DNA Ploidy Compared With Human Papilloma Virus Testing (Hybrid Capture II) and Conventional Cervical Cytology as a Primary Screening Test for Cervical High-Grade Lesions and Cancer in 1555 Patients With Biopsy Confirmation

Martial Guillaud, PhD[1]
John L. Benedet, MD[1,2]
Scott B. Cantor, PhD[3]
Gregg Staerkel, MD[4]
Michele Follen, MD, PhD[5,6]
Calum MacAulay, PhD[1]

[1] Department of Cancer Imaging, British Columbia Cancer Research Centre, Vancouver, British Columbia.

[2] Department of Gynecologic Oncology, British Columbia Cancer Centre, Vancouver, British Columbia.

[3] Department of Biostatistics and Applied Mathematics, University of Texas M. D. Anderson Cancer Center, Houston, Texas.

[4] Department of Pathology, University of Texas M. D. Anderson Cancer Center, Houston, Texas.

[5] Department of Gynecologic Oncology, Center for Biomedical Engineering, University of Texas M. D. Anderson Cancer Center, Houston, Texas.

[6] Departments of Obstetrics, Gynecology and Reproductive Sciences, University of Texas Health Science Center at Houston, Houston, Texas.

Supported by the National Cancer Institute under Program Project Grant 3PO1-CA82710-06.

The research group is indebted to the patients who participated in our studies. We also thank Branko Palcic, PhD, and Dave Garner, PhD, for substantative comments and Brian Crain (Center for Biomedical Engineering, University of Texas M. D. Anderson Cancer Center), for editing and article preparation. Finally, we also thank Deanna Haskins, Anita Carraro, Jagoda Korbelic (Department of Cancer Imaging, British Columbia Cancer Research Center); Rosa Morales, Juan Gomez, Maria Teresa Arbelaez, Nan Earle, Trey Kell (Center for Biomedical Engineering, University of Texas M. D. Anderson Cancer Center); Tatiana Aleexenko, and Chris King for their many contributions to this project.

Address for reprints: Michele Follen, MD, PhD, Center for Biomedical Engineering, University of Texas M. D. Anderson Cancer Center, Unit 193, 1515 Holcombe Blvd., Houston, TX 77030; Fax: (713) 792-4856; E-mail: mfollen@mdanderson.org

Received December 1, 2005; revision received February 24, 2006; accepted March 9, 2006.

BACKGROUND. Because 80% of cervical cancers arise in low-resource settings, many inexpensive strategies are being tested. In that spirit, the authors are testing large-scale genomic or DNA ploidy measurements as an inexpensive and semi-automated strategy.

METHODS. Patients entered either a screening or diagnostic study of several optical technologies: quantitative cytology, quantitative histopathology, and fluorescence and reflectance spectroscopy using a point probe, a multispectral digital colposcope, or a combination of the two. We calculated sensitivities, specificities, positive and negative predictive values, and their confidence interval testing conventional cytology, Hybrid Capture (HC) II testing, and DNA ploidy measured on the Feulgen-stained quantitative Pap smear.

RESULTS. The current investigation reports on 1555 patients for whom colposcopically directed biopsies were read 3 times by study pathologists. The final histopathologic diagnosis was high grade (cervical intraepithelial neoplasia [CIN] 2, CIN 3, carcinoma in situ [CIS], and cancer) in 16% of patients. Using high-grade squamous intraepithelial lesions (SILs) histopathology as the threshold and gold standard, the sensitivity and specificity, respectively, were: 0.47 and 0.96 for conventional cytology, 0.91 and 0.80 for HC II, and 0.59 and 0.93 for DNA ploidy. The positive and negative predictive values (PPV, NPV) for conventional cytology were 0.70 and 0.90, 0.46 and 0.98 for HC II, and 0.63 and 0.92 for DNA ploidy.

CONCLUSIONS. DNA ploidy shows comparable sensitivity, specificity, PPV, and NPV values to conventional cytology and HC II. Unlike conventional cytology, DNA ploidy is semiautomated and can be performed in less than 8 hours. Cost effectiveness studies are under way, but in the authors' laboratory DNA ploidy is inexpensive. ***Cancer* 2006;107:309–18.** © *2006 American Cancer Society.*

KEYWORDS: DNA ploidy, aneuploidy, cytology, cervical cancer, squamous intraepithelial lesions (SIL), cervical intraepithelial neoplasia (CIN), human papillomavirus, HPV DNA test, Hybrid Capture II, liquid-based samples, Cytyc, ThinPrep, quantitative measure cytology, stoichiometric staining quantitative image analysis.

DOI 10.1002/cncr.21993
Published online 13 June 2006 in Wiley InterScience (www.interscience.wiley.com).

TABLE 1
Results of Pooled Analyses from Sankaranarayanan et al.[3]

Screening methodology	No. of studies reviewed	No. of patients	Patients with HG and greater, %	Patients with HG range	Sensitivity pooled	Sensitivity range	Specificity pooled	Specificity range
VIA	11	54,981	16	7–27	0.77	0.56–0.94	0.86	0.74–0.94
VIAM	3	16,900	14	11–18	0.64	0.61–0.71	0.87	0.83–0.90
VILI	10	49,080	16	9–29	0.92	0.76–0.97	0.85	0.73–0.91
Cytology	5	22,663	6	2–14	0.58	0.29–0.77	0.95	0.89–0.99
HPV HC II	4	18,085	7	6–9	0.67	0.46–0.81	0.94	0.92–0.95

HG indicates high grade; VIA, visual inspection with acetic acid; VIAM, visual inspection with acetic acid and magnification; VILI, visual inspection with Lugol iodine, cytology, conventional cytology; HPV HC II, human papillomavirus Hybrid Capture II test (Digene, Gaithersburg, MD).

The basic element common to all successful cervical cancer screening programs is the cervical cytology or Papanicolaou smear. Screening programs based on cytology require an extensive, costly infrastructure. Because 80% of cervical cancers arise in low-resource settings, alternative methods are the focus of intense study.[1,2] Sankaranarayanan et al.[3] and others have demonstrated impressive results using visual inspection with acetic acid in single-armed and in a randomized controlled trial. These methods are in real-time, but require training. In Sankaranarayanan et al.'s provocative analysis, simple methodologies such as inspection with acetic acid (VIA), inspection with acetic acid and magnification (VIAM), or inspection with Lugol iodine (VILI) required little infrastructure and had better sensitivity and lower specificity than conventional cytology and Hybrid Capture II (HC II) testing.[4] Table 1 summarizes their pooled analysis for several populations in which the prevalence of high-grade cervical lesions and cancers ranged from 6% to 16%.

