Clinical Performance of Human Papillomavirus E6 and E7 mRNA Testing for High-Grade Lesions of the Cervix

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Infection with high-risk (HR) human papillomavirus (HPV) is the major cause of cervical cancer. However, relatively few infections progress to malignant disease. Progression to malignancy requires the overexpression of the E6 and E7 genes in the integrated HPV genome. It follows that the E6 and E7 transcripts could be useful markers of disease progression. The study presented here tests this possibility, using data from colposcopy and from cytological and histological tests to compare RNA assays for the E6 and E7 genes with DNA testing. A total of 180 women underwent colposcopy, cytology, and biopsy of suspected lesions (143 cases). Cervical brush specimens were analyzed for HPV DNA and for E6 and E7 mRNA. DNA from HR HPV was found in 57.8% of the specimens; E6 and E7 transcripts were found in 45%. The rates of detection of HPV DNA and of E6 and E7 transcripts were 33.3% and 25%, respectively, for specimens with normal findings; 51.4% and 31.9%, respectively, for specimens with cervical intraepithelial neoplasia grade 1 (CIN1); and 61.1% and 44.2% for specimens with CIN2, respectively. All specimens with CIN3 and 95.5% of specimens from patients with squamous cell carcinoma were positive by both assays. Thirty-seven patients with normal colposcopy findings did not undergo biopsy. HPV DNA and mRNA transcripts were found in 32.4% and 18.9% of these cases, respectively. Comparisons with cytological tests produced similar results. Overall, the mRNA tests showed a higher specificity than the DNA tests for high-grade lesions (72.7% and 56.2%, respectively) and a higher positive predictive value (59.3% and 49.0%, respectively). These findings suggest that mRNA assays could be more powerful than DNA testing for predicting the risk of progression and offer a strong potential as a tool for triage and patient follow-up.

Carcinomas of the anogenital tract, particularly cancer of the cervix, represent the second most frequent type of neoplasm worldwide (43, 57). The major cause of these cancers is infection with high-risk (HR) human papillomavirus (HPV). DNA from HPV has been detected in more than 99% of cervical squamous cell carcinomas (SCCs) and a smaller proportion of adenocarcinomas (3, 4, 31, 33). However, most HPV infections regress spontaneously or progress only after a long period of latency. As a result, the number of infections is far higher than the number of women who develop cancer.

The most common types of HPV found in cancer patients are types 16, 18, 31, 33, and 45 (5, 10, 40, 53). Persistent infection with these types is regarded as a significant risk factor (40). The role of HPV in the etiology of cervical cancer is tightly correlated with the overexpression of two oncogenes (E6 and E7) due to a specific opening in the E2 open reading frame in the integrated viral genome (23, 28). Studies of cervical cancer cell lines and cancer biopsy specimens have shown that the continuous expression of the genes is a necessary condition for the transformation and maintenance of neoplastic and dysplastic cells (46, 56, 57).

Cervical cancer is characterized by a well-defined premalignant phase that can be detected by cytological examination of exfoliated cervical cells and confirmed by histological examination of cervical material. Premalignant changes are reflected in a spectrum of histological abnormalities ranging from cervical intraepithelial neoplasia grade 1 (CIN1) or mild dysplasia, to moderate dysplasia (CIN2) and severe dysplasia or carcinoma in situ (CIN3 or CIS). Screening for these conditions has reduced the incidence of cervical cancer, especially in industrialized countries with effective screening programs. However, cervical cytology has limited sensitivity, specificity, and accuracy, especially in cases of low-grade or borderline lesions. As a result, cytological and histological examinations on their own are unable to distinguish the small number of women who will progress to invasive cancer from the vast majority of women whose abnormalities will spontaneously regress (8, 54).

