Squamous Cell Carcinoma of the Cervix

A Cytology-Histology-Human Papillomavirus Correlation in Clinical Practice

Ross A. Miller, MD; Lindsay L. Waters, MD; Dina R. Mody, MD; Kimberlee C. Tams, MD

• ***Context.***—**Cervical cancer mortality has declined by 74% in the United States since the implementation of the Papanicolaou (Pap) test. Nevertheless, more than 12 000 US women annually develop cervical cancer, and squamous cell carcinoma (SqCa) remains the predominant cervical malignancy.**

Objective.—**To evaluate screening techniques used in the detection of SqCa of the cervix and provide insights regarding which technique(s) is (are) most efficacious in our study population.**

Design.—**We retrospectively reviewed all available cytologic, human papillomavirus (HPV), and histologic malignancy burden data from patients diagnosed with SqCa. The clinical data were collected from 2 geographically and socioeconomically diverse hospital systems. Cases in which identified patients had a Pap test with a negative result/unsatisfactory specimen within 5 years of SqCa tissue diagnosis were considered Pap test screening failures. Cases in which patients were diagnosed with HPV-negative SqCa were considered HPV screening failures.**

Results.—**Eighty-eight cases (patients' ages ranging from 19 to 73 years) were identified. Of those, cytologic history was available for 64 cases present in our electronic medical history record. Three cases were cytology screening failures (one being an unsatisfactory specimen) and 3 cases were HPV screening failures (one being the cytologic unsatisfactory case). Although measuring sensitivity in practice has limitations, we calculated the SqCa detection sensitivity at 95.3% by Pap test alone and 97% when HPV DNA testing was incorporated.**

Conclusions.—**Our results highlight the necessity of combining Pap and HPV testing. Although the number of cases identified is relatively small, our data suggest detection failures will decrease as the practice of combining HPV and Pap testing increases.**

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Since the Papanicolaou (Pap) test was implemented, cervical cancer mortality rates have declined by 74%[1] in the United States. As such, the Pap test is arguably the most effective cancer screening and prevention test in medical history.[2] The Pap test–associated decline in cervical cancer incidence has predominantly been attributed to a reduction in squamous cell carcinoma (SqCa) and is achieved by both prevention and early detection.[3] Conversely, studies have shown that the relative reduction of cervical adenocarcinoma is less than that of SqCa,[4,5] while some data even suggest that the incidence of adenocarcinoma has escalated since the implementation of the Pap test.[6,7] Despite the effectiveness of the Pap test, more than 12 000 women in the United States annually develop cervical cancer,[2,8] and SqCa remains the predominate culprit.[9] Moreover, cervical cancer eradication has not been achieved in any patient population.[10,11]

To improve early detection of cervical cancer, efforts have been made in recent years to further advance screening methodologies through liquid-based cytology and computer-assisted imaging. Additionally, with various studies indicating virtually all cervical cancers are high-risk human papillomavirus (HPV) induced/related,[12,13] HPV testing was developed and has been incorporated into routine screening.[14] Screening recommendations have been, and continue to be, tailored on clinical trial results of these various screening methodologies. However, the best combination of screening tests has yet to be established.[15,16] The goal put forth by the US Preventive Services Task Force and the American Cancer Society/American Society for Colposcopy and Cervical Pathology/American Society for Clinical Pathology when making screening recommendations is to maximize benefit and minimize potential harm.[14]

Continuing research places screening recommendations under perpetual scrutiny; however, this scrutiny is anticipated to be advantageous by driving improvement of cervical cancer prevention strategies. For example, a current screening recommendation is to cease screening in women

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From the Department of Pathology, University of South Dakota, Sioux Falls (Dr Miller); the Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Houston, Texas (Drs Waters and Mody); and Sanford Health Pathology Clinic, Sanford USD Medical Center, Sioux Falls, South Dakota (Dr Tams). Dr Miller is now at the Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Houston, Texas. Dr Waters is now at the Department of Pathology, Medical City, Dallas, Texas.

