p16 and MIB1 improve the sensitivity and specificity of the diagnosis of high grade squamous intraepithelial lesions: Methodological issues in a report of 447 biopsies with consensus diagnosis and HPV HCII testing

Dirk Van Niekerk, Martial Guillaud, Jasenka Matisic, John L. Benedet, J. Adrian Freeberg, Michele Follen, Calum MacAulay

Department of Cancer Imaging, British Columbia Cancer Agency, Vancouver, British Columbia, Canada
Department of Pathology, British Columbia Cancer Agency and Vancouver General Hospital, Vancouver, British Columbia, Canada
Department of Bioengineering, Rice University, Houston, TX 77005, USA
Department of Gynecology, Obstetrics and Reproductive Sciences, The University of Texas Health Science Center at Houston, Houston, TX, USA
Department of Gynecology, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA
Center for Biomedical Engineering, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA

Received 6 July 2007
Available online 7 September 2007

Abstract

Objective. Many investigators are studying the additional value of biomarkers to improve histopathologic agreement, but few are using the same methodologies. Our objectives in this analysis to differentiate High-grade Squamous Intraepithelial lesions (HGSIL) from Low Grade Squamous Intraepithelial Lesions (LGSIL), atypia, and normal were: (1) to examine the rate of Human Papilloma Virus High-Risk positivity (HPV HR+), (2) to compare and grade the basal, parabasal, intermediate, and superficial layer staining of each marker, (3) to determine the optimal qualitative threshold for markers, (4) to compare p16 and MIB1 agreement, and (5) to examine the sensitivities and specificities using each markers alone and together.

Methods. A sample of biopsies from 208 patients were chosen from a total of 1850 patients and 3735 biopsies obtained during the course of ongoing optical trials. At least two independent blinded reviews were performed for each biopsy. A third review was performed if there was a disagreement between the two reviews. Both endocervical and ectocervical samples were stained for p16 and MIB1. A grading system that is delineated in the text ranged from 0 to 3 for both markers and each biopsy was scored by each cell layer. Frequencies, sensitivities, and specificities were calculated using Statistica. An ANOVA was used to compare p16 and MIB1 staining in the epithelial layers. Finally the sensitivity and specificity of each marker alone and together were examined.

Results. 453 specimens from 208 patients whose final diagnoses were normal (n=244), low-grade (LG) (n=59), and high-grade (HG) (n=144) were selected for analysis. 447 of 453 specimens were available for staining. Most LG and HG lesions were HPV HR positive. Endocervical samples stained positive less often than ectocervix and often results were discordant from ectocervical results. The analysis by layers showed pronounced increases in staining of both p16 and MIB1 as lesions progressed from normal to LG to HG. The cutoff or threshold for p16 was 0 versus 1–3 while for MIB1 it was 0–1 versus 2–3. Using the intermediate epithelial layer measurement of both p16 and MIB1 in HPV High-Risk Positive separated the normal tissue from LGSIL, normal from HGSIL, and LGSIL from HGSIL by a statistically significant margin (p<0.05). Each marker had sensitivities and specificities for the diagnosis of HGSIL versus LGSIL and normal of ~85–90% and this improved by 5% for both sensitivity and specificity when used together (p16 sensitivity 90%, specificity 85%; MIB1 sensitivity 89%, specificity 87%; together sensitivity 94%, specificity 90%).

Conclusion. Several important methodological issues have been studied. Overall, p16 and MIB1 are promising markers to help pathologists differentiate HG lesions from all else. The staining of the endocervix and the ectocervix do not always agree, and the ectocervix more often stains positive with the presence of HGSIL. Each marker is helpful and both are helpful together. In conclusion, both markers are useful for the confirmation of HG lesions.

© 2007 Published by Elsevier Inc.
Introduction

Several biomarkers are being studied to see if their readings increase agreement among pathologists. Readings of cervical intraepithelial neoplasia or squamous intraepithelial lesions have been the subject of many investigations. Kappa statistics for the inter- and intra-pathologist agreement range from 0 to 0.80, that is, chance to excellent agreement on the Landis scale [1,2]. Recent data from the ALTS study showed a kappa of 0.41, representing only moderate agreement on the Landis scale[2,3], suggesting that biomarkers could play a useful role.

