Cervical FISH Testing for Triage and Support of Challenging Diagnoses: A Case Study of 2 Patients

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CLINICAL HISTORY

Patients: A 29-year-old Caucasian woman (patient 1) and a 23-year-old Caucasian woman (patient 2).

Chief Complaints: Abnormal Pap tests with high risk HPV positivity.

History of Present Illness

Patient 1: In June 2014, this patient was diagnosed with atypical squamous cells of uncertain significance (ASCUS) after a routine screening cervical Papanicolaou (Pap) test. Reflex high risk human papilloma virus (HPV) testing was performed; the results were positive (genotyping was not performed). Subsequent endocervical curettage showed a small focus of immature, atypical squamous cells with abnormally positive p16 staining and an abnormally increased proliferative index staining pattern (evaluated with Ki-67) (**Images 1A-1C**). These findings strongly suggested a high-grade lesion; nevertheless, due to the minute and focal nature of these findings, a diagnosis was rendered of squamous dysplasia, cannot exclude high grade dysplasia. Additional follow-up was recommended, and the option of a fluorescent in situ hybridization (FISH) assay (HPV-4C, using reagent manufactured by Cancer Genetics Italia S.r.l.) was also suggested as a method of triage to be performed using the same Thinprep collection media used to create the Pap test. The results of the FISH assay were positive, with 6.6% of cells showing gain of the 3q26 region (**Image 1D**). With this knowledge, the gynecologist performed a cervical loop electrosurgical excision procedure (LEEP), which revealed moderate squamous dysplasia (cervical intraepithelial neoplasia grade 2 [CIN 2]) supported by strong and abnormal p16/Ki-67 co-expression (**Images 1E-1G**).

Patient 2: In June 2013, this patient was diagnosed with atypical glandular cells (AGUS) and was shown to have high-risk human papillomavirus (HPV) positivity. (Again, genotyping was not performed.) The subsequent biopsy showed mild reactive atypia of the glandular cells, which did not completely correlate with the atypical cells revealed by the Papanicolaou (Pap) test. Therefore, further follow-up was recommended. A fluorescent in situ hybridization (FISH) assay was performed on the specimen assayed via the AGUS Pap test; the FISH assay yielded positive results, showing many cells with a gain of 3q26 and 5p15 regions above the established cutoff values. A repeat Pap test was performed, which also was interpreted as indicating AGUS. Results of a second HPV-4C FISH assay showed numerous (14.6%) cells with a gain of 3q26 and 5p15 regions (**Image 2A**). Repeat cervical and endocervical biopsies showed scant atypical glands in otherwise-generous biopsies (**Image 2B**). Supported by abnormal p16 and Ki-67 immunohistochemical staining results (**Image 2C** and **2D**) and the knowledge of the abnormal FISH assay results, the pathologist diagnosed the patient with endocervical adenocarcinoma in situ (AIS). The results of a subsequent LEEP confirmed the diagnosis of endocervical adenocarcinoma in situ with negative resection margins.

Laboratory Findings: Abnormal Papanicolaou (Pap) test results with high risk of human papilloma virus (HPV) positivity and scant lesional tissue, as revealed by cervical/endocervical biopsies.

Keywords: gynecologic pathology, cervical cancer, squamous-cell carcinoma, adenocarcinoma, cytology, histology, FISH

Abbreviations

Pap, Papanicolaou; HPV, human papilloma virus; ASCUS, atypical squamous cells of uncertain significance; FISH,fluorescent in situ hybridization; LEEP, loop electrosurgical excision procedure; CIN 2, cervical intraepithelial neoplasia grade 2; AGUS, atypical glandular cells; H&E, hematoxylin-eosin; HSIL, high-grade squamous intraepithelial lesion; mRNA, messenger RNA

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Questions

1. What are the limitations of current cervical cancer screening methods?
2. How does fluorescent in situ hybridization (FISH) testing represent a possible solution to these problems?
3. Is fluorescent in situ hybridization (FISH) testing useful in the diagnosis of adenocarcinoma of the cervix?