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Reprints: Ross A. Miller, MD, Department of Pathology and Genomic Medicine, 6565 Fannin St, Ste B490, Houston, TX 77030 (e-mail: rmiller@houstonmethodist.org).

older than 65 years who have a negative past screening history.[14] Potentially disrupting this recommendation is a recent study that suggests cervical cancer incidence continues to climb until at least age 69 years in women with an intact uterus.[17] The same study indicates the actual incidence of cervical cancer may be higher than formally anticipated in the United States, as past incident rates did not account for the high rates of hysterectomy. Furthermore, recently the US Food and Drug Administration (FDA) Medical Devices Advisory Committee recommended the Cobas HPV test from Roche (Basel, Switzerland) as the first-line, primary screening tool in women older than 25 years.[18] This recommendation stemmed from the ATHENA trial, which included more than 47 000 women.[18] However, other studies[19–22] have recognized the existence of cervical cancers that test negative for HPV, cancers that would be potentially missed by stand-alone HPV testing.

Considering these conflicts between certain studies in the medical literature and screening recommendations, we retrospectively analyzed all available cytologic, HPV, and histologic malignancy burden data for patients diagnosed with SqCa by cytology or histology at our institutions. Clinical data were collected from 2 geographically and socioeconomically diverse hospital systems. We examined data in this manner as such study designs are considered effective for evaluating cancer prevention strategies.[23,24] Our objective was to evaluate and hopefully tailor screening practices in our institutions and encourage others to evaluate their clinical practices to optimize screening strategies they use.

MATERIALS AND METHODS

Institutional review board approval was obtained from Sanford USD (University of South Dakota) Medical Center (Sioux Falls, South Dakota) (IRB No. 03-12-108), The Sanford School of Medicine of the University of South Dakota (Vermillion, South Dakota) (IRB No. 2012.236), and Houston Methodist Hospital (Houston, Texas) (IRB No. Pro00000653). A retrospective study was subsequently performed by searching the computer database for cervical SqCa. Clinical data were collected at Sanford USD Medical Center from January 2002 to January 2013, and at Houston Methodist Hospital from February 2006 to March 2013. When available, surgical pathology, cytology, and HPV testing data were collected. Both Sanford USD Medical Center and Houston Methodist Hospital are referral centers for the areas they serve; therefore, some of the patients in our study are possibly referred to our institutions because of abnormal screening test results from outside facilities. These data are not available to us; and therefore, it is not known if all patient screening histories reviewed in our study were complete.

One of the objectives of this study was to evaluate Pap testing alone or in combination with other available techniques for patients from diverse geographic and socioeconomic areas. In this regard, Sanford USD Medical Center is a 500-bed tertiary care center. The Sanford Health Pathology Clinic receives specimens obtained from clinicians within the primary hospital and surrounding clinics, equating to more than 40 000 surgical specimens and 48 000 Pap smear specimens per year. The atypical squamous cell to squamous intraepithelial lesion ratio (ASC/SIL) has averaged 1.1% during the past 3 years, taking conventional and liquid-based Pap test methods into account. Conversely, Houston Methodist Hospital is a 975-bed quaternary care hospital and serves as the flagship hospital to the Methodist Hospital System for Houston and the surrounding suburbs. The Methodist system-wide pathology volume consists of approximately 60 000 histology specimens and 90 000 Pap smears between the hospital referral population and a contract with a commercial laboratory. During the study period, the hospital and the commercial laboratory total volume was 363 218 liquid-based Pap tests, about equally split between ThinPrep (Hologic, Bedford, Massachusetts) and SurePath (BD, Franklin Lakes, New Jersey). During the past 3 years, the average ASC/SIL ratio has been 0.4% for SurePath and 0.6% for ThinPrep Pap tests. Benchmarking the statistics with nationally reported numbers places the laboratory below the fifth percentile for ASC/SIL ratio and above the 95th percentile for squamous intraepithelial lesion (SIL) rates.[25] These numbers have been attributed to the large population of dysplasia clinics and charity care clinics that have a high SIL ratio.