In recent years, many studies have shown that testing for HPV DNA can improve the detection of high-grade squamous intraepithelial lesions (HSILs) and SCCs (33). This suggests that DNA testing can make a useful contribution to the triage of women with an equivocal cytology finding and to follow-up after the treatment of precursor lesions (9, 42, 45). However, the high prevalence of transient and asymptomatic HPV infections means that DNA tests have low specificities (38). Iden-
tification of the persistent infections likely to produce high-
grade lesions currently requires repeated monitoring of the
HPV DNA types (5, 19, 25). Commercial nucleic acid se-
quence-based amplification in a real-time format allows the
reliable type-specific detection of E6 and E7 mRNA from
HPV types 16, 18, 31, 33, and 45. Several authors have thus
suggested that RNA-based assays could be more effective than
data testing in risk assessment (16, 21, 22, 35, 36, 38, 48, 55). In
the current study, we test this hypothesis using cytological
and histological findings to compare the sensitivities, specific-
ities, positive predictive values (PPVs), and negative predictive
values (NPVs) of RNA and DNA testing.

MATERIALS AND METHODS

Study subjects and collection of specimens. Specimens were collected from
September 2007 to October 2008 from patients admitted for secondary screening
to the Colposcopy Outpatient Service and the Gynecological Oncology Unit
University Cattolica del Sacro Cuore, Rome, Italy) and the Department
Oncology (Università Cattolica del Sacro Cuore, Campobasso, Italy). The study
group consisted of 180 women between 20 and 77 years of age (median age, 35
years; interquartile range [IQR], 13). Forty percent were aged between 30 and 39
years, 27% were under 30 years of age, and 32% were over 40 years of age. The
age at first intercourse lay in the range of 13 to 39 years (median, 18 years; IQR,
3), with 49.7% of the patients having their first intercourse between 16 and 18
years of age. The number of partners was two to four for 45.6% of the women,
and 38.5% of the women were nonsmokers. The study protocol was approved by
the Ethical Committee of the Università Cattolica del Sacro Cuore, Rome, Italy.
Written informed consent was obtained from all participants. All participants
received a self-administered questionnaire requesting personal data, a gynecolo-

copy and Cervico-Vaginal Pathology). Histology was performed with specimens
collected by colposcopy-directed biopsy (traditional punch biopsy specimens)
and/or cone specimens collected by the loop excision procedure. Histology re-

tained to detect HPV at concentrations as low as 5 copies per sample.
HPV mRNA detection. Samples were analyzed for HPV E6 and E7 mRNA by
real-time multiplex nucleic acid sequence-based amplification. Transcripts of HR
HPV types 16, 18, 31, 33, and 45 were detected by the NucliSens EasyQ HPV
system. Colposcopy was performed by specialized gynecologists. The results were
reported following guidelines issued by SICPCV (the Italian Society of Colpos-

data analysis. Summary results are presented as counts and percentages,
with 95% binomial exact confidence intervals being used for categorical data and
medians and IQR values being used for continuous data. The concordance among
the DNA and RNA test results was evaluated by using Cohen’s kappa statistic,
as described by Fleiss (18). The sensitivities, specificities, and PPVs for
the HPV DNA and RNA assays were estimated by comparison with the cyto-

groups as normal-findings–low-grade squamous intraepithelial lesions (LSILs)
versus HSILs-SCC; histological results were grouped as normal–CINI versus
CINI-2-SCC (target condition, CIN2-SCC). The expected values and
95% confidence intervals for sensitivity, specificity, PPV, and NPV were calcu-
lated as described by Sebed and Tobias (47).

RESULTS

Cytological and histological findings. As mentioned earlier, all patients underwent a conventional Pap smear test. All
except two Pap smears (1.1%) were of satisfactory quality.

The results for 44.4% (n = 80) of the smears were normal; 54.5% showed various forms of cytological abnormality. A
total of 11.1% of cases (n = 20) showed atypical squamous cells of uncertain significance (ASCUS); LSILs were found
in 25.5% (n = 46) of the women, and HSILs were detected in
13.9% (n = 25) of the women. SCCs were detected in
3.9% (n = 7) of the women (Table 1).

