Cellular Oncology 27 (2005) 33–41
IOS Press

Detection of cervical cancer and high grade neoplastic lesions by a combination of liquid-based sampling preparation and DNA measurements using automated image cytometry

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Abstract. *Objective*: To establish if measurements of DNA ploidy could be used to assist cytopathologists and cytotechnologists in population based cervical cancer screening programs in countries where manually reading the slides is impossible due to the lack of sufficient skilled cytotechnologists. The goal of such program is to identify only clinically significant lesions, i.e. those where a clinical intervention to remove the lesion is required immediately. *Study design*: A total of 9905 women were enrolled in the study. Cervical samples were taken with a cervix brush that was then placed into a fixative solution. The cells were separated from mucus by mechanical and chemical treatment and then deposited onto microscope slides by a cytocentrifuge. Two slides were prepared from each case; one slide was stained by Papanicolaou stain for manual cytology examination, while the other slide was stained by a DNA specific stain. The latter slide was used to determine the relative amount of DNA in the cell nuclei. *Results*: A total of 876 women were followed by colposcopy examination where biopsies were taken from the visible lesions or from suspicious areas and histopathology diagnosed 459 as normal or benign cases, 325 as CIN1, 36 as CIN2, 25 as CIN3/CIS, and 31 as invasive cancer. Of these 876 cases, manual cytology called 655 normal or ASCUS, 197 as LSIL, 16 cases as HSIL, and 8 as cancer. DNA measurements found 704 cases having no cells with DNA greater than 5c, 98 cases where there were 1 or 2 cells having DNA amount greater than 5c, and 74 cases where there were 3 or more cells having DNA amount greater than 5c. If manual cytology were to be used to refer all cases of HSIL and cancer to colposcopy and biopsy, 23 lesions that had to be removed would have been discovered (2 CIN2, 11 CIN3/CIS, and 10 cancers), for a sensitivity of $25.0 \pm 5.2\%$ at specificity of $99.9 \pm 0.1\%$. If DNA assisted cytology were to be used instead, and all cases having 3 or more cells with DNA amount greater than 5c were to be referred to colposcopy and biopsy, then 50 lesions that had to be removed would have been discovered (10 CIN2, 15 CIN3/CIS and 25 cancers) for the sensitivity of $54.3 \pm 6.2\%$ at specificity of $96.9 \pm 0.6\%$. *Conclusions*: The study suggests that screening for high grade cervical neoplastic lesions and cervical cancer by DNA assisted cytology could be implemented with minimal use of skilled cytotechnologists, at least in those countries where it would be difficult to introduce population based screening for cervical cancer due to the lack of availability of such skilled cytotechnologists.

Keywords: Cervical intraepithelial neoplasia (CIN), liquid based sample preparation, image cytometry, DNA ploidy, cytotechnologist shortage, cytology, sensitivity, specificity, invasive cancer

1. Introduction

The Pap test has been a powerful tool for detecting cancerous and precancerous cervico-vaginal lesions. In countries where well organized population based screening programs are in place, the incidence of invasive cervical cancer and mortality due to cervical can-

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cer has dropped dramatically [2,14,24]. Unfortunately, there are many countries in the world where population based screening has not yet been implemented due to the shortage of skilled cytotechnologists and the relatively high cost. In addition, many cervical screening programs have a high false-negative rates ranging from 2% to 40% [10,17,22,26,30]. It is has been postulated that some of the key contributors to this false negative rates come from sample collection and sample preparation errors [9,13,19].

Over the past three decades numerous attempts have been made to reduce the high false-negative rates as well to introduce new technologies to assist in the cervical screening programs. The use of the cervix brush or cytobrush has been claimed to increase the yield of endocervical and metaplastic cells and has provided a more even distribution of cells on the microscope slides [18,20,35,36]. Liquid-based, thin-layer preparations were also developed and are currently being marketed as an improved alternative method to deposit cervical cells onto the slides [23,27,31,37]. For example, the use of ThinPrep (Cytyc, MA, USA) reported an increase of 13% in the rate of detection of cervical intraepithelial neoplasia (CIN), as compared with the non-liquid based Pap smear technique [37]. However, the high cost of this technology currently limits its widespread use in most developing countries.