All performed cytologic screening tests were evaluated at the Sanford USD Medical Center (46 cases) and the Houston Methodist Hospital (42 cases). Overall, 11 ThinPrep nonimaging, 6 conventional, and 5 ThinPrep imaging Pap tests were obtained from the Sanford USD Medical Center computer database; the remaining 24 cases had no prior Pap history in our databases. From the Houston Methodist archives, 1 conventional, 25 SurePath nonimaging, 8 ThinPrep nonimaging, 4 SurePath imaging, and 4 ThinPrep imaging Pap tests were accrued. Importantly, HPV testing associated with the early detection of cervical cancer is performed similarly between the 2 hospitals. At the Sanford USD Medical Center, HPV testing is performed when ordered by the clinician and occurs as follows: reflex testing (prompted by a cytology diagnosis of atypical squamous cells of undetermined significance [ASCUS] or above), concurrently with the Pap test in women older than 30 years, or cotesting regardless of age or cytologic diagnosis. The high-risk HPV testing platform used is the FDA-approved Hybrid Capture 2 (HC2) method from Qiagen (Hilden, Germany),[26] which tests for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 (both intermediate and high-risk types). At Houston Methodist Hospital, HPV testing is carried out in a similar manner by using HC2 at the hospital and HC2 or a polymerase chain reaction–based analysis at the commercial laboratory if less than 4 mLs of residual specimen is available. It should be noted that HC2 testing on SurePath-fixed specimens has been internally validated at the facility, as specified by the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and the College of American Pathologists (CAP) since the HC2 test has not been FDA approved for use with SurePath fixative.

The diagnoses of SqCa were established from histopathologic examination of endocervical curettage, cervical biopsies, and excisional specimens. The SqCa diagnoses at the Sanford USD Medical Center were rendered by general surgical pathologists who see a wide range of surgical specimens, including many gynecologic specimens. Prior Pap smears are routinely reviewed and evaluated with the surgical specimen at the time of diagnosis. At the Houston Methodist Hospital, real-time cytology/histology correlation is performed by cytopathologists, who prepare a statement in the biopsy report regarding their findings of correlation, lack thereof, and the reason for lack of correlation. These are tabulated at the end of the year for CLIA/CAP accreditation requirements.

In this study, SqCa cases that were identified to have a corresponding Pap test within 5 years of tissue diagnosis were assigned a stepwise discordance score on the most recent Pap test: 0 if atypical squamous cells favor high (ASCUS-H) or above, 1 for low-grade cancer (low-grade squamous intraepithelial lesion [LSIL]), 2 for atypical squamous cells (ASCUS), and 3 for negative for intraepithelial lesion or malignancy/unsatisfactory specimens. Those with a 3-step or greater discordance score were considered screening failures. Human papillomavirus–negative cases were considered HPV screening failures.

RESULTS

A total of 88 SqCa cases were identified in our study, with 46 cases from the Sanford USD Medical Center and 42 from the Houston Methodist Hospital. Of those 88 cases, 28 (31.8%) were diagnosed with microinvasive disease and 60 (68.2%) were diagnosed with invasive disease. The average age of patients diagnosed with microinvasive disease was

Human Papillomavirus (HPV)–Tested Cases						
Case No.	Age, y	Tissue Dx	Last Pap Dx	HPV Result	HPV Method	Pap Method
1	31	SISCa	HSIL	Pos	HCII	TPi
2	40	SqCa	ASC-H and AGC	Pos, 18	PCR HB	SPi
3	41	SqCa	HSIL, ATYEndocx	Pos, 16,18	PCR HB	SPi
4	49	SqCa	HSIL	Pos	HCII	TPi
5	53	SqCa	NILM	Pos, 16	PCR HB,16	SPi
6	37	SISCa	ASC-US	Neg	HCII	TPi
7	45	SqCa	HSIL	Neg	HCII	SP
8	64	SqCa	USAT	Neg	HCII	SPi
9	22	SISCa	LSIL	Pos	HCII	TPi
10	38	SISCa	HSIL	Pos	HCII	TPi
11	49	SqCa	HSIL	Pos	HCII	TPi
12	40	SISCa	ASC-US	Pos	HCII	TPi
13	57	SqCa	ASC-US, high	Pos	HCII	TP
14	41	SqCa	HSIL	Pos	HCII	TP

