Original Articles

Repeat conisation or HPV test? What should be done if histology of the primary conisation requires a second conisation?

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Summary

Objective: In our retrospective study we focused on the sensitivity of HPV DNA testing towards reducing the number of repeat (re)-conisations. Is the second HPV test (pre repeat conisation) an appropriate method to reduce the number of interventions in histologically positive cases? *Study:* 438 cervical conisations - loop electrosurgical excision procedure (LEEP) - were performed between March 2008 and August 2010 at our Gynaecology Department. Samples for high-risk HPV testing (Genoid, Hungary) were taken from the surface of the cervix and from the cervical canal before the LEEP procedure, and histopathological examinations were performed. Margin positivity was the indication for re-conisation (re-LEEP). *Results:* 119 (27.2%) out of 438 cases were re-conisations. In cases of histologically proven residual dysplasia (29 of 119) high-risk HPV infection was also detected by HPV testing. In 90 cases of 119 residual dysplasia was not seen by histological examination. In this high-risk group HPV infection had not been detected in 77 cases (85.5%) by the time the second HPV test was performed. HPV tests for high-risk types were positive only in 13 of 90 (14.5%) without residual dysplasia. Furthermore the same HPV type was detected only in three cases taken before the first and second conisation procedure. *Conclusion:* Pre re-conisation HPV testing might be useful in reducing the number of re-conisations where the high-risk HPV test is either negative or does not confirm the previously proven HPV type.

Key words: HPV test; Repeat conisation; Screening cervical cancer, Prevention cervical cancer; Cervical intraepithelial neoplasia.

Introduction

Cervical cancer

Cervical cancer is a significant cause of death and is – with precancerous lesions – a major cause of emotional and physical distress in women [1, 2]. Each year an estimated 500,000 new cervical cancer cases occur worldwide and 270,000 women die from the disease. The majority in the developing world but in the European Union a woman dies of cervical cancer every 18 minutes despite the well organised screening system.

Cervical screening programmes where they exist allow early detection of abnormal and precancerous cells and this might eventually lead to appropriate treatment. Most current mass screening programmes are based on Pap smear cytology assessment to detect precancerous cell changes [4, 5]. The limitations of cervical cytology, particularly in terms of sensitivity, are well known [1].

HR-HPV infection and CIN

High-risk human papilloma virus (HR-HPV) is a necessary prerequisite for most high-grade cervical intraepithelial neoplasias (CIN) and all cervical cancers (CC) [5-7].

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CIN is a very common disease especially in women of reproductive age and a balance is needed to maximise the prevention of CC and at the same time avoid over-treatment. Management strategies of CIN include decision-making regarding the appropriateness of a conservative approach versus treatment. Conservative strategies are appropriate for women with low-grade CIN, particularly in the younger age range. High-grade CIN (CIN 2 or CIN 3) should be treated. Conservative methods reduce over-treatment as low-grade CIN lesions may regress spontaneously.

When high-grade (HG) CIN is detected the treatment is mandatory. CIN 3, the true precursor of cervical cancer, will progress to cancer if left untreated at a rate of around 30% over two years [1].

CIN 1 has been reported to progress to CIN 2/3 at a rate of 15% over two years but some of these cases may harbour undetected CIN2/3 [1, 2].

Every procedure shortening the cervical canal may lead to miscarriage and preterm delivery.

Since the loop electrosurgical excission procedure (LEEP) procedure puts less burden on the patient than cold-knife conisation, the risk of preterm delivery rises due to the increasing number of LEEP interventions [3]. Recent studies have demonstrated that women who

previously suffered from CIN remain at high risk of recurrent CIN [1].

CIN will evolve again in nearly 50% of these patients. The following factors increase the risk of residual dysplasia: positive surgical margins, age, and post-treatment HPV test positivity. Opinions differ on the significance of the last element [2, 4, 8-10].

The aim of the study was to assess the second HPV test as an appropriate method to reduce the number of interventions in histologically positive cases.

