Interobserver Variability in Human Papillomavirus Test Results in Cervicovaginal Cytologic Specimens Interpreted as Atypical Squamous Cells

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Abstract

We studied interobserver variability in the proportions of human papillomavirus (HPV)-positive results for atypical squamous cells of undetermined significance (ASCUS) and atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H) diagnoses among 5 pathologists from the same laboratory during a 2-year period. These proportions were compared with individual pathologist’s ASCUS/squamous intraepithelial lesion (SIL) ratios.

Of 1,299 ASCUS diagnoses, 32.3% had HPV testing; 49.4% were HPV+. Positive findings by individual pathologists ranged from 38% to 67% (P = .057). There was a difference in the proportions of high-risk HPV results for individual pathologists (P < .001). For the pathologist who diagnosed 38% (23/61) of samples as HPV+, the ASCUS/SIL was 0.58; the pathologist who diagnosed 67% (28/42) as HPV+ had a ratio of 1.02. Of the ASC-H diagnoses, 32.9% were tested for HPV; 63% (46/73) were positive. Although the HPV+ proportion by pathologist ranged from 54% to 83%, no significant differences were identified.

Within the same laboratory, interobserver variability exists in the proportions of HPV positivity for ASCUS and ASC-H interpretations.

According to the Bethesda System, atypical squamous cells (ASC) is a term for which the definition has evolved over time. Generally, ASC is used to designate squamous epithelial cells in cervicovaginal cytologic samples that appear abnormal and have features suggestive but not fully diagnostic of squamous cell dysplasia (cervical intraepithelial neoplasia). Currently, the Bethesda System recognizes two levels of ASC: atypical squamous cells of undetermined significance (ASCUS) and atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H). Commonly, ASCUS refers to specimens that approach or resemble low-grade squamous intraepithelial lesions (LSILs), whereas in ASC-H, lesions have cellular features suggestive of high-grade SIL (HSIL). Although morphologic criteria have been promulgated for both categories, the actual application in day-to-day cytologic practice manifests considerable variability.

The central carcinogenic role of human papillomavirus (HPV) in the development of cervical epithelial abnormalities has been recognized for some time. Accordingly, a variety of tests have been created to identify the presence of HPV and the associated cancer risk type in cervicovaginal samples. Although some clinicians use these tests as part of the primary screening package for women, most professionals have used HPV testing in women with a cytologic interpretation of ASCUS.

As manifested by the results of the ASCUS/LSIL Triage Study and other investigations, viral testing has proven useful for determining whether women with such a diagnosis should be referred for colposcopy and biopsy or be followed up in screening programs. This determination is based on the knowledge that HPV, especially high-risk HPV types, is associated with invasive cervical carcinoma and its precursors that,
if untreated, are more likely to develop into malignancy at a relatively high rate.4

However, the value of HPV DNA in the quality assurance process of cervical cytology is almost totally unknown.1,14 Because HPV DNA should be positive in a prominent but as yet undetermined proportion of specimens interpreted as ASCUS and ASC-H, the results of concurrently performed cytologic and HPV DNA testing may provide a measure of validity of such morphologic interpretations by individual pathologists and laboratories. We acknowledge that low-risk HPV types are generally not associated with the development of HSILs and carcinomas, and, thus, testing for them on clinical grounds is usually not indicated. On the other hand, low-risk viruses may result in cytomorphologic features of ASCUS and, to a lesser extent, of ASC-H, which are indistinguishable from the features of high-risk HPV and, thus, detection of low-risk viruses is desirable and valid from a quality assurance perspective. This measure should prove to be more objective than cytologic and histologic interpretations, in which subjectivity and, thus, variability are known to exist.3,15

Materials and Methods

This study covered the 2-year interval from January 1, 2004, to January 1, 2006. During this period, 5 pathologists, 3 of whom are board certified in cytopathology by the American Board of Pathology, examined and interpreted all cytologic specimens referred from a cytotecnologist. This study was approved by the appropriate institutional review boards.