These biomarker studies are critically important for patient care. Since patients with lesions less than HGSIL are followed conservatively, while those with HGSIL are treated, it is important to differentiate them. Initially it was hoped that the presence of high risk HPV would help distinguish lesions, but we now know that the vast majority of HG lesions are HPV High-Risk Positive and those that are negative are probably falsely negative due to sampling or measurement error.

P16 is a cyclin-dependent kinase inhibitor involved in control of the cell cycle that has been demonstrated to correlate positively with HG lesions and negatively with normal cervical biopsies. Additionally, recent studies show that patients who are p16 positive with normal or low-grade lesions are likely to progress to high-grade lesions [4,5]. P16 is thought to be a surrogate marker of the integration of HPV .

Table 1 summarizes the current literature of p16 and MIB1 staining in the cervix. Sensitivities obtained in these studies are typically 70+% [4,6–8].

One of the hallmarks of neoplasia is uncontrolled proliferation. MIB1 is a marker of proliferation that has been extensively validated [9–12]. MIB1 has been studied both qualitatively and quantitatively in cancers from many organ sites including the cervix [12]. Table 1 also includes a study by Kruse [10] in which 121 MIB1-stained samples were algorithmically classified; HGSIL was distinguished from LGSIL and atypia with a sensitivity of 93%.

Few studies have evaluated the use of p16 and MIB1 staining together. Table 2 summarizes three recent efforts for three different organ sites: the cervix, the anal cavity, and the aerodigestive tract [13–15]. The study of these markers is labor intensive, and few studies use similar measures of these markers. There is also wide variation in the thresholds of positivity used in these studies. Research to date, in all tumor sites, has relied on relatively small sample sizes for the amount of variability noted in staining.

Methodology is increasingly shown to be of critical importance in studies. As markers are developed, new gold standards may be defined that improve diagnosis. For this study, the histopathologic assessment was the gold standard. In this report, we examined methodological issues in the staining and use of p16 and MIB1, both alone and together, to discriminate HGSIL from all lesser diagnoses.

Our objectives in this analysis to differentiate High-grade Squamous Intraepithelial lesions (HGSIL) from Low Grade Squamous Intraepithelial Lesions (LGSIL), atypia, and normal were: (1) to examine the rate of Human Papilloma Virus High-Risk positivity (HPV HR+), (2) to compare and grade the basal, parabasal, intermediate, and superficial layer staining of each marker, (3) to determine the optimal qualitative threshold for markers, (4) to compare p16 and MIB1 agreement, and (5) to examine the sensitivities and specificities using each markers alone and together.

Material and methods

Sample selection

Samples were chosen from among 1850 patients and 3735 biopsies collected during the course of a multi-center optical detection study for patients between...
18 and 85 of age. After reviewing the literature, we conducted this as a pilot study. One of the objectives of a pilot study is to observe variability so that a delta, alpha, beta, and power and be calculated for future studies. Seeking a distribution of lesion types and a sample size of approximately 400 biopsies, we chose 208 patients for whom 453 biopsies were available. Each biopsy was treated as an independent sample. These samples were chosen from British Columbia Cancer Agency, one of the four institutions involved in the study.

**Patient samples**

Non-pregnant women 18 years and older were enrolled in the study and an informed consent was obtained from each between 1999 and 2006. The Institutional Review Boards at all four institutions approved the protocols. In the trials, biopsies were taken from one to two colposcopically abnormal areas and from two colposcopically normal areas. Each patient had specimens collected for routine Papanicoloau testing on a slide, Hybrid Capture II (Gaithersburg, Maryland), and cytologic liquid based sample that was used for quantitative cytologic measurements and an endocervical cuttorage [16].

All biopsy specimens were fixed in buffered formalin and embedded in paraffin blocks. Three adjacent sections were cut at 4 μm and stained with hematoxylin and cosin (H & E). These sections were used for clinical histopathological interpretation. Two additional adjacent sections were stained for p16 and MIB1.

