Value of 3q26 Gain in the Triage of Low-grade Squamous Abnormalities

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ABSTRACT

Introduction: Approximately 10% of patients diagnosed with low-grade squamous intraepithelial lesion (LSIL) will progress to high-grade squamous intraepithelial lesion (HSIL), 60% will regress to normal and 30% will remain. Deciding which subset of patients with LSIL who would benefit from more aggressive follow up, i.e. colposcopy/biopsy, is the key. It has been postulated that the gain of 3q26 is associated with LSIL progression to cervical intraepithelial neoplasia (CIN) 2/3 and invasive squamous carcinoma. The purpose of this study is to correlate high-risk (hr) human papillomavirus (HPV) viral load (VL) results and histologic follow up with the status of 3q26 gain in patients with LSIL and ASC-US (hrHPV positive [+]) Pap test interpretations.

Materials and Methods: Between October 2011 and September 2012, 80 patients who had an index cytologic interpretation of LSIL or ASCUS-hrHPV+ were tested for gain in 3q26. Only ThinPrep Pap tests (Hologic, Marlboro MA) were included in the current study. hrHPV status (by hybrid capture (HC) II; Qiagen, Germantown MD) was available in 48 patients; hrHPV viral load results were reported as very low, low, moderate, and high. Additional ThinPrep slides were prepared and tested for the chromosome 3q26 region using Oncofish (Ikonisys, New Haven, CT), an automated qualitative fluorescent in situ hybridization (FISH) test. hrHPV DNA viral load data, 3q26 FISH results and histologic cervical biopsy data were reviewed.

Results: The average age of the patients in this study was 35 years (range 17-76 years). The majority 71/80 (89%) of the cases in this study were negative for 3q26 gain. Of the 3q26 gain negative cases with hrHPV data, 38 were hrHPV positive (38/42; 90%), and half of these had a moderate or high viral load (**Table 1**). Additionally, the majority of 3q26 gain positive cases with hrHPV data were positive for hrHPV (5/6, 83%). All patients with available follow up data who tested positive for 3q26 gain were found to have CIN 1 or 2+ on biopsy (4/4; **Table 2**). On the other hand, 6 out of 24 (25%) patients with available follow up who tested negative for 3q26 gain had CIN 2+ on subsequent biopsy (**Table 2**).

Conclusion: A positive 3q26 gain is associated with hrHPV positivity; however, there is no correlation between viral load and 3q26 status. In addition, a lack of 3q26 gain as determined by FISH did not predict the absence of CIN 2+ on subsequent follow up. Further study with greater numbers will help elucidate the role of this test in the management of low grade squamous abnormalities.

INTRODUCTION

Cervical cancer is the second most common cancer in women worldwide, the most common type being squamous cell carcinoma. Infections with high-risk (hr) human papillomavirus (HPV) appear to be an important factor in the etiology of cervical cancer with virtually 80% of cervical cancer being caused by specific types of HPV. Persistent infection of the cervix with HPV is necessary, although not sufficient, to cause cervical neoplasia. However, not all persistent infections progress to high-grade lesions, and not all high-grade lesions develop into cancer. Persistence of HPV in women who do not develop dysplasia or carcinoma suggests that additional genetic aberrations are required for tumor initiation and progression, and genomic integration of hrHPV appears to be an important genetic event in the progression of cervical dysplasia to invasive cancer (Hopman 2006).

In addition to infection with HPV, the majority of cervical carcinomas carry extra copies of the long arm of chromosome 3, in particular cytoband 3q26 which contains sequences for the *human telomerase RNA* gene (*hTERC*). Studies have demonstrated that progression is never observed in the absence of genomic amplification and inversely, extra copies of this gene are not present in lesions that spontaneously regress, suggesting that the detection of gain and amplification of 3q26 as an adjunct biomarker to cytologic screening can assist in identifying ASC-US or LSIL with a high progression risk (Heselmeyer 2005).

At population-based screening, management of women whose Pap test shows mild cellular abnormalities such as ASC-US and LSIL is potentially of concern since CIN2 or 3 or even carcinoma may be present at biopsy. Testing for hrHPV DNA as a technique for identifying women with equivocal cytology who require immediate follow-up has been endorsed but the clinical utility of viral load on histologic severity remains debated. It has been shown that the HPV viral load in ASCUS and LSIL cases significantly correlates with the probability of CIN 2 on cervical biopsies, however the correlation between viral load and amplification of 3q26 is unclear.

The purpose of this study is to correlate high-risk (hr) human papillomavirus (HPV) results, hrHPV viral load data, and histologic follow up with the status of 3q26 gain in patients with LSIL and ASC-US (hrHPV positive [+]) Pap test interpretations.

MATERIALS & METHODS

Eighty cases categorized as LSIL or ASCUS-hrHPV+ on liquid-based preparations from cervicovaginal specimens and tested for gain in 3q26 were identified in the pathology database at Yale New Haven Hospital between October 2011 and September 2012. Only ThinPrep Pap tests (Hologic, Marlboro MA) were included in the current study. hrHPV status (by hybrid capture (HC) II; Qiagen, Germantown MD) was available in 48 patients; hrHPV viral load results were reported as very low (V low), low (L), moderate (M), and high.

Additional cervicovaginal ThinPrep slides were prepared and tested for the chromosome 3q26 region using OncoFISH cervical probe kit (Ikonisys, New Haven, CT). After hybridization, the slides were analyzed using an automated system which uses digital microscopy to analyze hundreds of nuclei. It assesses amplification of the 3q26 region containing *hTERC* by use of of two FISH probes, one for the 3q26 locus and a control probe, centromeric chromosome 7. Enumeration and comparison of the 3q26 and control probes, in conjunction with the nuclear morphology, result in a 3q copy number for each of the nuclei analyzed and relative 3q gain (as defined by higher copy number of 3q compared to the control probe CEP7).

hrHPV DNA test results, viral load data, 3q26 FISH results and follow-up histologic cervical biopsy data were reviewed.

RESULTS

Table 1. Correlation Between hrHPV Viral Load and 3q26 Gain Results

3q gain	HPV results				Total
	Negative	V low/Low VL	Mod/High VL	Total Positive	
Positive	1 (17%)	3	2	5 (83%)	6
Negative	4 (10%)	19	19	38 (90%)	42

Table 2. Correlation Between 3q26 Gain Results and Histologic Follow-up

3q gain	Surgical Follow Up				Total
	Negative	CIN 1	CIN 2+	No FU	
Positive	0	1	3	5	9
Negative	7	11	6	47	71

RESULTS

In order to examine the data statistically using the cases with surgical follow-up, we divided the patients into two cohorts: those with CIN1 or negative biopsy results, versus those with CIN2 or higher on biopsy. In looking at the two cohorts as divided above, 3q26 gain as a test to predict follow up as above was 33% sensitive (95% CI 9%-69%), and 95% specific (95% CI 72%-100%) with a PPV and NPV of 75% (95% CI 22%-99%; 95% CI 53%-89%). The likelihood ratios for a positive 3q26 gain result was 6.3, and for a negative 3q26 gain result was 0.7 (Fisher exact test, p=0.0131)

CONCLUSIONS

- A positive 3q26 gain in our patient group and CIN2+ on follow up are associated with hrHPV, but not with viral load.
- A positive 3q26 gain is associated with CIN2+ on follow up.
- A negative 3q26 gain does not predict the absence of CIN2+.

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