The standard cytology smear is stained with the Papanicolaou stain, which, like hematoxylin and eosin, works well to identify nuclear and cytoplasmic features but requires considerable training and skill to interpret. The Feulgen-Thionin stain is a stoichiometric stain, meaning that it has a linear relation to the amount of DNA in the cell nucleus.[5] The Feulgen-Thionin stain can be used to detect DNA ploidy using computer-assisted image cytometry. There is abundant evidence that alterations in large-scale genomic status, or DNA ploidy, constitute early events in carcinogenesis.[6] Sudbo et al.[7,8] carefully documented aneuploidy as a surrogate marker for mortality in oral cancer. DNA ploidy has been used as a prognostic factor and in treatment planning in breast cancer.[9] Aneuploidy has been correlated with recurrence and decreased survival in many cancers and is the best validated surrogate endpoint biomarker of cancer.[10]

Our group is testing the use of DNA ploidy as an inexpensive, semiautomated screening test for the detection of cervical cancer and its precursors. In the developed world, DNA ploidy could reduce cost. Once well tested, DNA ploidy could be used in the developing world to reduce cost and infrastructure. To that end, we designed both screening and diagnostic trials that measure DNA ploidy in addition to conventional cytology and human papillomavirus (HPV) HC II testing, quantitative histopathology, and other optical technologies against the gold standard of clinical histopathology.[11] In this preliminary analysis, we examined the performance of DNA ploidy compared with conventional cytology and HC II testing as methods for the detection of histopathologically confirmed high-grade lesions in 1555 patients. The prevalence of high-grade lesions and cancers in this study population was 16%, making this analysis comparable to the pooled studies of Sankaranarayanan et al.

MATERIALS AND METHODS

Patients

The study described was conducted at the University of Texas M. D. Anderson Cancer Center (Houston, TX), the University of Texas Health Science Center (Houston, TX), and at the British Columbia Cancer Agency and Research Centre (Vancouver, British Columbia, Canada). Nonpregnant women age 18 and older were enrolled in the study after written informed consent was obtained. All study protocols were approved by the Internal Review Boards at M. D. Anderson Cancer Center, the University of Texas Health Science Center, the Lyndon Baines Johnson Hospital Health District, British Columbia Cancer Agency, and the University of British Columbia.[11] Figure 1 shows the flow of patients and their procedures through the studies.

Clinical Specimens

Two Pap smears were obtained with an Ayre spatula and a Cytobrush. After the conventional Pap smears, the HC II swab was obtained, as per instructions.

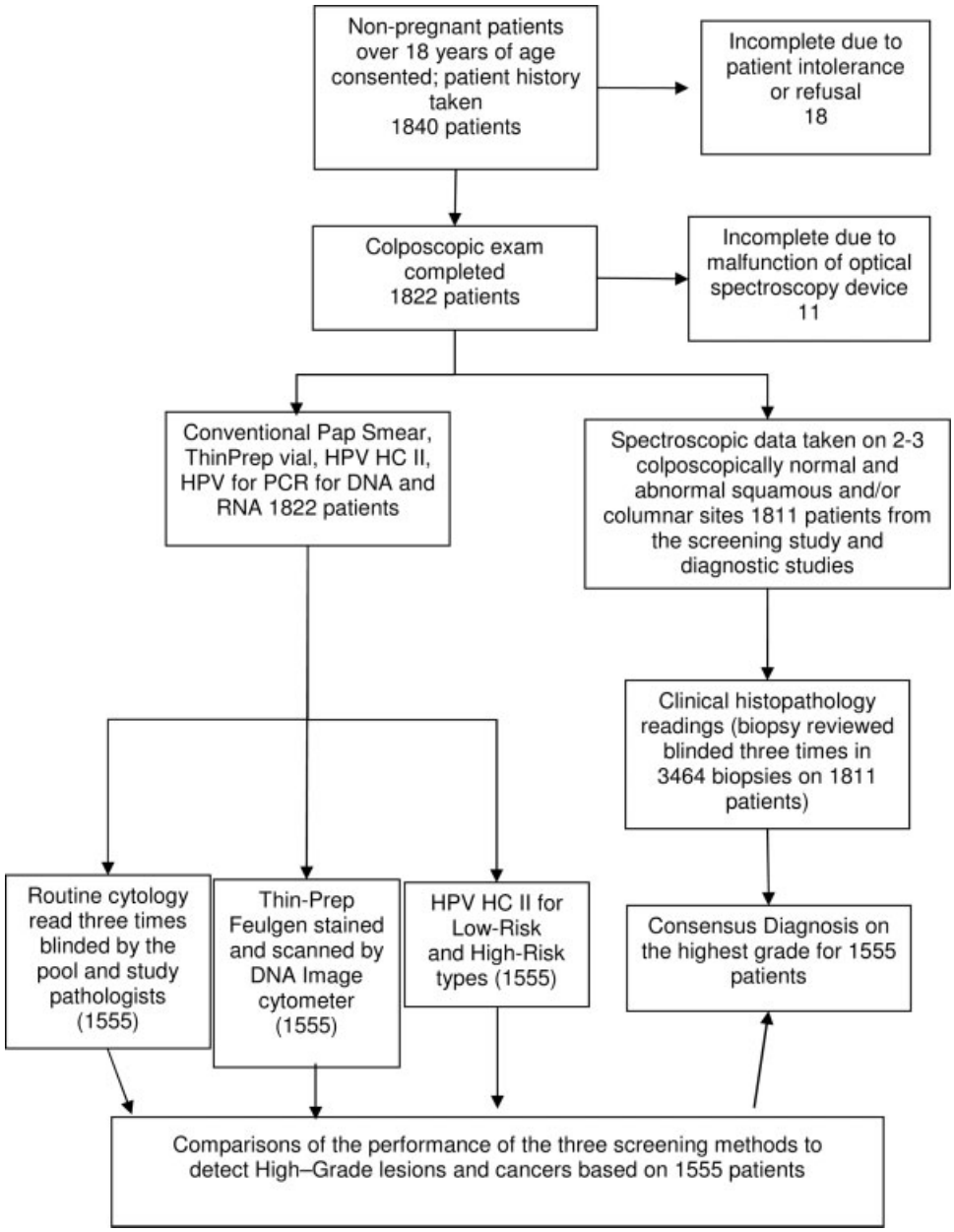

FIGURE 1. Optical technology study flow diagram.