**Criterion standard**

For the purpose of this analysis, the consensus histopathologic clinical diagnosis was used as the criterion standard. A study of inter-pathologist, intra-pathologist, and inter-institutional agreement has been published [1].

**Consensus diagnoses**

All histopathologic specimens were read at the British Columbia Cancer Centre, using both the WHO and the Bethesda criteria. The first pathology review was performed by one of the gynecological pathologists on clinical duty (hereafter called the "pool"). There were seven pathologists in the pool at BCCC, and the pool included the study pathologists. A second blinded review was performed by one of our two study pathologists. If the first and second readings agreed exactly in the WHO system, a third review was not performed. A discrepancy of two grades of the WHO criteria mandated a third blinded reading by both study pathologists. The kappas for the readings of the pathology are reported in Malpica et al. [1]. In summary, for all diagnoses ranging from normal to SIL to cancer, the kappa readings ranged from 0.4 to 0.80; those in the HG and cancer range were in the high 0.70–0.85 value kappa range. The Bethesda classification for cervical neoplasia is reported in this analysis for simplicity.

**HPV testing**

Endocervical samples were collected and tested for HPV using the Hybrid Capture II method (Digene Corporation ®, Gaithersberg, Maryland). A nucleic acid hybridization microplate assay with signal amplification for the chemiluminescent detection of HPV DNA, the Hybrid Capture II test identifies both low-risk HPV types (6, 11, 42, 43, 44) and high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). All samples were processed at LabCorp® using funds from public grants.

**p16 staining methodology**

The p16 immunostaining was performed on 4 μm thick sections from paraffin blocks using a commercially available kit (DakoCytoVision ®, Milan, Italy). The sections were dried overnight at 37°. This kit includes the monoclonal antibody E6H4 which identifies an epitope of p16 (between aa 134 and aa 156). The slides were heated and immersed in the kit retrieval solution in a calibrated water bath at 96° for 40 min. Nuclear and cytoplasmic activity if present was noted and considered positive.

**Assessment for p16**

Staining intensity was graded by counting cells within the epithelial layers present, using both nuclear and cytoplasmic staining. Fig. 1 shows examples of study biopsies stained with H & E, p16, and MIB1. The pathologist scoring the slide was blinded to the diagnosis. The absence of stained cells was considered negative. If less than 5% of cells were positively stained, it was called negative or zero (0); if between 5% and 25% of cells were positively stained, it was called 1+; if between 25% and 75% of cells were positively stained, it was called 2+,
and finally if more than 75% of cells were positively stained, it was called 3+.
This grading system was used to grade each epithelial layer (basal, parabasal, intermediate, and superficial layers). A sensitivity analysis of this qualitative scoring system was performed and will be the subject of a future work.

MIB1 staining methodology

Paraffin sections of 4 μm were mounted on slides and dried overnight at 37°. Sections were deparaffinized in xylene and rehydrated in alcohol. Following rehydration, the endogenous peroxidase activity was blocked by 3% H2O2 in phosphate-buffered saline. The sections were immersed in sodium citrate buffer and heated at 1000 W for 2 min and at 160 W for 15 min in a microwave. Before immunostaining, the slides were soaked in phosphate buffered saline. Sections were incubated with biotinylated swine anti-rabbit antibody (DAKO®, Glostrup, Denmark) at 1:100 for 30 min. Visualization of the complex was realized with diaminobenxidine/H2O2 for 10 min at room temperature. Two washes in phosphate buffered saline were performed prior to counterstaining with Mayer’s Hematoxylin. The sections were dehydrated using graded ethanol and mounted with a mixture of distyrene, plasticiser, and xylene (DPX) (Nustain®, Nottingham, UK).

MIB1 classification

Fig. 1 shows examples of study biopsies stained with H & E, p16, and MIB1. The pathologist scoring the slide was blinded to the diagnosis. When there was no parabasal layer staining, the specimen was classified as 0. Since the parabasal layer usually stains, these specimens were classified as 1+. Staining in the intermediate layer was classified as a 2+, and staining in the superficial or basal layers was denoted as 3+.