Possible Answers

1. The diagnosis of premalignant cervical lesions can be challenging. Although the role of the Papanicolaou (Pap) test in cervical cancer screening may represent the most successful cancer prevention program ever implemented, its sensitivity may be limited (especially regarding some high-grade squamous lesions, or glandular lesions of the endocervix). For example, cervical cytologic testing is approximately 55% sensitive for moderate squamous dysplasia/cervical intraepithelial neoplasia grade 2 (CIN 2).[1,2] The addition of high-risk human papilloma virus (HPV) testing increases the sensitivity of cervical cancer screening; however, this test is nonspecific for the presence of cervical dysplasia. The addition of genotyping for types 16 and 18/45 is recommended by screening guidelines to assist in triage of patients with HPV positivity who have negative cytologic abnormalities. However, not all patients with types 16, 18, or 45 will experience progression to cervical cancer (17% of patients with HPV type 16 will experience progression to cervical cancer during a 10-year period, compared with 13% who have types 18/45 and 3% who have other high-risk HPV types).[3,4] Also, some studies[5] have shown that amongst women in certain populations (African American, Hispanic, and patients of lower socioeconomic status), high-grade squamous lesions are less likely to be associated with the high-risk HPV types 16 and 18/45.

Persistently negative Pap cytology results, which reveal positivity for high-risk HPV, may be vexing for the health care professional, worrisome for the patient, and often followed up by HPV genotyping or repeat cytology in 1 year. This may mean unnecessary concern on behalf of the patient or a yearlong delay in the treatment of a high-grade lesion.

Hematoxylin-eosin (H&E) examination of tissue biopsies of the cervix and endocervical canal represents the gold standard for the assessment of cervical disease. However, based on experience gained in a busy gynecologic pathology practice, microscopic interpretation of the biopsies may be subject to sampling bias, scant volume, and technical difficulties such as tangential sectioning or biopsy artifact. Such limitations, even with the usage of ancillary studies (eg, p16, which is a biomarker for the presence of high-risk HPV, and Ki-67, a proliferative index stain) may lead to a less-than-satisfying diagnosis for the health care professional and pathologist. Although ambiguous diagnoses are rarely made, they are sometimes unavoidable and are often delivered with the recommendation to repeat the biopsy or to closely follow the patient using other methods (such as cytologic and/or HPV testing.)

Finally, squamous dysplasia is a moving target in that it may regress. A total of 40% of cases of CIN 2 may regress during a 2 year period, even without treatment.[6] This means that the affected patients may have a history of a high-grade cytology results (high-grade squamous intraepithelial lesion [HSIL]) and subsequent negative biopsy results (due to the regression of disease), which can be troubling for the health care professional and the patient. Also, even with biopsy confirmation of high-grade cervical disease, these lesions may then subsequently regress, meaning that the patient may be overtreated with cervical excision in the interim.

2. The limitations discussed in possible answer 1 show unmet prognostic/predictive and diagnostic needs in the process of cervical-cancer screening. Recently, human papilloma virus (HPV) testing has evolved, allowing evaluation for the presence of HPV messenger RNA (mRNA), rather than HPV DNA, the former of which looks for the presence of the oncogenic proteins E6 and E7 produced during integration of HPV DNA into the host genome. These tests may be more specific for a clinically meaningful, integrated HPV infection[7] (rather than merely the presence of transient HPV DNA). However, the presence of HPV mRNA does not guarantee progression to a high-grade squamous (or glandular) lesion. Therefore, a test that could help triage challenging clinical scenarios such as those described earlier herein would be desirable to guide treatment by healthcare professionals and to support the diagnoses made by pathologists.

HPV infection is only the first step of many in the development of cervical cancer. Advancement to a higher level of disease (ultimately becoming an invasive carcinoma) requires persistence of the infection; integration of the viral DNA into the host genome; and overexpression of the oncogenic proteins E6/E7, which then subvert normal cell-cycle control mechanisms and cause genomic instability, leading to further tumor development. Using FISH technology to assess for genomic instability in the form of nonrandom chromosomal abnormalities may provide the solution to challenging clinical settings. For example, these tests may serve as a next-level mode of triage after an atypical cytologic testing result or may support a difficult diagnosis based on scant tissue, as evidenced in the patient examples outlined herein.