The Ayre spatula and the Cytobrush samples were placed on a slide, fixed, and sent for Papanicolaou staining at the M. D. Anderson Department of Pathology and at the British Columbia Cancer Agency Department of Pathology. The second cytological sample was deposited in a vial with Cytyc solution followed by deposition onto slides using the ThinPrep method (Cytyc, ThinPrep, Marlborough, MA) for DNA ploidy analysis. All of these DNA ploidy samples were assessed at the British Columbia Cancer Research Centre Cancer Imaging Laboratory. The HC II swab was placed in the appropriate medium and sent to Labcorp (Houston, TX) for analysis.

Conventional Cytology and Histopathology

The first cytology and pathology reading was performed by 1 of the gynecological pathologists on clinical duty at each institution. A second blinded review was performed by 1 of the study pathologists (G.S., J.M., D.V.N.). In cases of disagreement between the two readings, the slide was read a third time by a study pathologist to provide a final consensus diagnosis. The range of agreement between the two institutions in a study of 1792 biopsies used the four-category Bethesda criteria: 0.70 for generalized, 0.69 for weighted, and 0.56 to 0.94 for unweighted binary categories.[12]

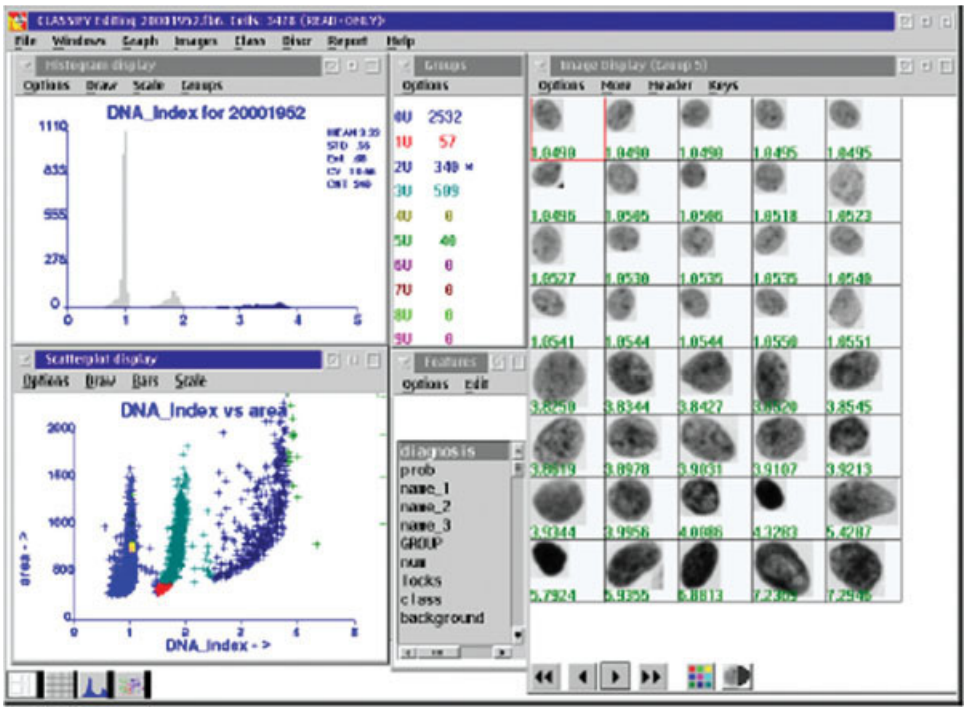

FIGURE 2. An image gallery from a Feulgen-stained ThinPrep slide visualized by the CytoSavant imaging software demonstrates the separation of different populations of cells based on DNA ploidy.

HPV Testing

Samples for HC II testing were collected after the Pap using the swab provided following the instructions provided with the test kit. All HC II samples were analyzed by Labcorp. Results were linked to patient bar codes and entered into the database. Costs of testing were paid from grant sources. For the purpose of this analysis, specimens were called positive if they were low-risk or high-risk positive. Both thresholds are reported: low-risk/high-risk and high-risk only.

DNA Image Cytometry on Liquid-Based Specimens

The second sample was processed using the ThinPrep technology. The specimens were stained by the Feulgen-Thionin method as detailed by Tezcan et al.[5] The sample was then analyzed using the CytoSavant, a fully automated high-resolution imaging cytometry system.[13] The CytoSavant was developed to be used as a semiautomated device for the British Columbia Cancer Screening Program.[14] The performance of the device has been extensively tested on air-dried cervical smears. This report is the first comprehensive report on liquid-based samples.

The cytometer employed a digital camera with a scientific CCD with approximately 1.4 million sensing elements of size of 6.8 μm $\times$ 6.8 μm square. The images of the cell nuclei were projected onto the CCD that was positioned in the primary image plane of the $\times$20 objective, resulting in an effective pixel size of 0.34 μm $\times$ 0.34 μm ($\sim$0.1 μm^2). A typical image of the nucleus of a cervical epithelial cell is represented by between 500 to 700 pixels.[15]

This system automatically loads each slide, scans the area of the ThinPrep deposition, collects images of every object detected, calculates a set of approximately 120 features for each object, and uses a multilevel decision tree to classify each object as either a cell nucleus or "junk." Figure 2 shows the interface of the CytoSavant program that allows one to interactively assess the image of cell nuclei with its corresponding morphometric features. The cytotechnologists visually reviewed each cell image and removed any object that did not fulfill the minimum quality requirements (bad mask, out of focus, pale nucleus, pyknotic nucleus, etc.).[16]

At least 300 (300–3000) epithelial normal cells were used in each specimen as the internal reference diploid population. The coefficient of variation (CV) of the DNA amount of these reference cells never exceeded 3%. This value is lower than the CV of 5% recommended by the European Society of Analytical Pathological consensus.[17,18] The DNA amount of each cell nucleus was normalized to account for batch-to-batch staining intensity variation.[19,20] All atypical cells were reviewed by an experienced cytotechnician and any equivocal or abnormal cells were visually reviewed by the senior cytopathologist at the British Columbia Cancer Agency (BCCA).