Method

Study design

Four hundred and thirty-eight cervical conisation procedures were performed between March 2008 and August 2010. The age range was between 22 and 65 years. In most of the cases the indication was cytological alterations. One hundred and nineteen (27.2%) out of the 438 cases were repeat (re-) conisations. The patients who were referred for re-conisation had CIN 2 or CIN 3 histopathological results with positive surgical margins. In cases where CIN 1 was confirmed we chose conservative management independently of the status of the surgical margins. The mean age of women referred for a second conisation was 34.7 years (range 22-65 years).

In every case the LEEP was used.

LEEP conisation was performed under local anaesthesia using wire loop electrodes, with a diathermy apparatus set to 50 W for cutting and 50 W for coagulation. Generally only one specimen was removed by a single excision. All specimens were fixed with 10% buffered formalin and submitted to histopathology examination. Prior to the biopsy a HPV test was taken from the cervical canal and from the surface of the cervix. HPV samples were analysed by the Genoid ELISA-PCR method.

The spectrum HPV Detection Kit (GenoID) was used according to the instruction manual. The cervical specimen was collected in PreservCyt medium, transferred to the laboratory and after isolation of the nucleic acids by a silica-based method, multiple HPV specific PCR was carried out. The amplicon was genotyped using a hybridisation based method; the biotinylated amplicons were captured in the solid phase, and labelled genotype specific oligonucleotides were used as probes. The assay is capable of detecting virtually all mucosal HPV types and also high-risk genotypes (16,18,31,33,45,51,52,56,58,66,68).

Pathological examination verified the histological grade. The formalin fixed preparations were sliced and embedded in paraffin for histological examination. The sections were stained with hematoxylin and eosin.

Statistical analysis of the data was performed according to the chi-square test; a p value of < 0.05 was considered significant.

We observed the relationship between the second HPV tests and residual dysplasia.

Characteristics of participants

The mean age of the 119 patients who underwent re-conisation was 34.7 years. According to this we divided the patients to two subgroups: under and over 35 years.

Of the patients 56.3% were in the younger age group while 43.7% fell into the older age group. Every patient had HG CIN with positive surgical margins at the first conisation. The re-conisation was done within eight weeks.

Results

Out of 438 cases 119 (27.2%) were re-conisations. In cases of histologically proven residual dysplasia (29 of 119) HR-HPV infection was also detected by HPV testing.

In 29 patients (25.4% of the total number of re-conisation patients), where residual dysplasia was confirmed HR-HPV infection was detected in 100%.

In 90 cases of the 119 re-conisation patients (75.6% of the total number of re-conisation patients), residual dysplasia was not detected at re-conisation in spite of surgical margin positivity at the first biopsy.

In 77 out of this 90 patient cohort repeated HPV testing did not confirm any HPV infection.

In 13 out of these 90 patients HPV infection was detected repeatedly but only in three cases could we confirm the same HPV type. In these three cases the first histology proved to be severe cervical dysplasia.

In most of the 13 patients a new HPV type was detected at the second HPV test, showing a break of continuity of persistent infection relating to a previously detected HPV type (Table 2).

Where the histology revealed persistent HG-CIN the repeated HPV test detected the same HPV type as occurred the first time (64%) (Table 3).

Futhermore in those cases where re-conisation detected a lower grade of dysplasia as seen previously, a new HR-HPV type was observed (36%).

Analysing the HPV distribution we realized that HPV 16, 31 and 33 types were very common (92%) in precancerous lesions.

We analysed the HPV results of the second tests according to age distribution. We hypothesised that patient age might be a prognostic factor for residual dysplasia.

All patients who were referred for re-conisation had positive surgical margins at the first biopsy. The group was divided into two subgroups according to their age, younger or older than 35 years. Residual dysplasia was confirmed in 29 cases. There was no significant difference in occurrence of residual dysplasia between the two subgroups (below and above 35 years) by chi-square test.