Colposcopy was performed for all 180 cases. In 37 patients
(20.5%), no suspect lesions were detected. These patients were
subjected only to cytological surveillance. For the remaining 143
(79.5%) of the women, a specimen was taken by colposcopically
directed biopsy. The majority of these patients had cytological
findings within the normal limits and/or low-grade disease (Table
1). Among the 80 patients with normal cytology findings, biopsies
were performed in 55 cases (68.7%), revealing 6 cases with normal
histology findings, 34 with CIN1, 9 with CIN2, 3 with CIN3,
and 3 with SCCs. High-grade lesions were found in 4 cases, and
SCCs were found in 3 of 20 patients with ASCUS.

By consideration of the 143 patients, 12 biopsy samples
(8.4%) were classified as normal/benign, 72 (50.3%) as CINI,
TABLE 1. Cytological and histological findings for the 180 women enrolled in the study

<table>
<thead>
<tr>
<th>Cytology or histology result</th>
<th>No. (%) of women</th>
<th>Age (yr) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsatisfactory</td>
<td>2 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>80 (44.4)</td>
<td>20–55 (33.5)</td>
</tr>
<tr>
<td>ASCUS</td>
<td>20 (11.1)</td>
<td>22–77 (33.5)</td>
</tr>
<tr>
<td>LSIL</td>
<td>46 (25.6)</td>
<td>22–59 (36.5)</td>
</tr>
<tr>
<td>HSIL</td>
<td>25 (13.9)</td>
<td>21–63 (35.0)</td>
</tr>
<tr>
<td>SCC</td>
<td>7 (3.9)</td>
<td>38–56 (43.0)</td>
</tr>
<tr>
<td>Total</td>
<td>180</td>
<td></td>
</tr>
</tbody>
</table>

Histology result

<table>
<thead>
<tr>
<th>Histology result</th>
<th>No. (%) of women</th>
<th>Age (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal-benign</td>
<td>12 (6.7)</td>
<td>20–58 (30.0)</td>
</tr>
<tr>
<td>CIN1</td>
<td>72 (40.0)</td>
<td>20–58 (33.0)</td>
</tr>
<tr>
<td>CIN2</td>
<td>18 (10.0)</td>
<td>22–59 (35.0)</td>
</tr>
<tr>
<td>CIN3-CIS</td>
<td>19 (10.5)</td>
<td>21–49 (34.0)</td>
</tr>
<tr>
<td>SCC</td>
<td>22 (12.2)</td>
<td>27–77 (44.5)</td>
</tr>
<tr>
<td>Total</td>
<td>180</td>
<td></td>
</tr>
</tbody>
</table>

* The data represent the range (median) ages.

18 (12.6%) as CIN2, and 19 (13.3%) as CIN3 or CIS. Twenty-two cases (15.4%) were classified as SCC (Table 1).

HPV DNA and mRNA tests. All cervical specimens were tested for DNA and RNA from HR HPV. The tests were performed by investigators blinded to the cytology and histology results. The HC2 DNA assay detected HPV infection in 57.8% of the cases (104/180 patients). HPV RNA was detected in 51.1% of the cases (92/180 patients). Mixed infections with LR types were detected in 11 cases (6.1%). Only one case was positive for LR HPV DNA (0.6%). The remaining 76 women (42.2%) tested HPV DNA negative.

The NucliSens EasyQ HPV assay, which detects E6 and E7 mRNA from HR HPV types 16, 18, 31, 33, and 45, identified specific transcripts in 81 of 180 (45%) samples.

To avoid false-negative results due to RNA degradation, all samples were tested with an RNA control (U1A) included in the HPV E6 and E7 mRNA test. All samples were positive.

In nine cases in which the HC2 test detected no DNA from HPV, the mRNA assay yielded positive results. These samples were retested by multiplex PCR. In each case, the assay confirmed the presence of the specific HPV genotype previously revealed by the RNA-based method.