Abbreviations: AGC, atypical glandular cells; ASC-H, atypical squamous cells—favor high grade; ASC-US, atypical squamous cells of undetermined significance; ATYEndocx, atypical endocervical cells; Dx, diagnosis; HB, Home Brew; HCII, Hybrid Capture II; HSIL, high-grade squamous intraepithelial lesion; i, imaging; Last Pap Dx: Pap diagnosis before tissue diagnosis of squamous cell carcinoma; LSIL, low-grade intraepithelial lesion or malignancy; Neg, negative; NILM, negative for intraepithelial lesion or malignancy; Pap, Papanicolau; PCR, polymerase chain reaction; Pos, positive; SISCa, superficially invasive squamous cell carcinoma; SP, SurePath; SqCa, squamous cell carcinoma; TP, ThinPrep; USAT, unsatisfactory specimen.

38.7 years (range, 19–64 years), while that for invasive disease was 47 years (range, 30–73 years). The overall average age was 44.4 years (range, 19–73 years).

A total of 24 cases (27.3%) had no prior cytologic data in our electronic medical records before tissue diagnosis, with 16 being invasive SqCa cases and 8 being microinvasive SqCa cases. The remaining 64 cases (72.7%) had cytologic data available within 5 years of tissue diagnosis, and the time between cytologic and tissue diagnosis ranged from 0 days to 4 years. For these 64 cases, the following tests were performed: SurePath, 25 cases; ThinPrep, 19 cases; ThinPrep imaging, 9 cases; conventional, 7 cases; and SurePath imaging, 4 cases. Sixty-one cases (95.3%) had prior abnormal cytologic test results, 2 cases (3.1%) had normal cytologic test results (one the same day of tissue diagnosis and one 3 years before tissue diagnosis), and 1 case (1.6%) had an unsatisfactory specimen that was obtained on the same day as tissue diagnosis and was due to inadequate cellularity. The most frequently observed previous Pap test diagnosis was high-grade squamous intraepithelial lesion (HSIL) or above (51 cases, 79.7%) before or at the time of tissue diagnosis.