All cervicovaginal cytologic specimens were SurePath liquid-based samples (TriPath Imaging, Burlington, NC). This laboratory used the 2001 Bethesda System terminology for reporting cervical cytology interpretations. All cervicovaginal specimens were initially screened by at least 1 cytotecnologist. A specimen judged not normal by the cytotecnologist was referred for interpretation to one of the pathologists.

During this 2-year period, the proportion of ASCUS specimens sent for HPV DNA testing varied and increased owing to increasing acceptance of reflex HPV DNA testing with a Papanicolaou (Pap) test interpretation of ASCUS. During this same period, some clinicians referred a portion of their specimens with an ASC-H interpretation for HPV DNA testing as well. When HPV DNA testing was requested, the fluid remaining in the patient’s vial was outsourced to a commercial laboratory (ESOTERIX, Austin, TX). The analyses performed used the polymerase chain reaction (PCR). The exact details of this assay are not well known to us because the laboratory was reluctant to release proprietary data. The DNA from the samples was amplified using a primer based on the L1 region of the HPV DNA genome. PCR products were separated by electrophoresis on a gel. If the test result was positive for HPV DNA, it was genotyped by restriction endonuclease digestion. Typing was based on the pattern of DNA fragments. If the pattern from a given patient sample did not match that of any known HPV types, the HPV was considered of an unknown risk level. As a control sample, human DNA was amplified by PCR to determine if sufficient DNA was present for testing.

Results were reported as negative for HPV DNA, positive for HPV DNA of a low-risk viral type, positive for HPV DNA of a high-risk viral type, positive for HPV DNA of an unknown risk type, and DNA, quantity not sufficient for analysis. For statistical analysis, results positive for HPV of an unknown risk type were coded as positive for HPV of a low-risk viral type, and samples with quantities insufficient for analysis were excluded.

The proportion of positive HPV DNA tests from samples interpreted as ASCUS, in which analyzable DNA was available, was calculated by pathologist and overall. The same calculation was done for ASC-H interpretations. A major thrust of this investigation was to evaluate the presence and degree of inter-observer variability (IOV) in the proportions of HPV DNA positivity in specimens interpreted as ASCUS or ASC-H. The χ² test was used to determine whether a statistically significant difference existed for HPV positivity vs negativity, high-risk positivity vs not positive for a high-risk HPV type, and low-risk positivity vs not positive for low-risk HPV type for ASCUS and ASC-H separately. A Spearman correlation was used to determine if there was a correlation between HPV positivity for ASCUS and ASC-H and the ASCUS plus ASC-H/SIL ratio by pathologist and between HPV positivity for ASCUS and ASC-H and the total number of Pap tests by pathologist. Statistical significance was assumed at a P value of .05 or less.

Results

A total of 20,354 cervicovaginal cytologic specimens were examined in this institution’s laboratory during the 2-year period. Because policy dictates that a cytotecnologist can sign out negative specimens without a pathologist’s review, a pathologist provided a diagnosis on 3,680 (18.1%) of these cases. Table I shows the breakdown of ASCUS and ASC-H by pathologist. A total of 1,299 cases had an interpretation of ASCUS, accounting for 6.4% of all specimens (35.3% of all pathologist diagnoses). An ASC-H interpretation was made in 222 samples accounting for 1.1% of the total (6.0% of all pathologist diagnoses). During this same interval, a diagnosis of LSIL or HSIL was made in 1,444 specimens (7.1% of total specimens; 39.2% of all pathologist diagnoses). Thus, the ratio of ASCUS plus ASC-H/SIL was 1.05. This ratio varied among the 5 pathologists from 0.58 to 1.53; the pathologist with the lowest ratio evaluated a total of 929 samples during this period, whereas the pathologist with the highest ratio...
evaluated a similar number, 924. We also looked at the ratio of ASCUS plus ASC-H to the total number of cervicovaginal specimens examined by each pathologist. As a percentage of the total, the spectrum ranged from 27.3% to 50.1%. The same pathologists, respectively, were responsible for those 2 ratios.