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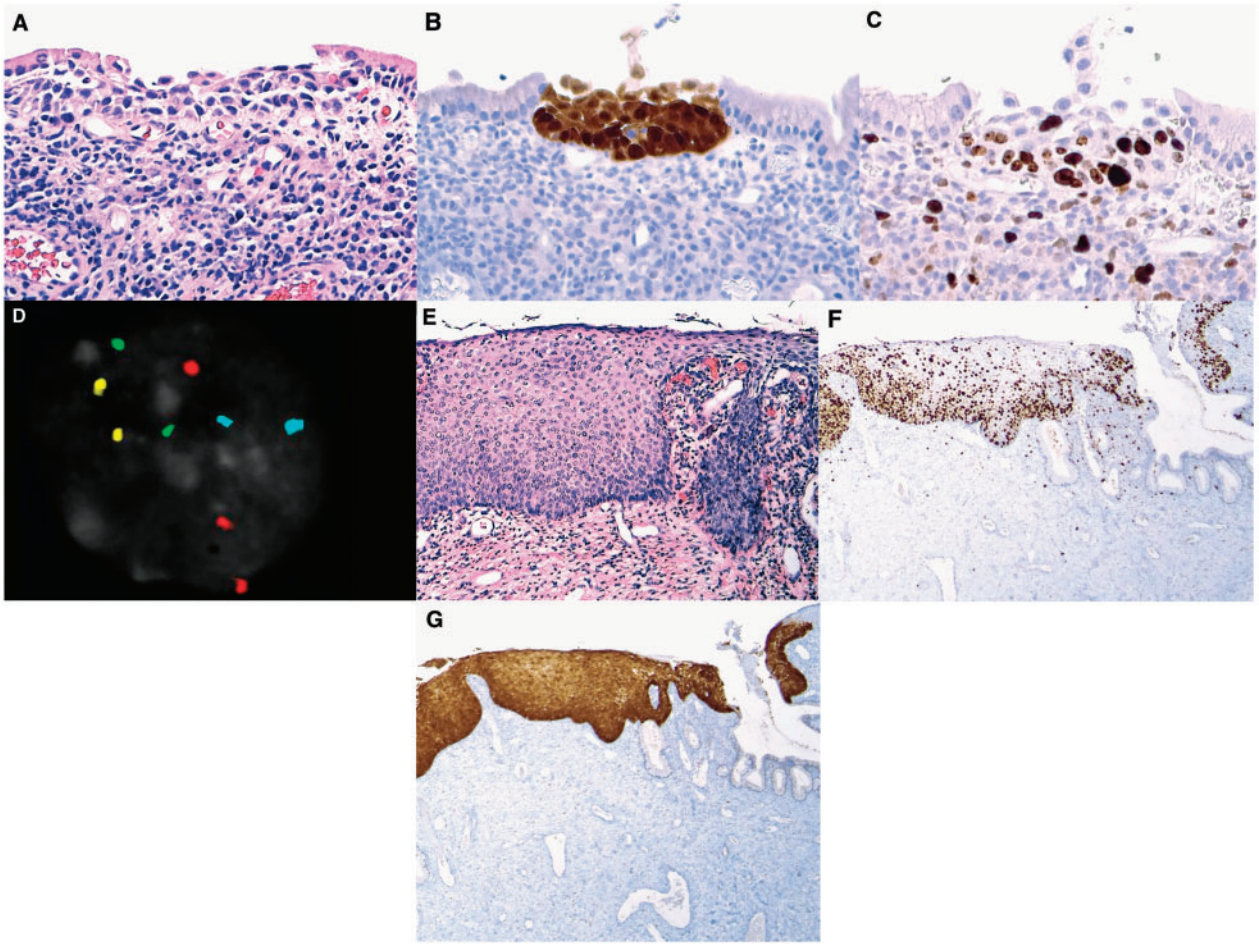