The detection and correct diagnosis of high grade cervical intraepithelial neoplasia, carcinoma *in situ* (CIS) and invasive cancer lesions is a difficult task in the cytological evaluation of cervico-vaginal smears [4,13,21]. The performance of several laboratories was summarized by van der Graaf et al. [34] who reported that only about half of biopsy-documented invasive cancers were appropriately recognized by cytology of cervical smears. To increase the diagnostic accuracy and to avoid mistakes by cytopathology, several cytometry based systems have been developed over the past three decades for the detection of abnormal DNA content of the cervical epithelial cells [5,6,12,25]. We have been using a high resolution, fully automated system (Cyto-Savant) that was originally developed at the British Columbia Cancer Agency [1,12,15]. Similar systems have also been used to investigate the malignant potential of cervical intraepithelial neoplasia [6], as well as for the detection of early cancers and neoplastic lesions of lung and other organs [16,29,32].

In this paper we report an investigation on whether or not a simple, low cost, liquid based sample preparation in combination with DNA ploidy status assessments by a fully automated image cytometer could be employed for a cytotechnologist resource effective, population based screening for cervical cancer and high grade cervical neoplastic lesions.

2. Materials and methods

2.1. Sample collection and sample preparation

The study enrolled a total of 9905 women from the Wuhan (population 7 million) region of Hubei Province (population 60 million), China, comprised of 3236 women undergoing their routine screening and 6669 women undergoing their first screening test. The women were invited to come to either Landing Early Cancer Detection Center (LECDC) in Wuhan or to one of the several local hospitals. In some cases samples were collected at the nearby villages where a specially equipped van served as a mobile examination and sample collection facility. More than half of the women of this study were from rural villages near Wuhan. The samples were obtained by employing a cervix brush and the brush tip was immediately immersed in 30 ml of fixative (SedFix, Surgipath Medical Inc., Richmond, IL) in 50 ml plastic vials. Vials from all collection sites were transferred to LECDC where the specimens were further processed by first adding dithiothreitol (DTT, Sigma) to the final concentration of 0.1% of DTT. The cell suspensions were then mildly agitated for 1 hour to release cells from the brush into the suspension and to disaggregate the cells from mucus and cell clusters. The cells were then washed twice with 50% alcohol. Two slides were prepared from each specimen by cytocentrifugation onto microscope slides forming a uniform thin deposition layer. This preparation contained, on average, 10,000 cervical cells deposited in a round spot of 7 mm diameter (39 mm^2) positioned in the center of the slide. One of the two slides was stained with the Papanicolaou stain for manual cytology reading of the slide, and the other slide was stained with the DNA specific and stoichiometric (Feulgen–Thionin) stain [33] for the DNA ploidy status assessment of the cells by automated image cytometry.

Women presenting any cytological abnormalities as determined either by manual cytology or by detecting any DNA ploidy atypia (see below) were asked to attend colposcopy examination. In addition 75 women came to colposcopy examinations due to clinical symptoms. Of over 1100 invited women, the total of 876 women attended colposcopy examinations, typically

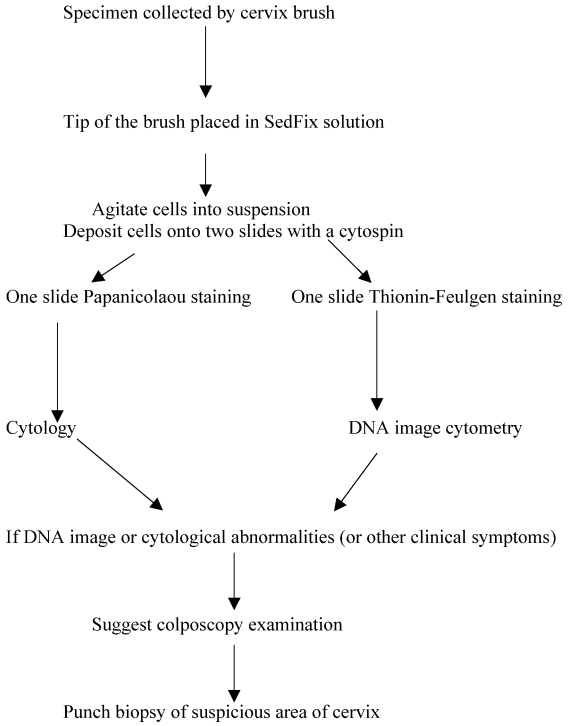

Fig. 1. Experimental protocol. Protocol used to prepare two sister slides for manual cytology and DNA assisted cytology from the same specimen and to select subjects for colposcopy.

within a month of the initial Pap test, and punch biopsy specimens (the standard of care in Hubei) were taken from the visible lesions or from suspicious areas. The full experimental protocol is illustrated in Fig. 1.