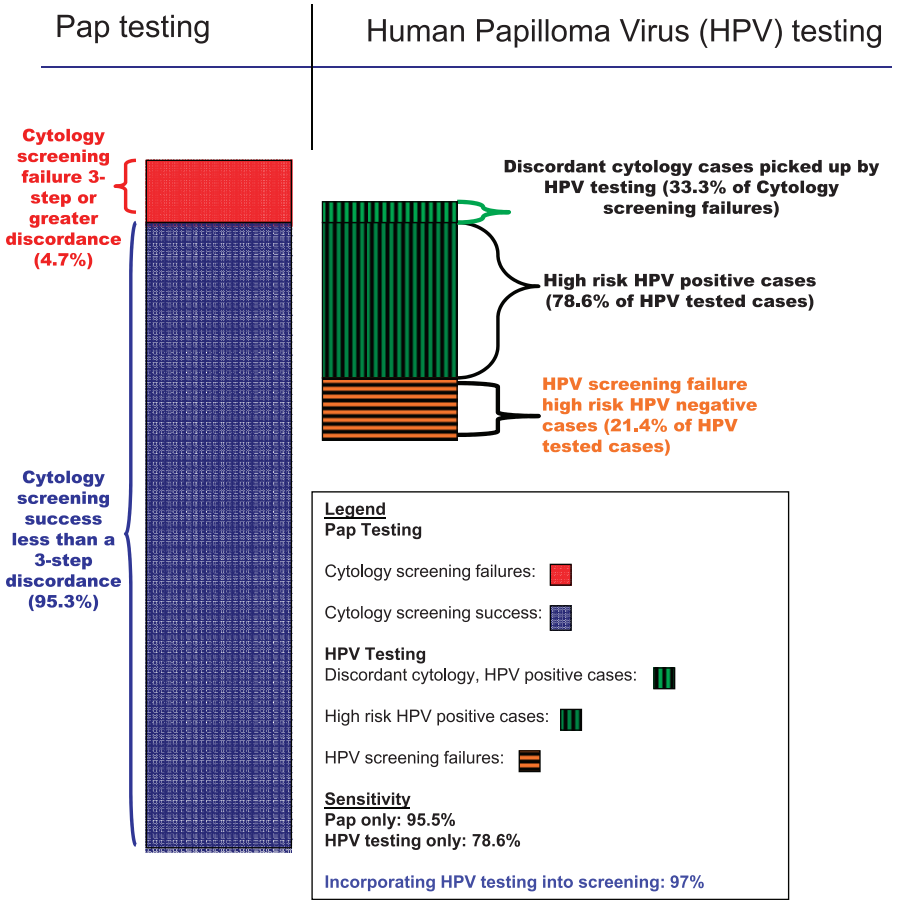
For a total of 14 cases, HPV testing was performed on the residual liquid-based specimen. Eleven cases (78.6%) were high-risk HPV positive and 3 (21.4%) were high-risk HPV negative (see Table). Of the 3 negative cases, one was on a ThinPrep imaging Pap test with a diagnosis of ASCUS 4 weeks before tissue diagnosis, one was on a SurePath Pap test with a diagnosis of HSIL 10 weeks before tissue diagnosis, and one was on a SurePath imaging Pap test and had a cytologically unsatisfactory specimen collected at the time of diagnostic tissue biopsy. The time from cytology to tissue diagnoses in the HPV-positive cases ranged from 0 days to 5 months (average, approximately 1 month) (see Figure for comparison of cytology and HPV data).

Although measuring sensitivity in gynecology cytology has limitations,[27,28] the measured sensitivity in this study was 96.8% by the Pap test alone if the unsatisfactory diagnosis is not considered versus 95.5% if the unsatisfactory Pap test is considered a screening failure. For HPV testing alone, the sensitivity was 78.6%, which rose to 97% if Pap and HPV testing were combined (see Figure). It must be taken into account that a negative HPV test result without cytology can result in a false sense of security, while an unsatisfactory Pap test would trigger a follow-up repeat, or, in a symptomatic patient, a concurrent or follow-up biopsy. Even with the newer HPV tests that contain an internal control, there is no guarantee that the control DNA came from the tumor as the DNA may have come from patients' lymphocytes/inflammatory cells. Additionally, studies[19–22] have shown cervical cancers exist that test negative for HPV. These factors can result in a false sense of security with the asymptomatic patient. Therefore, if screening intervals are based solely on HPV tests in asymptomatic women, there is a potential for serious delay in diagnosis with the extended screening intervals.

As part of this study, we evaluated discordance scores for cases with Pap tests obtained within 5 years of tissue diagnosis. The discordance scores were determined as follows: 0 if ASCUS-H or above, 1 for LSIL, 2 for ASCUS, and 3 for negative or unsatisfactory, which were considered screening failures. The overall average discordance score was 0.3 (discordance score range, 0–3). There were 9 cases with discordance: 2 (3.1%) with a 1-step discordance, 4 (6.3%) with a 2-step discordance, and 3 (4.7%) with a 3-step discordance. Of the 3-step discordance cases, one was a cytologically unsatisfactory specimen. Discordance scores varied little between microinvasive and invasive disease (0.4 versus 0.3, respectively). However, some variability was observed between methodologies: 25 cases had SurePath (no imaging) tests with an average discordance of 0.1 (range, 0–2); 19 cases had ThinPrep (no imaging) Pap tests with an average discordance of 0.3 (range, 0–2); 9 cases had ThinPrep tests with imaging, with an average discordance score of 0.1 (range, 0–2); 7 cases had conventional Pap tests with an average discordance score of 0.4 (range, 0–3); and 4 cases had SurePath tests with imaging with an average discordance of 1.0 (range, 0–3).