The commonest HPV genotype revealed by RNA testing was HPV-16 (50/180 cases [27.8%]), followed by HPV-45 (16/180 cases [8.9%]), HPV-31 (11/180 cases [6.1%]), HPV-18 (9/180 cases [5.0%]), and HPV-33 (6 cases [3.3%]).

In 70/180 cases (38.9%), the test detected infections with single genotypes; 11/180 cases (6.1%) involved infections with multiple genotypes (Table 2). The most common were mixed infections with HPV-16 and HPV-45.

Multiple infections were detected in 4 of 74 (5.4%) patients with CIN1, 1 of 18 with CIN2 (5.5%), 1 of 19 (5.2%) with CIN3-CIS, and 2 of 22 (9.1%) with SCC. Among 49 women with negative colposcopy findings who did not undergo biopsy (n = 37) or with normal histology findings (n = 12), there were 3 cases (6.1%) of double infection.

Comparing the data from our molecular assays with the cytological data, we found that the lowest prevalence rates for HR HPV DNA were in patients with normal cytology findings or ASCUS (31.2% and 60%, respectively) (Fig. 1). HPV DNA was also detected in one of the two smear samples unsuitable for cytological examination. A total of 76.1% (35/46) of the patients with LSILs and 96% (24/25) of the patients with HSILs or atypical squamous cells-cannot exclude HSILs (ASC-H) displayed simple or mixed infections with HR HPV.

HPV RNA was found in all cases of carcinoma (n = 7).

E6 and E7 transcripts were detected in 20 of 80 patients with normal cytology findings (25%) by the mRNA test. The proportion of patients with detectable transcripts increased progressively with the grade of the lesions observed, rising from 25% for patients with ASCUS (5/20 patients) to 50% for those with LSILs (25/46 patients) and 96% for those with HSILs or ASC-H (24/25 patients). All cases Pap smear positive for SCC were positive for HPV RNA (Fig. 1).

The concordance between the DNA and RNA tests was fairly good for patients with normal cytological findings (81.3%; kappa = 0.53) and LSILs (69.6%; kappa = 0.39) but was slightly lower for patients with ASCUS (55%; kappa = 0.18); in cases of HSILs (92%) and SCCs (100%), the concordance was so high that the kappa statistics were no longer useful. Detailed results are shown in Table 3.

In terms of the histology findings, HPV DNA was detected in 33.3% of the 12 patients with normal-benign specimens and 32.4% of the 37 women with negative colposcopy findings who did not undergo biopsy. Infection with HR HPV was found in 51.4% (37/72) of cases of CIN1, 61.1% (11/18) of CIN2, 100% (19/19) of CIN3, and 95.5% (21/22) of SCC (Fig. 2).

RNA tests showed a higher prevalence of E6 and E7 transcripts in patients with higher-grade lesions. Transcripts were detected in 25% (3/12) of the specimens with normal-benign findings, 31.9% (23/72) of those with CIN1, 44.4% (8/18) of those with CIN2, 100% (19/19) of those with CIN3, and 95.5% (21/22) of those with SCC. Among the 37 patients with normal colposcopy findings who did not undergo biopsy, HPV mRNA was present in 18.9% (Fig. 2).

The concordance between the DNA and the mRNA test results was fair for specimens from patients with normal colposcopy findings who did not undergo biopsy (64.9%; kappa = 0.10), very high for those with a normal-benign histology (91.7%; kappa = 0.80), and good for specimens classified as having CIN1 (72.2%; kappa = 0.45) or CIN2 (72.2%; kappa = 0.46). In the case of specimens classified as having CIN3-CIS or SCC, the concordance was so high (100% and 90.5%, re-
spectively) that kappa statistics were no longer useful. Detailed results are shown in Table 4.