The resulting DNA ploidy value is expressed as a "c" value for chromosome. A DNA ploidy value

of 2c indicates a normal diploid cell, 4c a tetraploid cell; 5c is a cutoff used for aneuploidy by most authors, whereas Bollman et al.[21,22] and Lorenzato et al.[23] favor 9c. The number of cells with a DNA index higher than 5c is often called the 5c-exceeding rate (5cER).[24]

A rigorous quality control process was implemented[25] and met the standard requirements of the consensus reports of the European Society for Analytical Cellular Pathology (ESACP).[19,20]

DNA Ploidy Analysis

In this study, DNA aneuploidy was defined as a function of 3 parameters: 1) the number of cells counted on a given slide; 2) the DNA ploidy index, above which a cell is called aneuploid; and 3) a threshold value corresponding to the number of cells, above which a specimen is called aneuploid.

Studies were performed to determine both the optimal number of cells on the slide and the level of aneuploidy that maximized sensitivity and specificity for the level of cells. The dataset was divided into 500-cell increments containing less than 500 cells, to specimen increments containing over 3000 cells. The sensitivity and specificity were calculated for each subset.

Similarly, we examined the sensitivity and specificity resulting from the two parameters used in the definition of aneuploidy. The DNA ploidy index was examined at ranges from 2c to 9c. Secondarily, the presence of aneuploid cells was examined at ranges from 1 cell to 50 cells. The combination of these two parameters was then subjected to sensitivity and specificity calculations in order to determine the optimal sensitivity and specificity.

Statistical Analysis

The sensitivity is the fraction of true-positives (test positive for high grade) / total number of patients with disease (high-grade squamous intraepithelial lesion [HGSIL] on histopathology) using histopathology as the gold standard. The specificity is defined as the true-negatives (test negative for high grade)/ the total nondiseased (low-grade SIL [LGSIL] and normal histopathology). Positive predictive values (PPV) and negative predictive values (NPV) were calculated using the Baye theorem, which includes the prevalence of disease. Ninety-five percent confidence intervals (CI) were calculated for sensitivity, specificity, PPV, and NPV. These calculations were made using software programmed in C++.

All statistical analyses were performed with Stat Soft (Tulsa, OK) STATISTICA v. 6 software to calculate differences among values of ploidy values. As the values were continuous variables, analysis of variance (ANOVA) and a Fisher least significant difference (Fisher LSD) test for post-hoc comparison between groups was performed and the significance of the testing was recorded.

RESULTS

Patient Population

The study population consisted of 1840 patients accrued from optical screening (54%) and diagnostic (46%) studies. The mean age of study participants was 40 years old, with a range of 18 to 85 years. Overall, 74% of patients were accrued at the Houston site and 26% at the Vancouver site. The study population consisted of 56% Caucasian, 21% Hispanic, 13% African-American, 7% Asian, 1% Native American, and 3% other. Patients were eliminated from analysis if they did not complete the spectroscopic testing (18 patients) or if the device malfunctioned (11 patients). The analysis was limited to the 1555 patients for whom all specimens were currently complete: conventional cytologic and histopathologic review, ploidy analysis, HC II testing, and biographical data. A formal analysis of the data from each study separately is planned at the end of the trial.

Study Samples

In this well-validated sample 1021 of 1555 (66%) of specimens had "negative" histology. Of the total sample, 288 of 1555 (19%) were low-grade lesions and 246 of 1555 (16%) were high-grade lesions. Six specimens showed invasive cancer and they were included in the high-grade category for this analysis. Table 2 shows the study samples stratified by cytologic, HC II, and histopathologic diagnoses. Placing the 6 invasive cancers in the high-grade or worse category gave a prevalence of 16% diseased.

Optimization of DNA Ploidy

In order to determine the optimal parameters for ploidy, we tested both cellularity and both chromosome and number of aneuploid cells as thresholds. We first tested the effect of the ThinPrep specimen cellularity on the performance of DNA ploidy. For this analysis, a specimen was called aneuploid if it contained at least 1 cell with a DNA index higher than 5c. The sensitivity and specificity of DNA ploidy is lower when specimens with low cellularity (less than 500 cells on the slide) were analyzed. Table 3 shows the sensitivity/specificity by cell count per slide at 8 levels ranging from less than 500 cells per slide to over 3000 cells per slide. As expected, the larger the number of cells scanned, the higher the per-

TABLE 2
Patient Specimens Classified by Conventional Cytology, HPV HC II, and Histopathology Using the Bethesda Classification

		Histology			
Cytology	HPV	Negative	LG-SIL	HG-SIL	Total
Negative	Negative	848	175	13	1036
Negative	Low-risk	24	6	3	33
Negative	High-risk	92	51	64	207
Total		964	232	80	1276
LG-SIL	Negative	8	6	3	17
LG-SIL	Low-risk	0	1	0	1
LG-SIL	High-risk	22	32	47	101
Total		30	39	50	119
HG-SIL	Negative	4	7	5	16
HG-SIL	Low-risk	0	0	0	0
HG-SIL	High-risk	23	10	111	144
Total		27	17	116	160
Total		1021	288	246	1555

HPV indicates human papillomavirus; HG-SIL, high-grade squamous intraepithelial lesion; LG-SIL, low-grade squamous intraepithelial lesion.

formance. The performance was optimized at a level of 2000 cells.