Image 1

Results of testing of several types of cells in patient 1, a 29 year old Caucasian woman. **A**, Small focus of atypical squamous metaplasia in endocervical cells collected via curettage (H&E; original magnification ×40). **B** and **C**, Abnormally strong p16 staining and increased Ki-67 co-expression of endocervical cells. **D**, Gain of 3q26 region (3 red signals) in fluorescent in situ hybridization (FISH) analysis (FISH DNA probes for 3q, 5p, 20q and Centromere 7; original magnification ×60). **E**, Subsequent loop electrosurgical excision procedure (LEEP) showed numerous regions of moderate dysplasia (cervical intraepithelial neoplasia grade 2 [CIN 2]) [H&E; original magnification ×20]). **F**, Immunohistochemical staining of loop electrosurgical excision procedure (LEEP); diffuse p16 positivity (p16 IHC stain; original magnification ×10). **G**, Endocervical cells with increased Ki-67 co-expression (Ki-67 IHC stain; original magnification ×10).

Numerous nonrandom chromosomal abnormalities are associated with the development of cervical carcinoma. Colloquially, some of the most common include gain of 1q, 3q, 5p, 8q, and 20q regions; gain of 1p, 9q, 15q, 17q, and 19q regions is observed less frequently. Typically, chromosomal losses are also observed, although gains are more easily assessed with FISH than losses due to artificial loss of a target with overlapping probe signals or other artifacts of analysis. The selection of the regions 3q, 5p, 20q and centromere 7 as targets in this test is based on the most frequent gains across numerous cases of HPV-associated carcinomas.[8]

3. Cervical adenocarcinomas make up 20% of all cervical cancers. The incidence of cervical adenocarcinoma is increasing, for unknown reasons. Cervical cancer screening