**Sensitivity, specificity, and predictive values.** On the basis of the results described above, we used the cytology and histology findings to estimate the sensitivities, specificities, PPVs, and NPVs of positive DNA and RNA test results. In the cytology-based analysis, the target conditions defining disease were diagnoses of HSILs or SCC; in the histology-based analysis, the target conditions were diagnoses of CIN2, CIN3, CIS, or SCC. The results of the DNA and mRNA assays for patients who did not undergo biopsy were very similar. In view of this similarity, patients with normal findings for the biopsy specimens and patients who did not undergo biopsy were considered a single group. The results, presented in Table 5, show that in terms of the cytology target condition—currently considered the “gold standard”—the RNA and DNA assays had the same sensitivities. However the RNA assay had a higher specificity (67.1%) than the DNA-based test (50.7%). The histology results were similar. Although the DNA test was slightly more sensitive than the RNA assay (86.4% and 81.4%, respectively), the confidence intervals overlapped. The RNA assay had a significantly higher specificity than the DNA assay (72.7% and 56.2%, respectively). In this case, the overlap of the confidence intervals was minimal.

**DISCUSSION**

Cervical cancer is strongly associated with HPV infection (2, 5, 53). Progression to cervical carcinoma often extends over decades and is partly driven by the overexpression of HPV oncogenes. Although little is known regarding the possible transient nature of such expression, it is certain that the E6 and E7 proteins are consistently expressed in neoplastic tissue and play a significant role in malignant transformation.

Against this background, the goal of the study reported in this paper was to assess whether tests for these transcripts could be a better predictor of disease progression than screening for HR HPV DNA.

Few of the specimens investigated in our study came from a primary screening program; rather, they came from the secondary screening of patients referred for the evaluation of preneoplastic lesions. The histological findings from our study confirm earlier suggestions (8, 11, 44) that the conventional Pap smear test commonly used for both primary and secondary screening has a relatively poor sensitivity for high-grade cervical lesions.

Compared to the findings of earlier studies (24, 36), the results presented here are for a relatively large number of samples evaluated histologically. To the knowledge of the authors, the only study with a larger sample (n = 383) is that of Lie et al. (29).

In women displaying cervical abnormalities of any grade, the RNA assay produced fewer positive results than the DNA test. Given that not all HR HPV infections express E6 and E7, this result was expected. Although the HC2 test used for DNA detection detects 13 types of HR HPV while the Nuclisens EasyQ HPV assay detects only 5 types (HPV types 6, 18, 31, 33,

![FIG. 1. Prevalence (95% confidence intervals) of positive results for HPV DNA and for E6 and E7 by cytological status of specimen.](http://jcm.asm.org/)
and 45), the HR genotypes included in the RNA assay are far more common than the rarer types in cancer specimens (10, 11, 26, 27, 30). It is thus very unlikely that differences in type coverage explain the gap in detection rates.

The results from the DNA and RNA assays associated well with the grade of lesion. The lowest rates of concordance were for patients with normal findings and low-grade lesions. In these cases, DNA from HPV was detected more frequently than the E6 and E7 transcripts. This result probably reflects the transient nature of most HPV infections, only a few of which produce precancerous lesions. However, the detection of E6 and E7 mRNA in a number of women with cytologically normal findings or low-grade lesions indicates that HR HPV may be oncogenically active before it produces detectable changes in cells and that the E6 and E7 transcripts could provide a sensitive, early predictor of persistent infection and subsequent severe dysplasia. Moreover, we cannot exclude the possibility that the condition in patients positive for HPV DNA but negative for mRNA will never progress to invasive cancer. Anyway, for this subset of patients, the timing of follow-up examinations can be less intensive. The value of repeated RNA testing as part of the follow-up examination remains to be assessed. We are currently studying these possibilities in a longitudinal follow-up study.

For patients with high-grade dysplasia and cancer (Fig. 1 and 2), the concordance between the RNA and DNA test results was high. This suggests that the presence of the E6 and E7 proteins is a specific marker for high-grade lesions and that positive RNA test results have a greater prognostic value than positive results from the DNA-based assays. In a single patient diagnosed with SCC, a DNA-positive specimen tested negative for RNA. It is possible that this negative result was due to a very low level of viral transcriptional activity. Alternatively, the DNA may have come from an HPV genotype not covered by the RNA test (27).

