Follow-up by combined cytology and human papillomavirus testing for patients post-cone biopsy: results of a long-term follow-up

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Abstract

Objective. The goal of this study was to evaluate the clinical implications of integrating human papillomavirus (HPV) testing into a long-term follow-up and management protocol for women postconization for high-grade cervical intraepithelial neoplasia (CIN2–3).

Methods. Sixty-seven women were followed-up by Pap smears and HPV type and load testing (mean follow-up, 63 months; range, 50–72). Patients with persistent abnormal cytology on two consecutive smears and those with positive HPV test results (whatever their cytologic findings) were referred for colposcopy-directed biopsy. Patients histologically diagnosed with CIN2–3 and those with high-load HPV (whatever their histologic findings) underwent repeat conization or hysterectomy for residual disease.

Results. At follow-up, 29 (43.2%) women had positive cytology or positive HPV results and were referred for colposcopy. Eleven (37.9%) had high-grade cervical intraepithelial neoplasia or high-load HPV results and were further treated by reconization/hysterectomy. The respective positive predictive values of high-load HPV and low-grade squamous intraepithelial lesions were 100 and 60% for any CIN and 90 and 15% for CIN2–3. Only five of nine cases with a final diagnosis of CIN2–3 were originally identified by cytology: the other four were detected only by parallel evaluation by HPV testing. High-load HPV results with normal cytology or low-grade lesions harbored an 80% risk for CIN2–3.

Conclusion. Adding HPV load assessment to the follow-up protocol of women postconization due to CIN2–3 lesions could help detect high-grade residual disease among low-grade lesions and normal cytology cases while concomitantly and safely bestowing the advantage of lowering the rates of colposcopic referrals and surgical procedures.

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Introduction

The main objective in the follow-up of patients post-cone biopsy due to high-grade cervical intraepithelial lesions (CIN2–3) is the early diagnosis of residual or recurrent cervical disease because of the significant risk of developing invasive carcinoma if treatment is not administered [1]. To achieve this aim, several issues relating to the practical aspects of the follow-up methodology need to be addressed: (1) What should be the preferred method of triaging patients for colposcopic evaluation: cytology alone, with its inherent false-negative and -positive rates [2], or combined cytology and HPV testing [3]? (2) Where should the follow-up take place, e.g., in a hospital or a cervical pathology unit, which are highly professional but more costly, or as an office procedure by a general practitioner or community gynecologist? (3) What should be the intervals between follow-up visits and the length of the follow-up period.

In the present prospective study, we attempted to resolve some of these issues by analyzing the results of a follow-up program longer than 5 years. The follow-up protocol was based on the combination of cytology and high-risk HPV DNA tests to triage for colposcopic examination. The histology of the colposcopy-directed biopsies and the load of the associated high-risk HPV DNA tests were used for the second triage for the presence of residual disease.
Materials and methods

Between January 1993 and December 1994, 72 consecutive patients with CIN2–3 lesions underwent cone biopsies at the Cervical Pathology Unit of the Tel-Aviv Sourasky Medical Center. Five patients were eventually excluded (2 failed to attend all follow-up visits and 3 were lost to follow-up), leaving a cohort of 67 patients who attended all the follow-up visits and for whom all cytology, histology, and HPV data were available. The follow-up visits took place at 6-month intervals during the first 3 years and then annually for another 2 years. Both a Pap smear and high-risk HPV type and load testing were carried out each time. The study was closed in January 2000.

Referrals for colposcopy and colposcopy-directed punch biopsies were based on the following criteria: (1) persistent abnormal cytology, i.e., high-grade squamous intraepithelial lesion (HSIL) or low-grade squamous intraepithelial lesion (LSIL), on two consecutive Pap smears, and (2) positive HPV test results whatever the cytology report.

Referral criteria for evaluating the presence of residual/recurrent disease were based on the histology of colposcopic biopsy or a high-load HPV (HLHPV) report whatever the biopsy result. Re-cone biopsy was chosen when fertility was to be preserved, while hysterectomy was carried out in women who had completed their family planning.

The standard cone biopsy consisted of colposcopy-guided loop excision of the transformation zone (LETZ) followed by laser vaporization of the crater base and side walls, as described in detail elsewhere [4].