Figure 3 shows the sensitivity and the specificity, respectively, of DNA ploidy for different values of two parameters: the DNA ploidy index (used for the definition of aneuploidy) and the minimum number of cells required to define a specimen as aneuploid (threshold values ranging from at least 1 cell to at least 50 cells). These two graphs show that the optimal values for sensitivity and specificity fall around a DNA ploidy of 5c and around a minimum number of aneuploid cells ranging from 1 to 5. The blue dot showing at least 1 cell was used to determine the maximum. Our data did not suggest that 9c was optimal, as did the work of Bollman et al.[21,22] and Lorenzato et al.[23]

Comparison of Technologies

Sensitivity, specificity, PPV, and NPV and the respective 95% CI were calculated for the three technologies. Table 4 shows the results of conventional cytology, HPV HC II testing, and DNA ploidy in our laboratory using these specimens with 2 to 3 blinded reviews. Each technology has relevant threshold values for the technologies. That is, atypical squamous cells of undetermined significance (ASCUS), low-grade and high-grade thresholds for cytology, high-risk thresholds for HC II testing, and 1 to 5 aneuploid cells present at an index of 5c for ploidy.

For the threshold of high-grade lesions, the highest sensitivity and specificity were achieved with HPV HC II, followed by DNA ploidy, then conventional cytology. In this population, with a 16% prevalence of disease, the highest PPV is achieved by conventional cytology, followed by DNA ploidy and HC II. The NPV value for HC II is high (0.92) but the PPV for HC II is 0.46. The values among the technologies are remarkably similar.

TABLE 3
Effect of the Cellularity on the Diagnosis of DNA Ploidy

No. of cells	No. of specimens	TN	FP	FN	TP	Sensitivity	Specificity
<500	99	84	4	4	7	unchanged	
>500	1456			unchanged			
<1000				unchanged			
>1000	1343	898			unchanged		
<2000				unchanged			
>2000	1114	725			unchanged		
<3000				unchanged			
>3000	621	351			unchanged		

TN indicates true negative; FP, false positive; FN, false negative; TP, true positive.

The potential of DNA ploidy to discriminate lesions of different histopathologic grades is illustrated in Figure 4, in which the number of nondiploid cells (DNA index higher than 2.2) is displayed by histopathologic diagnosis. The number of nondiploid cells is statistically higher in the high-grade biopsy specimens compared with the negative specimens ($P < 0.0005$) and compared with the low-grade specimens ($P < 0.0005$). The difference between low-grade specimens and high-grade specimens is not statistically significant but shows a similar trend in a significant direction ($P = 0.09$).

Potential Use of DNA Ploidy to Improve Diagnosis

In order to explore an additional dimension of DNA ploidy, that is, to see if cells the cytometer classified as aneuploid could be used to better predict histopathologic outcome and to see if aneuploidy could be used to better differentiate among HPV-positive lesions, we selected patients in whom the cytology was negative but the HPV HC II showed high-risk virus. In this group of patients, we calculated the number of aneuploid cells as a function of the histological diagnosis.

In those patients in whom conventional cytology was negative and HC II was positive, we were able to discriminate histologically negative specimens from high-grade specimens ($P < .01$), and low-grade specimen from high-grade specimens ($P = .02$) at statistically significant levels. This preliminary analysis suggests that DNA ploidy measured with liquid-based samples could be a more sensitive marker of true