COMMENT

Our retrospective study is relatively small, with only 88 cases of SqCa identified within the evaluated time frame, presumably owing to enhanced early detection of cervical cancer in the United States. Despite the small size of the study, some important information can be gathered. For example, of the 88 cases identified, patients diagnosed with



The figure illustrates the 64 cases having cytologic data available within 5 years of tissue diagnosis. The left column represents the Papanicolau (Pap) test results from these 64 cases. Sixty-one cases (95.3%, solid blue color) had previously abnormal cytologic test results. Two cases (3.1%) had normal cytologic test results and 1 case (1.6%) had an unsatisfactory specimen; these 3 cases were considered cytology screening failures (4.7%, solid red color). The right column represents the 14 cases in which patients underwent human papillomavirus (HPV) testing in addition to Pap testing. Of these 14 cases, 11 (78.6%, horizontal green-black lines) were high-risk HPV positive and 3 (21.4%, vertical orange-black lines) were high-risk HPV negative. Fortunately, the corresponding Pap test in the 3 HPV-negative cases showed a cytologic abnormality. One of the cytology screening failures was HPV positive on testing (horizontal light green–black lines). This graph represents the complementary nature of the Pap test and HPV test.

microinvasive disease were on average 8.3 years younger than those with invasive disease (38.7- versus 47-years-old), which could be attributed to the time needed for disease progression. Another issue highlighted in our study is the variability in patient screening history among women diagnosed with SqCa of the cervix at our institutions. Specifically, 24 cases in our cohort had no Pap test data in our systems before histologic diagnosis. Because Sanford USD Medical Center and Houston Methodist Hospital are referral centers for the areas they serve, the actual number of patients never receiving cervical cancer screening cannot be determined. Some of these patients with SqCa may have been referred owing to abnormal Pap test results from outside facilities, data unfortunately unavailable to us. Regardless, we feel it is essential for the medical community to continue patient educational efforts regarding the importance of cervical cancer screening, particularly in underserved populations/areas. Additionally, our study results demonstrate room for improvement with regard to screening and prevention of SqCa of the cervix in our clinical settings.

Although the FDA approved the first HPV test in 1999, in our cohort only 14 patients (15.9%) who had previously undergone Pap tests had prior HPV testing (see Table). Eleven of these cases were high-risk HPV positive and 3 were high-risk HPV negative. For patients who were HPV positive, 3 had ASCUS/ASCUS-H, 1 had LSIL, and 1 had negative results on cytology. The HPV test in these patients provided important information to the clinician and likely triggered future triage and management decisions. As an example, 1 cytologically negative/HPV-positive case concerned a 53-year-old woman who may have gone 3 years without further screening if the most recent cervical cancer screening guidelines had been followed.[14] On the other side of the spectrum, 3 cases were HPV negative by the HC2 method, with HPV-negative tests performed on average 4.7 weeks before tissue diagnosis (range, 0–10 weeks). Given these data, the measured sensitivity is 95.5% by the Pap test alone, 78.6% by HPV testing alone, and 97% with combined Pap and HPV testing (see Figure). These data suggest cytology is superior at detecting abnormalities in the setting of SqCa. Moreover, our results indicate that Pap/HPV cotesting provides superior sensitivity and yields important clinical information in a portion of ASCUS/LSIL cytologic diagnoses. This approach may also identify high-risk HPV cases in a percentage of patients with negative cytology results, which would assist clinicians with tailoring future screening measures. Despite its promise, we reiterate that only 14 patients were tested for HPV, and the benefits of Pap-HPV cotesting could be further pronounced if cotesting increased. However, currently this concept is purely hypothetical given our results, and further trials would be necessary to fully evaluate this proposal.