Table 2 shows the HPV results for ASCUS diagnoses by pathologist. A total of 431 specimens with a cytologic interpretation of ASCUS were sent for HPV DNA testing by PCR. Twelve of these had insufficient DNA for analysis. Thus, 32.3% of all cytologic interpretations of ASCUS had HPV DNA results.

For the laboratory overall, 15.9% of all ASCUS interpretations were positive for HPV DNA. However, 49.4% of all ASCUS cases with sufficient samples were positive. Of the DNA positives, 78.7% were for high risk HPV, 11.0% were for low risk HPV, and 10.0% were of unknown risk. Of all cervicovaginal specimens examined by pathologists, 5.6% were positive.

For each pathologist, HPV+ findings ranged from 11.3% to 24.1% of the number of ASCUS cases (P = .016), 38% (23/61) to 67% (28/42) of all HPV tests with sufficient sample for ASCUS cases (P = .775), and 0.6% to 1.6% for all cases examined (P < .001). There was significant variability among pathologists for high-risk viral types (P < .001), in all likelihood related to the high proportion of all positives that were high-risk HPV. There was no significant variability in the ASCUS interpretation for low-risk HPV (P = .957) or low-risk HPV combined with positive HPV of unknown type (P = .836).

Table 3 shows the HPV results for ASC-H diagnoses by pathologist. A total of 73 specimens with a cytologic interpretation of ASC-H were sent for HPV DNA testing by PCR. Thus, 32.9% of all cytologic interpretations of ASC-H had HPV DNA results.

For the laboratory as a whole, 63% (46/73) of these ASC-H specimens were positive for HPV DNA. Of the positive test results with known risk, 98% (45/46) were high-risk types. Of those with known risk, 1 was a low-risk HPV type. For each pathologist, HPV+ findings ranged from 12% (6/50) to 28% (7/25) of all ASC-H cases (P = .340), 54% (7/13) to 83% (5/6) of all HPV tests with sufficient sample for ASC-H cases (P = .775), and 0.6% to 1.6% for all cases examined (P = .388). Also, there was no significant variability among pathologists for high-risk (P = .338) or low-risk (P = .564) viral types or low-risk HPV combined with positive HPV of unknown type (P = .713). The lack of a statistically significant relationship despite an appearance of a difference is most likely due to the relatively small number of ASC-H specimens.

No statistically significant correlation was found between the proportion of positive HPV interpretations for ASCUS and...
ASC-H by pathologist with individual ratios of ASCUS plus ASC-H/SIL ($P > .999$). Similarly, there was no statistically significant correlation between the proportion of HPV DNA–positive ASCUS and ASC-H interpretations by pathologist with the total number of cervicovaginal cytologic specimens examined by each during the 2-year period ($P = .505$).

**Discussion**

More than a decade has passed since Sherman et al\(^1\) reported using HPV testing as a quality monitor for Pap smear interpretations of ASC, but little use has been described since then. In 2002, Stoler\(^14\) reemphasized the objective nature of HPV testing in quality assurance. The study by Sherman et al\(^1\) used 190 conventional smears initially interpreted as squamous atypia (borderline atypia).\(^1\) These were reclassified independently by 5 pathologists. Of the specimens reclassified as ASCUS, 30% were positive for high-risk HPV using 2 different assays, one of which used PCR. These authors did not provide the exact proportion of ASCUS specimens that were associated with low-risk HPV, but from their Figure 4C, it appears as if between 10% and 20% were positive for low-risk HPV. Similar proportions of specimens reclassified as normal and as SIL were also positive for low-risk HPV. Accordingly, between 40% and 50% of their ASCUS specimens would be positive for HPV of all known risk types. These authors concluded that owing to the association of high-risk virus with ASCUS, HPV testing may have a significant role in cervical cytologic quality assurance. We generally agree with this conclusion and have used HPV testing in one of our laboratories for such a purpose.