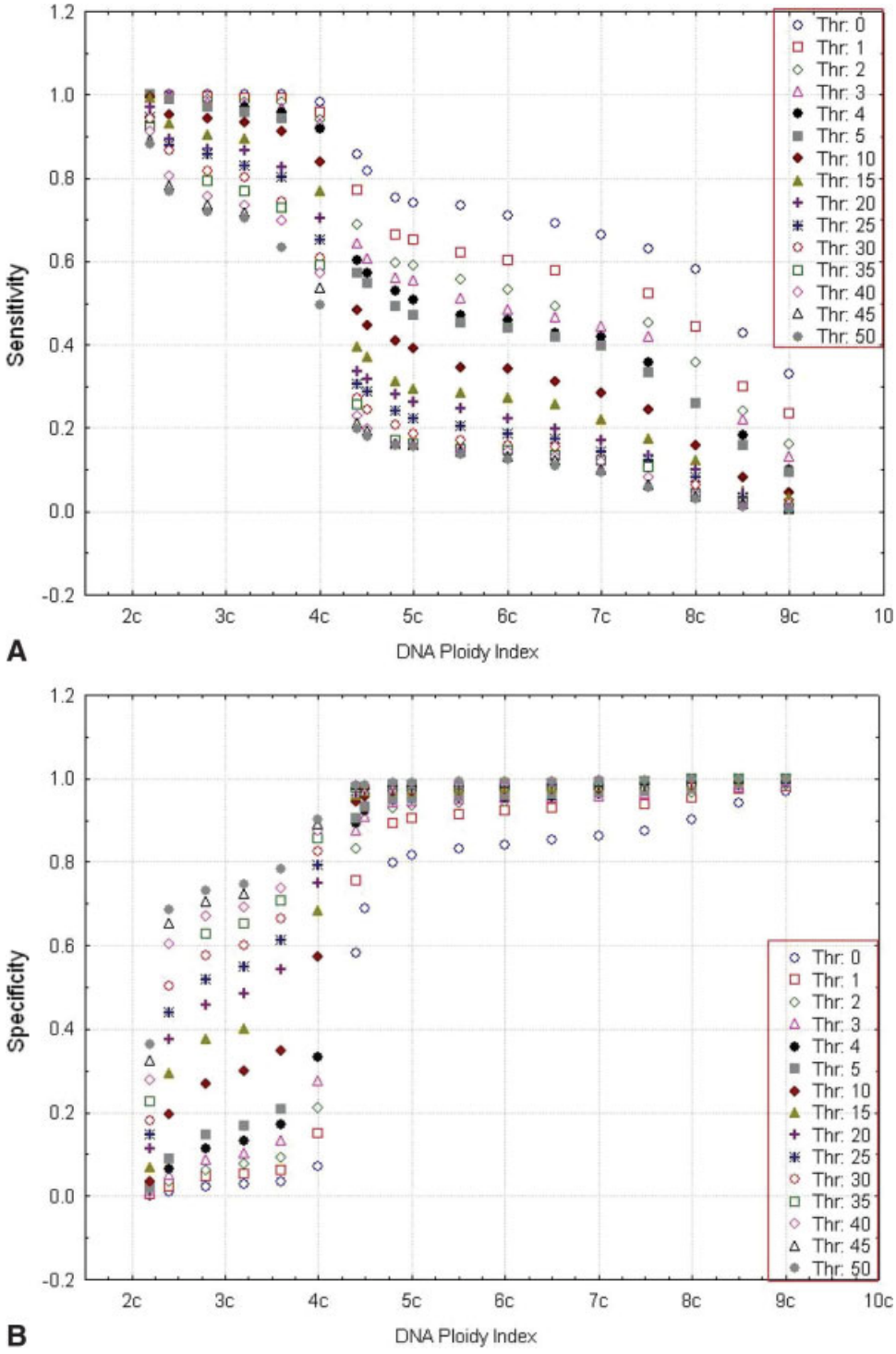

FIGURE 3. The (A) sensitivity and (B) specificity of DNA ploidy as function of two parameters: cut-off values for DNA ploidy index (ranging from 2.2c to 9c) and minimum number of cells used as threshold (ranging from 1 cell to 51 cells) using colposcopically directed biopsies showing high-grade lesions and cancer histopathology as the gold standard. Example: Blue circles represent the sensitivity and specificity of DNA ploidy when aneuploidy relates to at least 1 cell with a DNA ploidy index higher than 2.4c, 2.6c, etc., until 9c.

pathological state than conventional cytology. Additionally, although these patients were all HC II-positive, only one-third of them have high-grade disease (56 of 187). DNA ploidy resulted in increased specificity for high-grade lesions among HPV-positive, cytology-negative patients in this sample (see Fig. 5).

DISCUSSION

The introduction of new HPV vaccines could well diminish the incidence of cervical cancer to negligible levels within the next 20 years. Ideally, the vaccine should be given to prepubertal children and this will take some time to implement.[26] Meanwhile,

TABLE 4
Comparison of True Negative (TN), False Positive (FP), False Negative (FN), and True Positive (TP) Samples for Conventional Cytology, HC II, and DNA Ploidy

Prevalence of High-Grade Lesions and Cancers = 246/1555 = 0.158 (16%)	Threshold for positivity*	Total	TN	FP	FN	TP	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
Cytology														
	ASCUS+	1555	1212	97	102	144	0.59	0.52–0.65	0.93	0.91–0.94	0.60	0.53–0.66	0.92	0.90–0.94
	LG-SIL+	1555	1200	109	100	146	0.59	0.53–0.65	0.91	0.90–0.93	0.57	0.51–0.63	0.92	0.90–0.93
	HG-SIL+	1555	1264	45	137	109	0.44	0.38–0.50	0.96	0.95–0.97	0.7	0.63–0.77	0.9	0.88–0.99
HPV														
	High-risk	1555	1048	261	21	225	0.91	0.87–0.94	0.8	0.77–0.82	0.46	0.41–0.50	0.98	0.98–0.99
Ploidy														
	At least 1 5cER cell	1555	1068	241	64	182	0.74	0.67–0.79	0.82	0.79–0.83	0.43	0.38–0.47	0.94	0.92–0.95
	At least 2 5cER cell	1555	1183	126	86	160	0.65	0.58–0.71	0.9	0.88–0.92	0.56	0.50–0.61	0.93	0.91–0.94
	At least 3 5cER cell	1555	1223	86	101	145	0.59	0.52–0.65	0.93	0.92–0.94	0.63	0.56–0.68	0.92	0.9–0.93
	At least 4 5cER cell	1555	1238	71	110	136	0.55	0.48–0.61	0.95	0.93–0.95	0.66	0.59–0.71	0.92	0.90–0.93
	At least 5 5cER cell	1555	1245	64	121	125	0.51	0.43–0.56	0.95	0.93–0.96	0.66	0.59–0.72	0.91	0.89–0.92

HPV indicates human papillomavirus; HG-SIL, high-grade squamous intraepithelial lesion; LG-SIL, low-grade squamous intraepithelial lesion; ER, exceeding rate.
* From these values the sensitivity and specificity of each technology at the specified threshold were calculated. Using the prevalence of high-grade lesions and cancers of 16%, the positive predictive value (PPV), and negative predictive value (NPV) were calculated using Bayes Theorem. The gold standard for comparison of these technologies was the histopathology of colposcopically directed biopsies.

it is unclear at this time that the vaccine will benefit those patients who are already HPV-positive and those with precancerous and cancerous lesions. Thus, improving early detection of high-grade and cancerous lesions remains a priority.

Different problems arise in high- and low-resource settings. In high-resource settings, HPV testing with HC II has been demonstrated to be useful in the triage of ASCUS smears and is now an established part of clinical care.[27,28] Arbyn et al.[29] have demonstrated the usefulness of HC II in the follow-up of treatment failures of high-grade lesions. HC II testing has been proposed in cervical screening limited to women over 35 years of age, in whom HPV positivity confers a higher risk of the development of cervical cancer compared with those women under the age of 35, in whom HPV positivity implies transient infections.[30]

The possibility of using cervical cytology to diagnose uterine cancer was first put forth in 1928, but the concept of using it as a screening test was not developed until the historic article by Papanicolaou and Traut in 1941.[31] Over the next 3 decades, those countries that embarked on comprehensive cytology screening programs were able to show a 70% reduction in the incidence and mortality of cervical cancer, leading to the recognition that the cervical cytology smear was the best cancer screening test developed.[32] Unfortunately, the benefits available from screening programs have not been shared among all women, particularly those from the developing world, where cervical cancer continues to be the most common cause of death from malignant disease. The lack of appropriate resources has been the major barrier to successful screening programs in these countries.

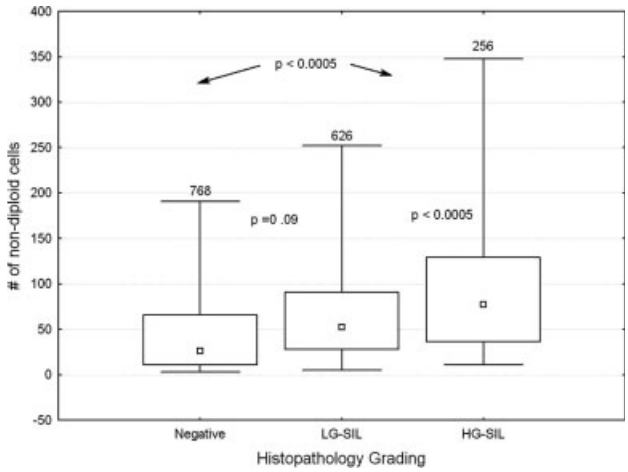

FIGURE 4. The number of nondiploid cells in samples classified by histopathology according to the Bethesda system. Nondiploid cells have a DNA index higher than 2.2. The black squares represent the median of the number of nondiploid cells, the boxes represent the 50th percentile, and the error bars represent the 5th and 95th percentiles of the number of cells.

