of the Kassenärztliche Vereinigung of Lower Saxony 1983-2004 were searched for invasive cancer patients with an age of 20 years or less. A sexual debut at the age of 14 years identified cases who underwent further analysis.

Paraffin-embedded tumour tissue blocks from all patients selected for further evaluation were used for detailed HPV-testing as follows: Five 7 µm sections of each tissue were deparaffinized with xylole in an Eppendorf tube and ethanol precipitat-

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ed. DNA extraction using the Qiagen EZ-1 robot (Qiagen, Germany), sample preparation and sample analysis procedures were carried out in separate rooms. All samples were tested for DNA integrity by PCR using primers PC04/GH20 for the human ß-globin gene. Two different primer combinations were used (PPF1/CP5, CP4/CP5) producing amplicons ranging in size from 270-450 bp, all located in the highly conserved helicase region of the E1 gene. The sensitivity and performance of this PCR system for different HPV types has been described elsewhere [8]. Direct automated sequencing of the PCR products was done using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA). Sequencing was performed in 47 cm capillaries with the use of an ABI 310 sequencer (PE Biosystems). Sequences with < 5% unidentifiable bases were processed and compared with those of known HPV types available through the GenBank database (NCBI, Bethesda, MD) using the Blast program. A nucleotide sequence was regarded as a distinct HPV type if it demonstrated < 90% homology with a known type. In addition, all samples were tested with the LiPAv2 genotyping system (Innogenetics, Belgium) and the Papillocheck genotyping test (Greiner BioOne, Germany) according to the manufacturer's instructions.

All slides were read by a second pathologist to exclude misclassification. In cases without a consensus diagnosis of primary cervical cancer, immunohistochemistry was used for the final diagnosis.

Results

Among 178 prospectively recruited patients with a referral diagnosis of cervical cancer, ten tested negative for HPV-DNA using HC2. Three of the ten HC2-negative carcinomas with a primary diagnosis of a cervical cancer at referral were reclassified with diagnoses not associated with HPV (two cases of Stage II endometrial cancer and one case of metastasis of a primary jejunum cancer), while another four cases of early cervical cancer had been completely excised by the cone biopsies prior to examination at our institutions. These cases were excluded from further analysis. Two invasive carcinomas were late Stage III-IV tumours with extended involvement of the pelvis. A detailed analysis of these two tumours has been published elsewhere [7]. In brief, immunohistochemistry was more consistent with a primary carcinoma of the ovary in one case and with bladder cancer in the other, although a primary cancer of the uterine cervix could not be ruled out completely. The remaining HC2-negative case was a FIGO Stage Ib1 adenocarcinoma of the uterine cervix. Radical hysterectomy was performed and of the 34 lymph nodes removed, none showed any involvement (case no. 1).

The majority (167/168) of cases of invasive cervical carcinoma that tested positive for HPV DNA reported intervals between first sexual intercourse and the diagnosis of cervical cancer that exceeded ten years. In one woman with FIGO Stage Ia1 microinvasive squamous cell carcinoma the interval between first sexual intercourse and the diagnosis was eight years and four months.

Among 1,034 cases of invasive cervical cancer in the MHH cancer registry we identified one case of a 14-year-old virgin with a FIGO Stage Ib1 clear cell adenocarcinoma of the cervix (case no. 2).

Out of 5,878 cases of invasive cervical cancer in Lower Saxony and Bremen we identified three further cases. Case no. 3 was an 18-year-old woman with a primary invasive Stage Ib1 endometriod adenocarcinoma of the uterine cervix, case no. 4 a 20-year-old patient with Stage Ia1 clear cell adenocarcinoma of the cervix (depth of infiltration 2 mm), and case no. 5 a 19-year-old woman with a microinvasive cervical cancer Stage Ia1 without further specifications. Another 20-year-old registered patient was excluded because the primary histology was sarcoma of the uterine cervix. All remaining 5,873 registered patients with primary cervical carcinoma were older than 20 years.

For cases no. 4 and 5 there was not sufficient tissue material available for further evaluation. Detailed DNA analysis did not reveal any HPV-DNA in the paraffin-embedded tissue blocks of the 14- and the 18-year-old patients (cases no. 2 and 3), while the HC2 negative adenocarcinoma (case no.1) was found to be HPV-18 positive in PCR analyses. To exclude poor tissue quality as a cause of false-negative HPV results, all samples were examined for the presence of ß-globin. ß-globin could be amplified in all samples examined, with the exception of one tissue block from the 14 year-old patient.