As part of this study, we also evaluated discordance levels between different methodologies used. The average discordance was 0.3, where average discordance ranged from 0.1 to 1.0 depending on the cytologic method used for screening. Owing to the limited number of cases, especially on SurePath imaging Pap tests (only 4 cases, one being unsatisfactory), we feel the discordance between methodologies cannot be reliably compared. Therefore, we conclude that the discordance seen in this study should not

reflect on the individual performances of SqCa detection methodologies discussed. Interestingly, regardless of the methodology used, the most frequent prior Pap test diagnosis was HSIL or above (51 cases, 79.7%). Furthermore, cases diagnosed as ASCUS-H and above were given a discordance score of 0 in this study and therefore 55 cases (85.9%) had no observed discordance.

Finally, quality of the sample collected during the Pap examination is an important factor that can determine potential diagnostic outcome. In this study, there was only 1 cytologically unsatisfactory case that was deemed unsatisfactory owing to inadequate cellularity. As illustrated in the new technology for cervical cancer (NTCC) trial, patient compliance with repeated cytologic testing is only approximately 70%.[29,30] As such, performing HPV testing on the remaining sample material has been the solution to this relative lack of follow-through. The unsatisfactory case from our cohort illustrates that unsatisfactory specimens are not all equal. Some are unsatisfactory owing to inadequate cellularity and some are obscured by elements such as blood or inflammation. Those with inadequate cellularity most likely do not have adequate DNA content in the material to perform accurate HPV testing. Therefore, in this setting, it may be best to have the patient undergo a repeated Pap test, as opposed to testing the inadequate specimen for high-risk HPV. This is important because many gynecologists do not recommend repeated Pap testing in the presence of negative HPV results,[31] and lack of cellular content during a HPV test might result in a false-negative result. For this same reason, we believe that the newer HPV tests that contain an internal DNA control may provide a false sense of security, since the DNA detected may not come from the tumor, but rather from contaminating inflammatory cells. Additionally, cervical cancers testing negative for HPV have been recognized in the medical literature.[19–22] Therefore, we believe using HPV testing as a sole, primary screening test will result in a high rate of false negatives in patients with invasive carcinoma. The Predictors 2 study[32] illustrated a wide range of varying sensitivities among different HPV testing platforms (range cited, 74.1%–96.3%, depending on the HPV testing methodology used). This is noteworthy, considering that the FDA Medical Devices Advisory Committee now recommends the Cobas HPV test from Roche as the first-line, primary screening tool in women older than 25 years.[18] Although our study used either the HC2 or a polymerase chain reaction–based HPV detection method, and not the Cobas HPV test, 3 of 14 women (21.4%) would have gone with an undetected SqCa for a period of time had their clinical samples been tested with the HPV testing alone. Yet, in our study, since all 3 of these HPV-negative women were also tested cytologically, their clinical diagnosis was improved. Therefore, while one cannot directly compare the results of our study with those obtained with the Cobas HPV test, data from the Predictors 2 study show a slightly higher HC2 sensitivity (96.3%) than that of the Cobas method (95.2%).[31] Overall, we strongly believe this pilot study, albeit with limited sample numbers, provides an important rationale for combining the Pap test with a concurrent HPV test in order to improve early detection of cervical cancer.

CONCLUSION

Although the number of patients in our study was relatively small, our results suggest that detection failures will decrease with increased utilization of combined Pap-HPV testing in clinical practice. The Pap and HPV tests are truly complementary, which was illustrated by our HPV-alone testing, which lacked detection sensitivity in our clinical practices. Additionally, we found that patients diagnosed with microinvasive disease in our study cohort were on average 8.3 years younger than those diagnosed with invasive disease. For these patients, the most frequently observed Pap test result before tissue diagnosis of SqCa of the cervix was HSIL or above.

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