In 9 of the 81 HPV E6 and E7 RNA-positive cases, the HC2 test detected no HPV DNA. As reported earlier, subsequent retesting by multiplex PCR consistently confirmed the presence of DNA from the E6 region of the viral genome. This suggests that the virus was present only at very low copy numbers and/or that only a specific region of viral DNA was integrated into the host genome. The loss of the L1 gene and its impact on viral replication after integration could lead to relatively low viral loads (7, 15, 24, 32). Given that malignant phenotypes require continuous expression of the E6 and E7 oncogenes (13, 27, 39, 57) and that they produce transcripts throughout the epithelium and the surface layers (12, 15), it is not surprising that RNA assays detect more clinically significant infections than DNA testing, especially when samples come from the surface layers of the cervical epithelium, as in the present study.

In our series, the most frequent single type that caused infection (42/81 samples) was HPV-16, followed by HPV-45 (9/81), HPV-31 (8/81), HPV-18 (6/81), and HPV-33 (5/81).

![FIG. 2. Prevalence (95% confidence intervals) of positive results for HPV DNA and for E6 and E7 mRNA by histological status of specimens.](http://jcm.asm.org/)

### TABLE 4. Concordance between HPV DNA and HPV E6 and E7 mRNA tests by histological status of specimen

<table>
<thead>
<tr>
<th>Histology result</th>
<th>No. of specimens with the indicated result by:</th>
<th>No. of specimens</th>
<th>Concordance</th>
<th>%</th>
<th>Kappa value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No biopsy</td>
<td>HPV DNA test only</td>
<td>37</td>
<td>24/37</td>
<td>64.9</td>
<td>0.101</td>
<td>0.2565</td>
</tr>
<tr>
<td>Normal</td>
<td>HPV DNA and mRNA test</td>
<td>12</td>
<td>11/12</td>
<td>91.7</td>
<td>0.800</td>
<td>0.0023</td>
</tr>
<tr>
<td>CIN1</td>
<td>HPV DNA and mRNA test</td>
<td>72</td>
<td>52/72</td>
<td>72.2</td>
<td>0.450</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No biopsy CIN1</td>
<td>HPV DNA and mRNA test</td>
<td>121</td>
<td>87/121</td>
<td>71.9</td>
<td>0.405</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CIN2</td>
<td>HPV DNA and mRNA test</td>
<td>18</td>
<td>13/18</td>
<td>72.2</td>
<td>0.458</td>
<td>0.0200</td>
</tr>
<tr>
<td>CIN3-CIS</td>
<td>HPV DNA and mRNA test</td>
<td>19</td>
<td>19/19</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCC</td>
<td>HPV DNA and mRNA test</td>
<td>22</td>
<td>20/22</td>
<td>90.9</td>
<td>0.448</td>
<td>0.5884</td>
</tr>
<tr>
<td>CIN2-SCC</td>
<td>HPV DNA and mRNA test</td>
<td>59</td>
<td>52/59</td>
<td>79.4</td>
<td>0.563</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*The data represent the number of specimens for which the test results were concordant/total number of specimens tested.*
These findings are consistent with the HPV DNA prevalence rates reported by other Italian groups (1, 6, 14, 51) and match the rates of prevalence from combined DNA- and RNA-based studies (2, 7, 10, 13, 24).

As reported earlier, the study detected a number of mixed infections (Table 4). The most common were mixed infections with HPV-16 and HPV-45 (6.1% of cases). Although previous studies have suggested that disease progression may depend on the overexpression of E6 and E7 RNA by a single dominant type (7, 13), the precise role of these infections in carcinogenesis remains unclear.