Statistical analyses

Data from grading each layer in each slide were entered into the research database. Primary data were used to enumerate specimens for descriptive statistics. Statistica (Statsoft®, Tulsa, OK) was used to calculate frequencies, sensitivities, and specificities for the number of specimens, the number of HPV HR + and HR −, and the amount of tissue stained in the various layers in the epithelium. High-grade lesions were the gold standard used for positive as the endpoint in the analysis. An ANOVA was used to compare staining intensities of different levels of epithelium. Statistica was used to graph the histograms and levels of immunostaining and calculate p values.

Fig. 1. Histologic biopsy specimens showing different diagnosis from the consensus diagnosis set of specimens. (a) Hematoxylin and eosin stained samples, (b) p16 staining, and (c) MIB1 staining histopathologic consensus diagnosis.
Results

Table 3 provides an overview of all the samples, their diagnoses, and those available for marker staining for each analysis in these results. Consistent with all studies using biopsies, sample sizes differ by category because there are: missing samples, insufficient tissue on recutting, samples missing another test (HPV), or samples missing a cell layer (e.g. the intermediate layer). 447 of 453 biopsies from 208 patients were available for staining, 257 were available for the ectocervical and endocervical staining, and 408 were available for the final analysis of sensitivity and specificity.

In Table 4, showing HPV High-Risk positivity, the specimens were: (1) classified by diagnoses normal, LGSIL, and HGSIL, (2) stratified by HPV High-Risk + and HPV High-Risk − status, and (3) stratified by p16 and MIB 1 positivity. The correlation between SIL and High-Risk positivity suggests that the specimens used comprise a representative sample.

Table 5 shows the poor agreement of endocervical and ectocervical specimens. For the p16 staining, there was an agreement in 38% of the specimens, while for the MIB1 staining, there was an agreement for only 12% of the specimens. The endocervix samples for both markers were rarely positive. This suggests that neither marker is often present in the endocervix perhaps limiting the use of an endocervical curettage assessment.

A labor intensive review was carried out to look at staining among the basal, parabasal, intermediate, and superficial layers for both p16 and MIB1. Each sample was quantified, blinded to diagnosis, and recorded. These data can best be seen graphically in Fig. 2 showing p16 and Fig. 3 showing MIB1. Each graph shows increases in the respective marker as the histology worsens; both also confirm the reliability of separating these diagnoses using the layers in the samples. Analyses of p16 and MIB1 stains of the layers differed by HPV HR positivity. For HPV negative samples with p16 staining, only the basal layer was significant for separating diagnoses. For the HPV HR positive samples with p16 staining, the basal, parabasal, and intermediate layers all showed statistically significant separation. The HPV negative samples subjected to MIB1 staining showed significant separation of the diagnoses at the superficial layer only. For the HPV HR positive specimens with MIB1 staining, the basal, intermediate, and superficial layers significantly separated normal from LG from HG. Thus, the basal and intermediate layers appear most useful in the analysis when examining the overall data for both the p16 and MIB1 staining.

In Table 6, we examine the agreement of p16 and MIB1 positivity. After careful study, a sensitivity analysis using two different thresholds for both p16 and MIB 1 positivity was performed. Another manuscript will further delineate the sensitivity analysis performed for deciding the cutoff or threshold for each marker. The highest values for sensitivity and specificity in the analysis were noted for p16 threshold “0 staining as negative” versus “1–3+ staining as positive”. In contrast, for MIB1, the highest sensitivity and specificity were achieved for “0–1+ staining as negative” and “2–3+ staining as positive”. Using these thresholds, there is 90% agreement of specimens either negative or positive for those lesions for the diagnosis of HGSIL.