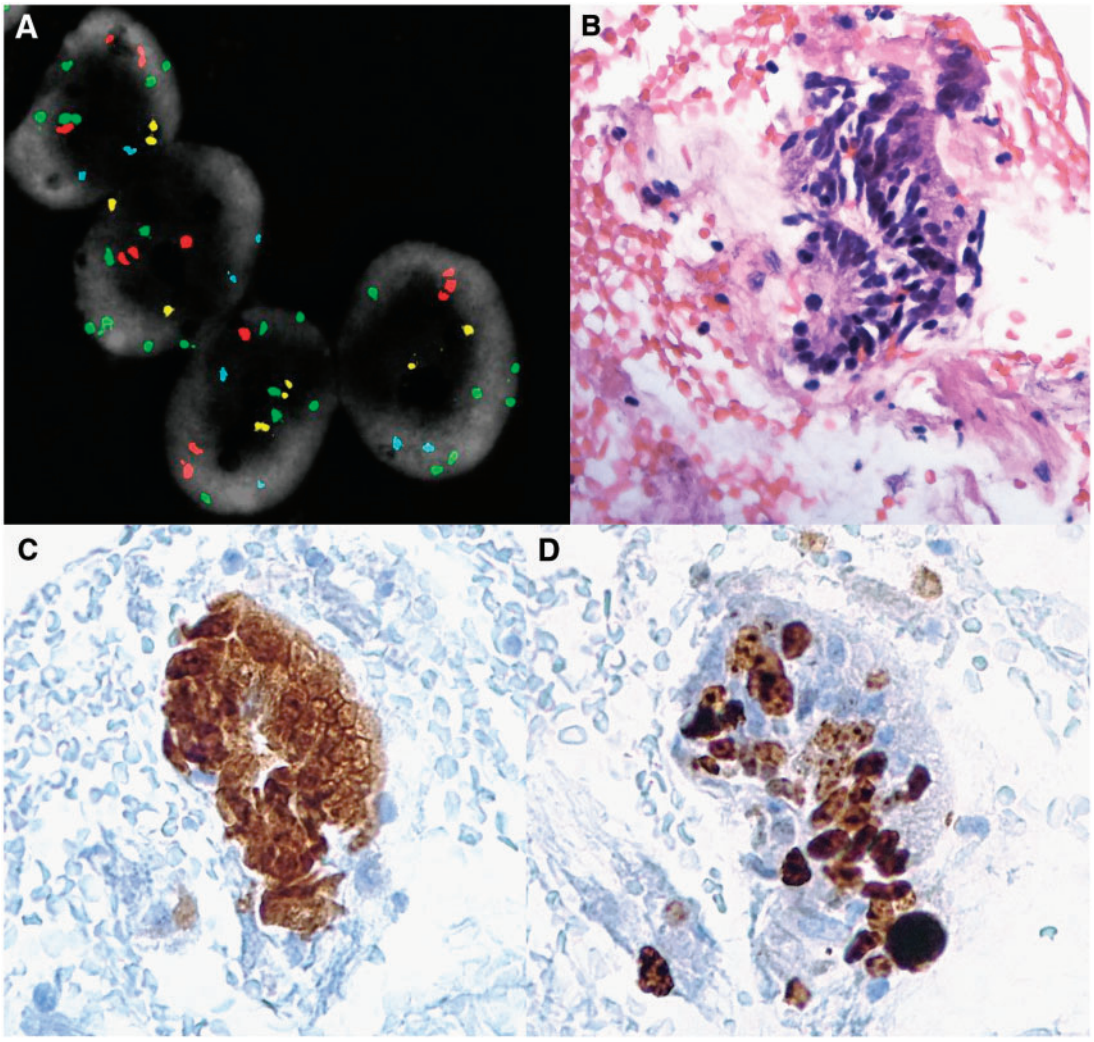

Image 2

Results of testing of several types of cells in patient 2, a 23 year old Caucasian woman. **A**, Four endocervical glandular cells, all with gain of 3q26 (red) and 5p15 (green) (FISH DNA probes for 3q, 5p, 20q and Centromere 7; original magnification ×60). **B**, Scant small strips of atypical endocervical glandular cells with enlarged, hyperchromatic nuclei and coarse chromatin (H&E; original magnification ×40). **C**, Strong p16 staining in the atypical glandular cells (p16 IHC stain; original magnification ×40). **D**, Increased Ki-67 co-expression Ki-67 IHC, magnification ×40 in the endocervical glandular cells, consistent with endocervical adenocarcinoma.

programs have had a significantly greater effect on cervical squamous carcinomas than adenocarcinomas. This result may be occurring because glandular neoplasia is more difficult to identify morphologically on cervical Papanicolaou (Pap) tests and because it is more difficult to obtain specimen material from the endocervical canal (in terms of anatomical location and the fact that glandular neoplasia may exist deep within endocervical crypts.) Human papilloma virus (HPV) DNA has been identified in a wide range of cervical adenocarcinomas (30% to 90%), unlike squamous-cell carcinomas, of which 99% of cases are associated with HPV.[9-14] HPV type 18 is the principal type associated with cervical adenocarcinomas (type 16 is the most predominant in squamous-cell carcinomas.)

A study[15] examined 12 cervical adenocarcinomas (8 of which tested positive for high-risk HPV); all 12 showed gains in 3q26. This finding suggests that amplification of the *TERC*

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gene, a biomarker for advanced cervical disease, has mechanisms of development in cervical adenocarcinomas that are similar to those found in high-grade squamous lesions (even in adenocarcinomas, which tested negative for high-risk HPV, in this study).

In summary, the HPV-4C assay, which uses the 4 probes 3q26, 5p15, 20q, and centromere 7, designed by Cancer Genetics Inc, has the ability to identify abnormal gains of 4 of the most common nonrandom chromosomal abnormalities observed in the development of cervical cancer (squamous and endocervical glandular types). Using this test can serve as an additional and more specific personalized method of triage in challenging clinical scenarios, particularly when histologic or cytologic testing provide unclear results.

Conflict of Interest

This FISH test (HPV-4C) is offered by PathAdvantage, with probes and reagents obtained from Cancer Genetics Italia S.r.l.

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