For patients with histological diagnoses of CIN2, we found E6 and E7 transcripts in only 44.4% of the cases. It is likely that many of these lesions will regress. This prediction is supported by the results of earlier studies (20, 41) that showed that 32% of lesions resolve, even in the presence of high-grade dysplasia. For reasons of safety, these lesions are usually surgically resected. Our findings support previous suggestions that some women who undergo surgical resection may be overtreated (12, 26, 27, 29, 32).

As reported earlier, comparison of the results of the RNA assay with the results of cytology—the current gold standard—confirms the superior sensitivity and higher specificity of the RNA assay. This finding is supported by the results of earlier studies (2, 24, 26, 37, 49, 50). Keegan et al. (24) actually found a stronger difference (75.8% and 43.7% for the RNA assay and cytology, respectively), with the discrepancy possibly being due to differences in the specificity and the sensitivity of cytological diagnoses.

Comparison with histology findings confirms the superior specificity of the RNA assay. Given the positive impact on PPVs, it is likely that in subjects with a high expected prevalence of disease (e.g., groups at risk, symptomatic patients, and patients with persistent cytological abnormalities after negative colposcopy results), RNA assays will provide better risk predictions than DNA tests.

Our results so far suggest that the RNA assay has approximately the same sensitivity as DNA assays and a higher specificity. The test can provide sensitive, early-stage detection of persistent infections at risk of progression and can also identify lesions that are likely to regress. Under these conditions, it is possible that a single test by use of an RNA assay could be more effective at detecting an HR HPV infection than repeated DNA testing (13). If this is true, RNA assays could take on a valuable role in the triage of patients with abnormal cytology findings and the follow-up of patients who have been treated for neoplastic lesions. Potential benefits include reductions in the number of cases referred for colposcopy, improved patient well-being, and significant reductions in costs.

Assessment of this possibility requires investigation of the prospective sensitivities and specificities of RNA-based tests. To date, there have been few studies in this direction. The most significant, from Molden et al. (35), compared the value of HPV mRNA and DNA testing for the prediction of CIN2 or worse at 2 years after the detection of HPV DNA or RNA. The study, which involved 77 women from a set of 4,136 cases, showed that the RNA assay is more specific than DNA testing (84.8% and 50%, respectively). With the support of these preliminary findings, we have recently begun a longitudinal study of RNA-positive patients to better assess the role of RNA testing as a predictor of clinical outcomes.

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REFERENCES


### Table 5

<table>
<thead>
<tr>
<th>Finding and test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
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<tbody>
<tr>
<td>Cytology finding of HSIL-SCC (sample prevalence, 18.0%)</td>
<td>96.9 (83.8–99.9)</td>
<td>50.7 (42.3–59.0)</td>
<td>30.1 (21.5–39.9)</td>
<td>98.7 (92.8–100.0)</td>
</tr>
<tr>
<td>mRNA test</td>
<td>96.9 (83.8–99.9)</td>
<td>67.1 (58.9–74.7)</td>
<td>39.2 (28.4–50.9)</td>
<td>99.0 (94.5–100.0)</td>
</tr>
<tr>
<td>Histology finding of CIN2, CIN3, CIS, and SCC (sample prevalence, 33.0%)</td>
<td>86.4 (75.0–94.0)</td>
<td>56.2 (46.9–65.2)</td>
<td>49.0 (39.1–59.0)</td>
<td>89.5 (80.3–95.3)</td>
</tr>
<tr>
<td>DNA test</td>
<td>81.4 (69.1–90.3)</td>
<td>72.7 (63.9–80.4)</td>
<td>59.3 (47.8–70.1)</td>
<td>88.9 (81.0–94.3)</td>
</tr>
<tr>
<td>mRNA test</td>
<td>81.4 (69.1–90.3)</td>
<td>72.7 (63.9–80.4)</td>
<td>59.3 (47.8–70.1)</td>
<td>88.9 (81.0–94.3)</td>
</tr>
</tbody>
</table>

* The data represent point estimates (95% confidence intervals).