To compare (a) our liquid based sample processing procedure to the smear deposition and (b) sampling by Ayer's spatula versus the cervix brush, a small study was carried out enrolling a sub-population of 50 women consecutively chosen from the start of the study. In this study one sample was taken with an Ayer's spatula and two slides were made by smearing the cells onto the slide. A second sample was then taken by a cervix brush and two smear slides were made first by "painting" the cells onto the slides and then the tip of the brush was transferred into vials with SedFix solution to make another two slides by a cytocentrifuge as described above. This process produced three pairs of slides that came from the same subject. One slide from each pair was stained with Papanicolaou stain for subjective evaluation of the quality of the cytology preparation. The sister slide from each pair was stained by Feulgen–Thionin stain for image cytometry measurements.

2.2. Cytology

All Papanicolaou stained slides were examined independently by two cytopathologists. The smears were classified into one of the five groups according to the Bethesda system: (i) within normal limits or benign; (ii) with ASCUS (atypical squamous cells of undermined significance); (iii) low-grade squamous intraepithelial lesion (LSIL); (iv) high-grade squamous intraepithelial lesion (HSIL)/carcinoma *in situ* (CIS); and (v) squamous cell carcinoma. A total of 39 cases of atypical glandular cells (AGC) and one case of endocervical adenocarcinoma *in situ* (AIS) were also found and these cases were excluded from the study. All women with any form of atypia were invited to colposcopy as a part of the study protocol.

2.3. Image cytometry

All Thionin–Feulgen stained slides were scanned by the Cyto-Savant high resolution image cytometer [11,28] which is equipped with a slide loader and scans slides in a fully "walk away" automated fashion. The cytometer employed a digital camera with a scientific CCD with approximately 1.4 million sensing elements of effective 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 20 times 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 between 500–700 pixels. The image of each cell nucleus was captured in an exact focus and the nuclear material was segmented from the background in a fully automated manner using algorithmic approaches [28]. For each nucleus over 100 nuclear features were calculated including morphological features, photometric features, discrete texture features, Markovian and non-Markovian texture features, run-length features and fractal features [11]. These features were used to identify objects as true cell nuclei or "junk" (overlapping cell nuclei, out of focus cell nuclei, cellular debris, etc.) as well as to classify the nuclei to belong to different cell types in a fully automated way. The mean integrated optical density (IOD) value of the 2c diploid cells from each slide was used to automatically normalize the optical density features to compensate for any stain intensity variations between the slides.

On average, from each slide approximately 6000 quality images of isolated cell nuclei were collected and stored in the computer memory of the cytometer.

The nuclei, determined by the system to be epithelial cells, were used to calculate and plot the DNA distribution histograms. The histograms were called normal if they corresponded to diploid cells with a low proliferation fraction (S+G2+M) according to the classification of Auer et al. [3]. All other histograms suggesting the presence of:

(i) any cells with DNA $>$ 5 c;
(ii) diploid cells with a very high proliferation rate where 10% or more of the total cells were found in the proliferation fraction; and
(iii) a population of aneuploid stem cells,

were called suspicious or potentially abnormal (see examples in Fig. 2) and the women corresponding to any of such histogram were called for a colposcopy examination. Of all women who were called for and who attended colposcopy on the basis on the DNA histogram analyses only 8 women were not also invited to attend colposcopy based also on the manual cytology call.

By the study protocol, all cytometry images of objects appearing to have a DNA amount greater than 5c were examined microscopically by a cytotechnologist to eliminate any artefacts such as dust, air bubbles, overlapping cells, etc. from the $>$5c cell galleries. Approximately 7% of all 9905 slides required such examination, taking on average less than 5 minutes per slide.

2.4. Pathology

Biopsy specimens were taken from suspicious areas for histo-pathological diagnosis from 876 women comprised of 793 cases with some form of cytological atypia and 83 cases of clinical suspicion despite normal cytology, of which only 8 cases had an atypical DNA histogram. Pathology reports of each specimen were generated independently by two experienced pathologists.