In another study that demonstrated the relationship of ASCUS and ASC-H to HPV positivity, Pirog et al\(^16\) compared the proportion of ASCUS and ASC-H cases that were positive for HPV by PCR. They found that 56% and 71%, respectively, were HPV+. Our laboratory values were somewhat lower, at 49.4% and 63%, respectively. These authors did not compare IOV among pathologists in correlating morphologic interpretations with HPV results.

Zuna et al\(^17\) also demonstrated the use of HPV testing as a quality assurance measure in cervical cytology. They considered this testing a good alternative to the “gold standard” of histologic examination. In their study, 43.7% of ASCUS interpretations were positive for high-risk HPV.

We have described one laboratory’s 2-year experience using HPV testing as a quality assurance metric to evaluate the accuracy of ASCUS and ASC-H interpretations. Overall, our laboratory values are equivalent to reported data that show a definite spectrum in the percentage of positivity for HPV DNA. Variability among laboratories may be due to the test used for the detection and typing of HPV, the cytomorphologic criteria emphasized, and the population of patients tested or screened. Our experience eliminates the elements of the exact test used and the patient population; thus, our IOV is largely the result of the morphologic criteria used by 5 independent pathologists, all of whom had perfect scores for the Clinical Laboratory Improvement Amendments–mandated gynecologic cytology proficiency testing for both years, 2004 and 2005. Although there was a spectrum of HPV+ ASCUS and ASC-H interpretations among the 5 pathologists, in neither situation was statistical significance reached. Whereas low-risk HPV types are usually not culprits in the evolution of HSILs or invasive carcinomas, we believe it is valid to include data on such viruses in a quality assurance investigation because low-risk types may result in the cytomorphologic attributes of ASCUS and ASC-H that microscopically are indistinguishable from such changes produced by their high-risk counterparts.

Several potential explanations exist for this IOV. Pathologist E had the highest proportion of HPV+ ASCUS interpretations, with a rate of approximately 67%. It is possible that this pathologist is the most accurate in the group in the ability to identify HPV-related morphologic changes in cells that do not quite reach the level of nuclear alterations sufficient for a diagnosis of SIL. Alternatively, this pathologist may tend to undercall true SIL cases, which would show a
greater proportion of HPV+ results, as ASCUS; perhaps this is related to a lower degree of confidence in making a frank diagnosis of SIL.

The opposite may be true for pathologist D, who had the lowest proportion of HPV positivity associated with ASCUS. Perhaps this pathologist overcalls ASCUS specimens as SIL and/or undercalls ASCUS as negative. This might reflect a reluctance to make more equivocal interpretations.

Overall, concerning the interpretation of ASC-H, 63% of the specimens were positive for HPV DNA. This finding is consistent with the spectrum reported in the literature. As expected, essentially all these cases were high-risk virus types. For specimens in which there was sufficient sample for DNA testing, the proportion of positive cases among the 5 pathologists ranged from approximately 54% to 83%. This difference was not statistically significant, in large part owing to the relatively small number of specimens with this morphologic interpretation with HPV testing done.

We have demonstrated that when controlling for the patient population and for the type of HPV test used, IOV in the proportion of HPV+ equivocal squamous lesion interpretations exists, although in our study, these differences were not statistically significant. We believe that is the first time such objective IOV has been shown. A major factor in this variability is likely due to the use of different cytomorphic criteria for identifying these atypical interpretations. Furthermore, these differences do not seem to be related to the total number of specimens examined by individual pathologists or to the ASCUS/SIL ratio of individual pathologists. A subsequent investigation evaluating postcytologic biopsy specimens may reveal differences in accuracies among the pathologists for predicting definite squamous abnormalities, especially HSIL. An educational tool may be in order to narrow the range in the proportion of cases deemed HPV+ by pathologists.

References