Table 3
Description of patient biopsy samples

<table>
<thead>
<tr>
<th>Description</th>
<th>Patients</th>
<th>Biopsy specimens with HCII HPV testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>208</td>
<td></td>
</tr>
<tr>
<td>Total biopsies</td>
<td>453</td>
<td></td>
</tr>
<tr>
<td>Consensus histologic diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>Atypia</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>LGSIL</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>HGSIL</td>
<td>144</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Insufficient tissue on recut</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>453</td>
<td></td>
</tr>
<tr>
<td>p16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>453</td>
<td></td>
</tr>
<tr>
<td>MIB1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>453</td>
<td></td>
</tr>
<tr>
<td>Biopsy Specimens for p16 and MIB1 staining</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ectocervix and endocervix</td>
<td></td>
<td></td>
</tr>
<tr>
<td>257</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ectocervix only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>154</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocervix only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsatisfactory</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>447</td>
<td></td>
</tr>
<tr>
<td>Biopsies available for ectocervical staining</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ectocervix with intermediate layer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>408</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ectocervix missing intermediate layer</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>411</td>
<td></td>
</tr>
<tr>
<td>Total biopsies available for final sensitivity and specificity analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>408</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4
Biopsy specimens stratified by HPV High-Risk +/-, histology, and biomarker (p16 and MIB1) positivity for ectocervical samples

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>HPV histology</th>
<th>p16 ectocervix</th>
<th>MIB1 ectocervix</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC-II</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>Normal</td>
<td>HR−</td>
<td>62</td>
<td>12</td>
</tr>
<tr>
<td>Normal</td>
<td>HR+</td>
<td>106</td>
<td>38</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>218/222</td>
<td>217/222</td>
</tr>
<tr>
<td>LGSIL</td>
<td>HR−</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>LGSIL</td>
<td>HR+</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>56/59</td>
<td>55/59</td>
</tr>
<tr>
<td>HGSIL</td>
<td>HR−</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>HGSIL</td>
<td>HR+</td>
<td>4</td>
<td>122</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>128/144</td>
<td>127/144</td>
</tr>
</tbody>
</table>

*Not all patients had HPV typing results and atypias excluded.

Table 5
Agreement of endocervical and ectocervical biopsy specimens

<table>
<thead>
<tr>
<th>Agreement for p16</th>
<th>Agreement for MIB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agree positive</td>
<td>21/2127 9%</td>
</tr>
<tr>
<td>Agree negative</td>
<td>74/74257 29%</td>
</tr>
<tr>
<td>Ecto + endo −</td>
<td>152/152257 59%</td>
</tr>
<tr>
<td>Endo + ecto −</td>
<td>10/10257 4%</td>
</tr>
<tr>
<td>Total</td>
<td>257 257</td>
</tr>
</tbody>
</table>
Finally, we analyzed how using both markers individually and together would impact the sensitivity and specificity for HG diagnosis. In Table 7, we show the result of the sensitivity and specificity of p16 in the intermediate layer, using each marker alone and together. The sensitivity for either marker was approximately 90%, and using both p16 and MIB1 together increased sensitivity by 5%. Similar gains are seen in specificity when both markers are used. Thus, if cost effective, both markers used together yield the highest sensitivities and specificities.

Discussion

Our objectives in this analysis to differentiate HGSIL from LGSIL, atypia, and normal were: (1) to examine the rate of HPV HR positivity, (2) to compare and grade the basal, parabasal, intermediate, and superficial layer staining of each marker, (3) to determine the optimal qualitative threshold for markers, (4) to compare p16 and MIB1 agreement, and (5) to examine the sensitivities and specificities using each marker alone and together.
We demonstrated that most HG specimens were HPV HR+, p16 +, and MIB 1+. We also demonstrated that staining of the endocervix and ectocervix was most often discordant and that the ectocervix stained positively much more often than the endocervix. Since these were endocervical curetage specimens from patients with squamous pathology, we acknowledge that the study of adenomatous lesions could yield different results.

We performed a layer by layer analysis to show that the basal and intermediate layers most often represented positivity on a global assessment. We performed a sensitivity analysis to determine the threshold or cutoff for positive versus negative within each marker (data not shown). Using those thresholds, we graded each sample. We showed that using both markers increased the sensitivity and specificity of the diagnosis of HG by 5% respectively to a sensitivity of 94% and specificity of 90%.