We used the type I Hybrid Capture Test System (Digene Corp., Silver Spring, MD, USA). Samples for the presence of a cocktail limited to high-risk HPV types 16, 18, 31, 33, 35, 45, 51, 52, and 56 were collected from the external cervical os and the exocervix with a special brush applicator and transferred to the lab in a container with transfer medium provided in a specific kit by Digene. In the type I Hybrid Capture assay, light is emitted during cleavage of the tested substrate. The intensity of the emitted light is proportional to the amount of DNA present in the examined specimen. A relative light unit (RLU) measurement greater than or equal to the cutoff value indicates the presence of HPV DNA, whereas a RLU measurement less than the cutoff value indicates the absence of an HPV DNA sequence. Based on the above criteria, the following scale of HPV load was used: RLU < 0.3 unit = a negative HPV test, RLU between 0.4 and 3 units = borderline test (BLHPV), RLU between 4 and 9 units = low-load HPV DNA test (LLHPV), and RLU > 10 = high-load HPV DNA test report.

Statistical analysis

Significant differences in proportions were assessed by Fisher’s exact test. All statistical analyses were performed using SPSS for Windows Version 8.0 (SPSS Inc, Chicago, IL, USA).

Results

Sixty-seven consecutive patients with CIN2–3 lesions who had undergone cone biopsies were studied. Their mean age was 38.8 (range, 17–54). The mean follow-up period was 63 months (range, 50–72). The mean interval between initial cone biopsy and the second surgical intervention was 13 months (range, 11–25). The correlation between cytology and high-risk HPV test report (Table 1) revealed that 40 patients (59.7%) had negative HPV results, among them 2 patients with LSIL reports. Twenty-seven patients (40.3%) had positive HPV results, among them 4 patients (5.9%) with normal cytology (2 had HLHPV reports) and 18 patients (26.8%) with LSILs (3 had HLHPV reports). Five patients (7.4%) had HSILs (all had HLHPV reports).

In 6 cases there was a discrepancy between the cytology results and the HPV load evaluation; i.e., 2 had LSILs with negative HPV findings and 4 had normal cytology with a positive HPV load (Table 1).

Based on the follow-up protocol, the 27 patients with positive HPV and the 2 with LSILs and negative HPV were referred for colposcopic evaluation. Correlations between the histology reports of the colposcopy-directed punch biopsies and the HPV DNA load tests of the above 29 patients are presented in Table 2. Ten of the twenty-nine patients referred for colposcopy were negative for pathologic evidence of dysplasia (PVV 65.5), including the 2 patients with LSILs associated with negative HPV results, and 8 of the other 18 patients with LSILs and positive HPV results (2 with BLHPV and 6 with LLHPV reports). There were two patients with CIN1 who had HLHPV reports and one patient with CIN2–3 who had an LLHPV report. Eight of the nine patients with CIN2–3 had HLHPV reports, while none of the high-grade lesions were associated with a negative high-risk HPV report. Moreover, 8 of the 27 patients who were
positive for high-risk HPV did not have pathology (PPV 70.3).

The correlation between the histology of the 11 patients referred for the presence of residual disease is presented in Table 3. Nine patients underwent repeat cone biopsy and two underwent hysterectomy. Of the 2 patients referred with CIN1, cone biopsy confirmed CIN1 associated with LLHPV in one, while the other was found to have CIN2–3 associated with HLHPV lesions. The single case referred with CIN2–3 and an LLHPV report (Table 2) was found to have CIN1 residual disease. Two of the ten patients with high levels of virus had only CIN1 (Table 2) but one was later found to have CIN2–3 residual disease on reconization (Table 3).

The rate of clearance of the HPV DNA throughout the follow-up period is presented in Fig. 1. There was a steady decrease in the number of patients harboring HLHPV DNA during the first 24 months. Only cases of BLHPV and LLHPV had positive HPV reports after 2 years of follow-up. The single persistent case of positive HPV was a woman who had a BLHPV report and negative cytology and who underwent endocervical curettage and colposcopic tests. While the high-load cases were treated after a mean interval of 13 months (range, 11–25), most of the low-load cases were treated expectantly by follow-up alone, meaning that their clearance rate represents spontaneous recovery.

Discussion

The objective of the current study was to assess the efficacy of the proposed protocol in identifying patients at high risk of developing recurrent invasive disease. In conducting such a protocol, several issues relating to practical aspects of the follow-up methodology arose and we attempted to address each of them.

The need for another test in addition to cytology

Physicians tend to repeat low-grade cytological tests at various intervals (e.g., immediately, every 3 or 6 months, etc.) to reduce the inherent false-negative rates of Pap tests. Since repeated smears do not improve the sensitivity and specificity of cytology [5], an additional test, such as HPV DNA, was used as a measure of safety [6]. It should be noted that the positive predictive value (PPV) of high-grade cytology (HSIL) for the presence of high-risk residual or recurrent disease is usually high [7]. The PPV of HSIL in the present study was 100%, which is similar to values reported by others [8], and this makes the addition of HPV testing pointless in such circumstances. However, this is not the case for LSIL reports which are associated with relatively high false-negative and false-positive rates [9]. Moreover, the PPV was low: it was 50% (10/20) for any CIN, 45% (9/20) for CIN1, and 15% (1/20) for CIN2–3 in the present study which is similar to that of other reports [8,10].