A major problem for established programs is the moderate sensitivity of traditional Pap smear testing. Whereas most cancers seen in countries with comprehensive screening programs occur in women who neither participate in or who have smears infrequently, approximately 40% of cancers are diagnosed in women who have participated in screening pro-

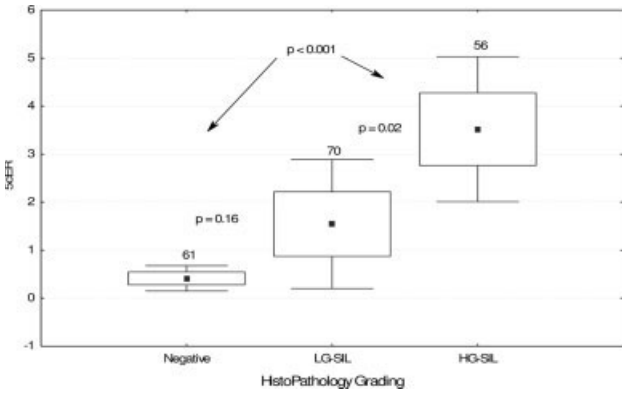

FIGURE 5. The number of cells that have greater than or equal to 5c DNA content, also called the 5c exceeding rate, versus the histopathologic grade, in patients in whom the conventional cytology was negative and the HC II positive for high-risk virus. The black boxes represent the mean of the number of cells, the box represents the standard error of the mean, and the error bars represent 2 times the standard error of the mean number of cells.

grams. Two major developments that were supposed to help with this problem of false-negative smears are, first, the use of liquid-based cytology collection and, second, the development of tests to identify high-risk strains of HPV. A recent metaanalysis shows that liquid preparations do not make more smears satisfactory nor do the preparations capture higher-grade cells. The real benefit of liquid-based preparations is in automated reading.[33,34] Several studies have been conducted to show the superior qualities of liquid-based smears compared with conventional smears. The role for HPV testing in patients with atypical cells of uncertain significance is established.[27,28] Whereas these strategies may help results in developed countries, they are beyond the reach of most countries with emerging economies.[35,36]

Several strategies have recently been described in an attempt to improve both the sensitivity and specificity of cervical cancer screening programs in low-resource settings. Sankaranarayanan et al.[3,4] performed a weighted analysis of several primary screening methodologies. These strategies included variations of clinical exam, such as VIA, VIAM, and VILI. Using VILI, their pooled analysis of 49,080 patients reported a sensitivity of 0.92 (range, 0.76–0.97) and specificity of 0.85 (range, 0.73–0.91). These studies are impressive, but suffer from a lack of histopathologic review. Thus, they may overstate the performance of histopathology in these settings. Other groups are exploring optical technologies such as fluorescence and reflectance spectroscopy. Yet others are experimenting with improvements in cytologic sampling such as shorter intervals between screens, liquid-based cytology preparations, HPV testing, DNA ploidy, and various molecular markers associated with integration of HPV (e.g., P16). More recently, the use of HPV testing alone or in combination with cervical cytology has been proposed as a means of improving the sensitivity and specificity of cytology screening programs.[30] The addition of tests improves sensitivity and specificity, but also increases cost.

DNA ploidy as used in this study could be used for primary cervical cancer screening, particularly in low-resource settings, as is it can be performed by a semiautomated system with a sensitivity, specificity, PPV, and NPV comparable to the those values obtained with either conventional cytology or HPV HC II testing. In that spirit, our collaborators have trained staff in China to use this technology in less than 2 months and have shown that this technology is preferable to conventional cytology.[37] The CytoSavant is being rapidly implemented there for primary cervical cancer screening.

Although detailed cost comparisons and effectiveness studies have yet to be published, many features of DNA ploidy make it an attractive technology for established programs wanting to improve quality control. The low cost, minimal training requirements, speed of analysis, and semiautomated features of DNA ploidy measurement make it ideal for primary cervical screening in low-resource settings where the impact would be the highest.

REFERENCES

1. Parkin DM, Bray FI, Davassa SS. Cancer burden in the year 2000. *Eur J Cancer*. 2001;37:S4–66.
2. International Agency for Research on Cancer – IARC Screening Group. Accuracy of screening test. Available at URL: http://screening.iarc.fr/study_acc.php?lang=1 Accessed November 11, 2005.
3. Sankaranarayanan R, Rajamanickam R, Theresa R, et al. Initial results from a randomized trial of cervical visual screening in rural south India. *Int J Cancer*. 2004;109:461–467.
4. Sankaranarayanan R, Gaffkin L, Jacob M, Sellors J, Robles S. A critical assessment of screening methods for cervical neoplasia. *Int J Gynecol Obset*. 2005;89:S4–S12.
5. Tezcan A, Garner DM, Lam P, Korbelik J, Palcic B. Analysis of thionin, gallocyanin, and hematoxylin for automated quantitative image cytometry of cervical samples. 8th Annual Meeting, Clinical Applications of Cytometry 1993:15–18.
6. Sudbo J, Reith A. The evolution of predictive oncology and molecular-based therapy for oral cancer prevention [Review]. *Int J Cancer*. 2005;115:339–345.
7. Sudbo J, Lippman SM, Lee JJ, et al. The influence of resection and aneuploidy on mortality in oral leukoplakia. *N Engl J Med*. 2004;350:1405–1413.
8. Sudbo J, Bryne M, Johannessen, Kildal W, Danielson HE, Reith A. Comparison of histological grading and large-scale genomic status (DNA ploidy) as prognostic tools in oral dysplasia. *J Pathol*. 2001;194:303–310.