To our knowledge, this is the largest study of both p16 and MIB1 in ectocervical and endocervical samples stratified by HPV High-Risk positivity. The simple analysis of specimens outlined here shows that most HG specimens and many LG specimens were HPV HR positive. As we know, HPV HR positivity does not help in distinguishing HG lesions, yet HPV HR positivity may help in confirming a true HG lesion.

Clearly both p16 and MIB1 are often associated with HG histology. This study confirms the work of others. From the analysis of each marker by layer (basal, parabasal, intermediate, and superficial), we see definite increases as the lesions progress from normal to LG to HG. Additionally, since the data are stratified by HPV HR positivity, we can see that the presence of HR HPV increases the detection of p16 and MIB1.

One could speculate that HG lesions which are HPV HR positive can further confirm a “true” HG diagnosis, while suspected HG lesions which are HPV HR negative may be falsely HPV negative, insufficient for diagnosis, or falsely positive HG (i.e. <HG lesions). In any case, the differences in staining suggest that the specimens differ. The HPV HR negative sample size is also smaller than that of the positives, which increases the confidence intervals.

Another statistical issue for all studies of biomarkers is the use of these samples as independent samples when some samples came from the same patient. We have looked for confounding (data not shown) and did not find it a problem; however, we realize the theoretical issues in the design. We plan a future study using only one biopsy from each patient.

Further examination of the associated figures makes it clear that both the intermediate and parabasal layers are significantly different than the other layers. This led us to examine how the sensitivity and specificity could improve if the information in layers is used to refine the diagnosis. Not surprisingly, the intermediate layer positivity provided the greatest increase in sensitivity or specificity in diagnosing HG lesions, irrespective of HPV status.

Another issue is whether both markers are needed. One might argue intuitively yes, since both markers give different information. In our simple analysis of sensitivities and specificities when both markers had been tested, each resulted in sensitivities and specificities of ~85–90% and using them together improved the diagnosis by 5% for both sensitivity and specificity. This suggests that the parabasal or intermediate layer is useful and may be helpful with fragmented or small specimens.

One of the important reasons to use p16 and MIB1 is to decrease the inter-observer and intra-observer readings in pathology. We believe these observations support that hypothesis and in fact, could improve the diagnosis of HG lesions. One of the goals of our research team is to introduce quantitative pathology for both routine samples (stained with feulgen) and for immunostained markers such as these.

In a subsequent publication, we hope to show additional value in quantification of these markers as well as information from their quantitative analysis of nuclear texture and architecture [17–19]. Automating the reading of pathology could improve the accuracy and agreement of diagnoses in the developed world and perhaps decrease infrastructure requirements in the developing world.

Conflict of interest statement
We declare that we have no conflict of interest.

Acknowledgments

Special thanks go to all the patients who participate in our trials, Trey Kell, Deanna Haskins, Anita Carraro, Maria Theresa Arbalaez, Rosa Morales, Nan Earle, and Brian Crain.

References


Table 6
Agreement of biopsy specimens for MIB1 (0–1 versus 2–3) and p16 (0 versus 1–3) using intermediate layer positive

<table>
<thead>
<tr>
<th></th>
<th>Agree positive</th>
<th>Agree negative</th>
<th>P16 − and MIB1 +</th>
<th>P16 + and MIB1 −</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>p16 +</td>
<td>227</td>
<td>138</td>
<td>18</td>
<td>25</td>
<td>408</td>
</tr>
<tr>
<td>MIB1 +</td>
<td>227/408</td>
<td>138/408</td>
<td>18/408</td>
<td>25/408</td>
<td></td>
</tr>
<tr>
<td>p16 + and MIB1 +</td>
<td>56%</td>
<td>34%</td>
<td>5%</td>
<td>6%</td>
<td></td>
</tr>
</tbody>
</table>

Table 7
Biomarker sensitivity and specificity in the intermediate layer for HGSIL using 408 specimens from Table 6

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>p16 +</td>
<td>0.90</td>
<td>0.85</td>
</tr>
<tr>
<td>MIB1 +</td>
<td>0.89</td>
<td>0.87</td>
</tr>
<tr>
<td>P16 + and MIB1 +</td>
<td>0.94</td>
<td>0.90</td>
</tr>
</tbody>
</table>