The contribution of HPV testing

In the present study, the main objective of adding HPV testing to cytology was for it to serve as an extra safety measure by increasing the sensitivity of triaging patients for colposcopy at the first stage of follow-up. It later enabled a safe reduction in the number of patients referred for reconization/hysterectomy procedures. For example, the two
patients with normal cytology and the three patients with LSILs, all with HLHPV reports (Table 1), would be classified in most protocols as low-risk cases and would have been referred for further cytology follow-up only. Adding HPV testing to cytology enabled us to identify these five patients as harboring HLHPV; it classified them as high-risk patients and led to their referral for further evaluation procedures that revealed CIN2–3 residual/recurrent disease in four of these five cases (Tables 2, 3). In addition, by concentrating on the HPV load, it was possible to differentiate between the patients who were at a low or high risk for developing CIN2–3 lesions (the 18 patients with either normal or CIN1 lesions who had negative, BLHPV, or LLHPV reports from the 11 patients with CIN1 and CIN2–3 who had LLHPV [n = 1] and HLHPV [n = 10] (Table 2). That feature enabled us to spare 62% (18/29) of the low-risk patients from further unnecessary surgical procedures.

The sensitivity and specificity of the HLHPV result were high (100 and 95%, respectively): 9 of 11 referrals were found to have high-grade residual/recurrent disease (Table 3). The PPV of HLHPV to CIN2–3 was 90% (9/10) and the PPV of HLHPV in the presence of normal or LSIL cytology was 80% (4/5). The negative predictive value of HLHPV to CIN2–3 was 100%.

**Rate of clearance of HPV**

The impact of high-risk HPV DNA concentration on cervical tissue has recently been discussed by Sun et al. [11], who demonstrated the potential influence of viral load on the size and severity of cervical lesions. The effect of the duration of high load of high-risk HPV infection on the development of cervical carcinoma in situ was demonstrated by Yitalo et al. [12]. Both groups of investigators concluded that women at high risk could be identified by the use of a quantitative HPV test in addition to cytology. In the present study, all cases of high-grade residual disease had HLHPV reports, and there was not a single case of high-grade residual lesion with a negative HLHPV test. Most of the reconization/hysterectomy procedures took place within the first 12–24 months of the follow-up period, which coincides with the major time of clearance of HLHPV cases.
HPV and the status of the patients

HPV, in addition to other factors such as the virulence of the curves in Fig. 1. It is also possible that the reason there have affected the total numbers and clearing rates of each of HLHPV to LLHPV and BLHPV reports, and this would (Fig. 1). It is possible that there had been a shift from recurrent disease and that HLHPV cleared within the first 24 months may indicate that the first 2 years represent a critical time frame and that a more intensive monitoring protocol should be implemented during that time. The small numbers in our study cohort preclude our speculating on an optimal length of follow-up for our high-risk patients (post-cone biopsies due to CIN2–3).

Given that colposcopy is an integral tool in the follow-up of these patients within these first 3 years, the follow-up during that period might best be carried out by trained cervical pathology personal in hospitals or special cervical pathology units. We recommend that high-risk patients should be kept in that setting for at least 3 to 5 years and then join the low-risk patients in follow-up by cytology alone in the community.

Duration and location of conducting the proposed follow-up

The observations in the current study that only patients with HLHPV were at high risk of developing residual or recurrent disease and that HLHPV cleared within the first 24 months may indicate that the first 2 years represent a critical time frame and that a more intensive monitoring protocol should be implemented during that time. The small numbers in our study cohort preclude our speculating on an optimal length of follow-up for our high-risk patients (post-cone biopsies due to CIN2–3).

Rationale for the proposed follow-up protocol

We demonstrated that HPV testing appears to be an accurate and sensitive tool for differentiating between low- and high-risk patients post-cone biopsy. The presence of the following parameters should be considered as defining “high risk” for predicting high-grade residual/recurrent disease and be used in the triage for colposcopic evaluation: a cytologic report of HSIL whatever the HPV status, a cytologic report of LSIL with an HLHPV report, a diagnosis of CIN1 and an HLHPV report, and a diagnosis of CIN2–3 whatever the HPV status. The flowchart of the suggested follow-up in Fig. 2 is based on these considerations.

According to our findings, we recommend adding HPV load testing to the follow-up protocols of women post-conization due to CIN2–3 disease because of its very high positive predictive values. HPV load assessment will be of benefit in identifying undetected high-grade residual disease in cases with LSILs and normal cytology while lowering the numbers of referrals for colposcopy and surgical procedures.

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References