9. Fitzgibbons PL, Page DL, Weaver D, et al. Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med*. 2000;124:966–978.
10. Follen M, Crain S, MacAulay C, et al. Optical technologies for cervical neoplasia: update of an NCI program project grant. *Clin Adv Hematol Oncol*. 2005;3:41–53.
11. Follen M, Schottenfeld D. Surrogate endpoint biomarkers and their modulation in cervical chemoprevention trials. *Cancer*. 2001;91:1758–1776.
12. Malpica A, Matisic JP, Niekirk DV, et al. Kappa statistics to measure interrater and intrarater agreement for 1790 cervical biopsy specimens among twelve pathologists: qualitative histopathologic analysis and methodologic issues. *Gynecol Oncol*. 2005;99:S38–52.
13. Garner D, Harrison A, MacAulay CF. Cyto-Savant^{TM} and its use in automated screening of cervical smears. In: Wied GL, editor. Compendium on the computerized cytology and histology laboratory. Chicago, IL: Tutorial of Cytology, 1994. p 346–352.
14. Anderson G, MacAulay CF, Matisic J, Garner D, Palcic B. The use of an automated image cytometer for screening and quantitative assessment of cervical lesions for screening. Columbia Cervical Smear Screening Programme. *Cytopathology*. 1997;8:298–312.
15. Palcic B, Garner DM, MacAulay CF, J Matisic J, Anderson GH. Oncometrics Imaging Corporation and Xillix Technologies Corporation. Use of the Cyto-Savant in quantitative cytology. *Acta Cytol*. 1996;40:67–72.
16. Doudkine A, MacAulay C, Poulin NB, Palcic B. Nuclear texture measurements in image cytometry. *Pathologica*. 1995; 87:286–299.
17. Haroske G, Giroud F, Reith A, Bocking A. 1997 ESACP consensus report on diagnostic DNA image cytometry. Part I. Basic considerations and recommendations for preparation, measurement and interpretation. *Anal Cell Pathol*. 1998; 17:189–200.
18. Giroud F, Haroske G, Reith A, Bocking A. 1997 ESACP consensus report on diagnostic DNA image cytometry. Part II. Specific recommendations for quality assurance. *Anal Cell Pathol*. 1998;17:201–201.
19. Guillaud M, Cox D, Malpica A, et al. Quantitative histopathological analysis of cervical intra-epithelial neoplasia sections: methodological issues. *Cell Oncol*. 2004;26:31–43.
20. Guillaud M, Cox D, Adler-Storthz K, et al. Exploratory analysis of quantitative histopathology of cervical intraepithelial neoplasia: objectivity, reproducibility, malignancy-associated changes, and human papillomavirus. *Cytometry*. A2004;60:81–89.
21. Bollman R, Mehes G, Speich N, Schmitt C, Bollman M. Aberrant, highly hyperdiploid cells in human papillomavirus-positive cytologic samples are associated with progressive lesions of the uterine cervix. *Cancer Cytopathol*. 2005;105:96–100.
22. Bollman R, Mehes G, Torka R, Speich N, Schmitt C, Bollman M. Human papillomavirus typing and DNA ploidy determination of squamous intraepithelial lesions in liquid-based cytologic samples. *Cancer Cytopathol*. 2003;99: 57–62.
23. Lorenzato M, Clavel D, Masure M, et al. DNA image cytometry and human papillomavirus (HPV) detection help to select smears at high risk of high-grade cervical lesions. *J Pathol*. 2001;194:171–176.
24. Bocking A, Nguyen VQ. Diagnostic and prognostic use of DNA image cytometry in cervical squamous intraepithelial lesions and invasive carcinoma. *Cancer (Cancer Cytopathology)*. 2004;102:41–54.
25. Chiu D, Guillaud M, Cox D, Follen M, MacAulay C. Quality assurance system using statistical process control: an implementation for image cytometry. *Cell Oncol*. 2004;26: 101–117.
26. Shaw AR. Human papillomavirus vaccines in development: if they're successful in clinical trials, how will they be implemented? *Gynecol Oncol*. 2005;99:S246–248.
27. ASCUS-LSIL Triage Study (ALTS). Group results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. *Am J Obstet Gynecol*. 2003;188:1383–1392.
28. Wright TC, Cox JT, Massad LS, Twiggs LB, Wilkinson EJ. ASCCP-Sponsored Consensus Conference. 2001 consensus guidelines for the management of women with cervical cytological abnormalities. *JAMA*. 2002;287:2120–2129.
29. Arbyn M, Paraskevaidis E, Martin-Hirsh P, Prendiville W, Dillner JA. Clinical utility of HPV-DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN: an update of pooled evidence. *Gynecol Oncol*. 2005;99:S7–11.
30. Wright TC, Schiffman M. Adding a test for human papillomavirus DNA to cervical cancer screening. *N Engl J Med*. 2003;348:489–490.
31. Papanicolaou GN, Traut HF. The diagnostic value of vaginal smears in carcinoma of the uterus. 1941. *Arch Pathol Lab Med*. 1997;121:211–224.
32. Benedet JL, Anderson GH. Cervical intraepithelial neoplasia in British Columbia: a comprehensive program for detection, diagnosis, and treatment. *Gynecol Oncol*. 1981; 12:S280–291.
33. Davey E, Barratt A, Irwig L, et al. Effect of study design and quality on unsatisfactory rates, cytology classification, and accuracy in liquid-based versus conventional cytology: a systematic review. *Lancet*. 2006;367:122–132.
34. Obwegeser J, Schneider V. Thin-layer cervical cytology: a new meta-analysis. *Lancet*. 2006;367:88–89.
35. Anderson GH, Boyes DA, Benedet JL, et al. Organisation and results of the cervical cytology screening programme in British Columbia, 1955–85. *Br Med J (Clin Res Ed)*. 1988;296:975–978.
36. Fahey MT, Irwig L, Macaskill P. Meta-analysis of Pap test accuracy. *Am J Epidemiol*. 1995;141:680–689.
37. Sun XR, Wang J, Garner D, Palcic B. Detection of cervical cancer and high grade neoplastic lesions by a combination of liquid-based sample preparation and DNA measurement using automated image cytometry. *Cell Oncol*. 2005;27:33–41.