Discussion

In recent years numerous publications have attempted to assess the predictive relevance of HPV status in the risk of persistent CIN [11-13]. All investigators showed a relationship between positive post-treatment HPV testing and persistent disease where surgical margins were positive.

In our study the second HPV test confirmed the same HPV type, detected before the first conisation and together with positive surgical margins were the indicators of re-conisation. According to our results there were two coherent indications for re-conisation: 1) positive surgical margin; 2) identical HPV test before and after the conisation. In these cases the existence of CIN2/3 residual dysplasia was confirmed.

Table 1. — *Residual dysplasia and HPV status correlated to age*.

Age	HPV pos. Res. dysp.: pos. (N = 29)	HPV pos. Res. dysp.: neg. (N = 13)	HPV neg. Res. dysp.: neg. (N = 77)
≤ 35 y	14	6	47
> 35 y	15	7	30

(Res dysp = residual dysplasia).

Table 2. — *Second positive HPV test without residual dysplasia*.

Cases	First HPV test	History of LLETZ	Second HPV test	History of re-LLETZ
1	HPV33	CIN3	HPV 31	Neg.
2	HPV 16	ICC Stage Ia1	HPV66	Neg.
3	HPV18	CIS (adeno)	HPV18	Neg.
4	HPV33	CIS	HPV33	Neg.
5	HPV 58, 33	CIN3	HPV59	Neg.
6	HPV16	CIN3	HPV31	Neg.
7	HPV51	CIN2	HPV59	Neg.
8	HPV16	CIN3	HPV33	Neg.
9	HPV58	CIN2	HPV52	Neg.
10	HPV 31	CIN3	HPV31	Neg.
11	HPV16,66	CIN2	HPV31	Neg.
12	HPV31	CIN2	HPV39	Neg.
13	HPV16	CIN2	HPV52	Neg.

LLETZ: Large loop conisation of the transformation zone; HPV: Human papilloma virus.

Table 3. — *Persistent HPV infection in relationship with residual dysplasia*.

Cases	First HPV test	History of LLETZ	Second HPV test	History of re-LLETZ
1	HPV16	CIN3	HPV16	CIN3
2	HPV31	CIS	HPV31	CIN3
3	HPV33,31	CIN2	HPV33	CIN2
4	HPV45	CIS	HPV45	CIN2
5	HPV16	CIN3	HPV16	CIN2
6	HPV33	CIN2	HPV33	CIN2
7	HPV16,31	CIN3	HPV16	CIN2
8	HPV16	CIN3	HPV16	CIN2
9	HPV16	CIS	HPV16	CIN3
10	HPV33	CIN2	HPV33,31	CIN2
11	HPV16	CIS	HPV16	CIN2
12	HPV18	CIS	HPV18	CIS
13	HPV31	CIN2	HPV31	CIN2
14	HPV33,16	CIN3	HPV16	CIN2
15	HPV45	CIN2	HPV45	CIN2
16	HPV33	CIN2	HPV33,16	CIN3
17	HPV16	CIN3	HPV16	CIN3
18	HPV16,52	CIN3	HPV16	CIN2

LLETZ: large loop conisation of the transformation zone.

In our present series the sensitivity of the second HPV test was 94%, and the second HPV test had a negative predictive value of 100% for detecting residual dysplasia.

Second HPV testing might be useful in reducing the number of re-conisations in those cases where HR-HPV testing is either negative or does not confirm the same HPV type as before.

These data enable us to define patient subgroups at different risks of persistent dysplasia on the basis of second HPV testing and surgical margin status in order to minimise the number of reconisation procedures. Vice versa patients with negative second HPV tests might be followed-up only, without any subsequent treatment.

However, all patients treated for HG CIN must be carefully followed-up for at least ten years because a British study revealed that the risk of developing invasive cervical cancer among these women during the following eight years is about five times higher than that of the general population [14].

In conclusion, second HPV testing might be useful in reducing the number of re-conisations in those cases where the HR-HPV test is either negative or does not confirm the same HPV type as previously.

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