Discussion

We did not detect any truly HPV-negative cervical cancer in our prospective cohort and no case of rapid onset with a latency of less than eight years between first intercourse and a diagnosis of cancer. With one exception, all primary cervical cancers tested positive for high-risk HPV-types using HC2. This result was not expected, but is in line with a higher sensitivity of HC2 to detect CIN3 compared with PCR. This may be a consequence of the use of the full-length HPV DNA in the HC2 assay, in contrast to the limited sequence detected by PCR primers, and therefore possible vulnerability of PCR to deletions in the HPV-DNA during carcinogenesis. Another possibility is that cross-reactivity enables HC2 to detect the complete spectrum of high-risk HPV types even if they are not included in the probe cocktail.

To help place these results in context, we searched MEDLINE and ISI databases as well as selected older publications for cases of invasive cervical cancers in patients younger than 18 years and/or in virgins as well as in cases with an interval of less than eight years between first intercourse and invasive cancer. As a result of this search, we identified another 14 cases of invasive cervical cancer in virgins and/or adolescents younger than 18 years. It should however be noted that the histology results from historical data cannot be confirmed. The histological type of tumour was not stated in three cases. Only two cases were classified as a squamous cell carcinoma. The remaining nine cases were described either as adenocarcinoma without any further specification, as clear cell adenocarcinoma, or, in one case, as endometriod adenocarcinoma (Table 1). No other case was associ-

Table 1. — *Patients with cervical cancer found by a literature search: Virgin, age younger than 18 years or cervical cancer less then eight years after first intercourse* (n = 14).

Author/year of publication	Age	Histologic subtype
Glöckner 1908*	7	adenocarcinoma
Lisa 1926*	16	squamous cell carcinoma
Bonner 1927*	13	adenocarcinoma
MacDonald 1929*	10	adenocarcinoma
Morse 1930*	10	cervical cancer (no further differentiation)
Ludwig 1936*	16	adenocarcinoma
Bowing 1941 [14]	13	adenocarcinoma
Shaw 1941 [15]	15	adenocarcinoma
Stadler 1966 [16]	17	clear cell adenocarcinoma
Andrews 1978 [17]**	17	cervical cancer (no further differentiation)
Dekel 1982 [18]	15	squamous cell carcinoma
Palit 1998 [19]	9	endometrioid adenocarcinoma
Graf 1998 [20]	17	clear cell adenocarcinoma

Sorted by year of publication. *described in Bowing [14].
**Two 17-year-old patients.

ated with an interval of less than eight years after first intercourse.

The development of cervical cancer in virgins or in an interval less than eight years after first intercourse may indicate the existence of a rare type of cervical carcinoma that is not related to HPV. The association between exposure to diethylstilböstrol (DES) *in utero* and development of clear cell adenocarcinoma (CCAC) of the vagina or cervix is well known. However, Seki et al. did not find any case of DES exposure in 32 patients with CCAC (age 0-79 years) [9]. Hanselaar described ten patients younger than 18 years with CCAC of the vagina or cervix [10]. The results of this study were excluded from our research because there was no further detailed classification of the patients with cervical carcinoma. Nevertheless, the development of cervical carcinoma in a patient at the age of one year described by Seki *et al.* indicates tumour development independent of HPV.

Waggoner's and Pirog's observation of only three high-risk HPV-positive cases among 18 patients with CCAC is in line with our own findings [11, 12]. Out of a total of 16 patients with rapid onset cervical cancer from the prospective and retrospective cohort, 68% were classified as adenocarcinoma, and a subgroup of 25% were identified as CCAC, but the true prevalence of CCAC might be even greater. Only two patients had squamous cell carcinoma. It seems very likely that a substantial number of CCAC was termed adenocarcinoma while others might be found among patients without specified histology.

The accumulation of CCAC and other rare adenocarcinoma subtypes among rapid onset cancers indicates that the genesis of these cancers is different from the majority of cervical cancers.

A meta-analysis as well as a randomised trial showed that HPV testing in primary screening for cervical cancer is superior to Pap smear screening and might help to increase screening intervals for HPV negative women [13]. However, concerns that HPV testing might miss some invasive tumours could make the implementation of such a screening strategy more difficult. Our results demonstrate that neither truly HPV-negative cervical cancers nor truly rapid onset carcinomas are relevant entities for screening strategies because they are very rare and would not be detected by any currently available screening method. Furthermore, results from our prospective cohort proved that the risk of false-negative HPV results is low for HC2 and might be even lower with the next generation HPV-tests.

In conclusion, clear cell and some other rare types of adenocarcinoma of the uterine cervix seem to represent a separate entity of cervical cancer with an HPV-independent genesis. With two exceptions, all identified cases of cervical cancer that did not fit in the concept of an HPV-induced carcinogenesis with a minimal interval of seven years either belonged to this rare histological type or lacked a detailed histological classification. These rare tumours might explain occasional rapid onset and HPV-negative primary cervical cancers. It is very likely that this small tumour entity escapes all existing screening strategies based either on Pap smear or HPV testing.

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