2.5. Statistical analysis

Values are expressed as means $\pm$ SEM. Statistical significance was set at $P < 0.05$ and determined by a two-way analysis of variance followed by the Newman–Keuls test. Sensitivity and specificity are calculated using the following formulae:

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}} \times 100\%,$$

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative} + \text{False positive}} \times 100\%,$$

and were reported with $\pm$ one standard deviation.

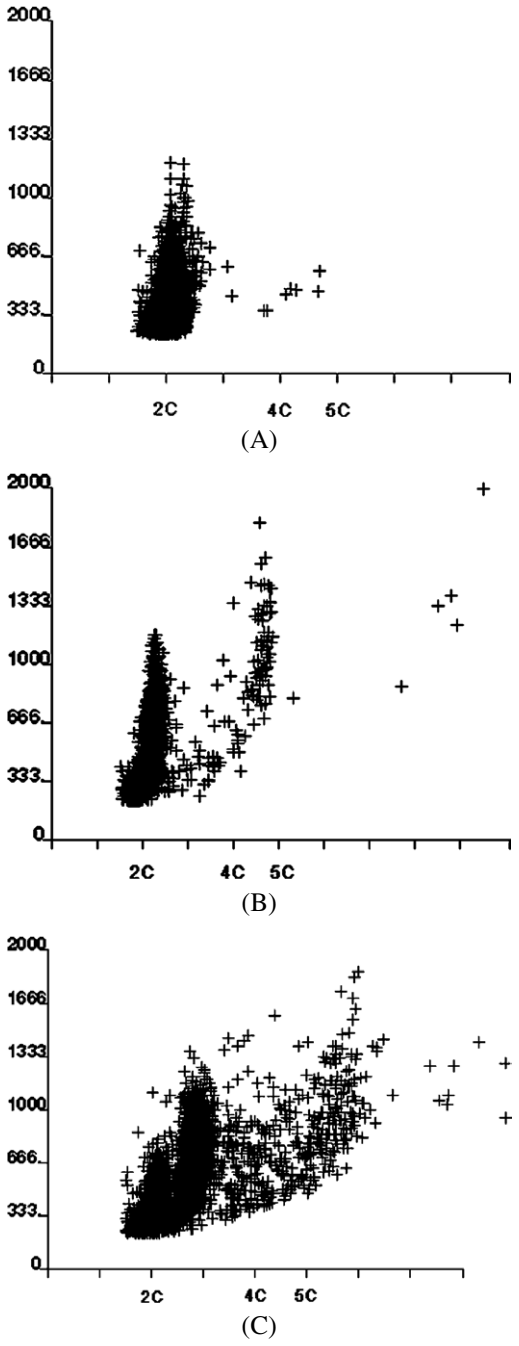

Fig. 2. DNA amount vs. nuclear size scattergrams. Cervical cell nuclei were stained by Thionin–Feulgen stain. The horizontal bar on the y-axis indicates the nuclear area in number of pixel (one pixel representing approximately 0.1 μm^2 area). (A) Histogram of the DNA amount distribution of normal cervical cells without any 5c cells (DNA index, DI 2.5 = 5c). (B) Histogram of the DNA amount distribution of a CIN3 case with a few cells having DNA amount greater then 5c. (C) DNA histogram of a case of invasive cervical cancer with aneuploid stem cells and a large number of cells with DAN amount greater than 5c.

3. Results

3.1. General results

Of the total of 9905 smears, manual cytology found 8867 (89.52%) smears were within normal limit, and 1038 (10.48%) had some form of cytological abnormality, including 632 (6.38%) with ASCUS, 368 (3.72%) with LSIL, 30 (0.3%) with HSIL and 8 with cancers (0.08%).

Of the 9905 cases, DNA histograms were considered normal for 9,482 (95.7%) cases and suspicious in 423 (4.3%) cases. Of these, 172 had at least one cell with DNA amount greater than 5c and the rest were found with too high proliferation index due to some other form of inflammation, repair processes, etc. There were a couple of stem cell-like aneuploid cases but they had also a large number of cells with DNA amount greater than 5c.

3.2. Comparison of conventional sample taking and sample deposition with liquid-based, monolayer sample preparation

Table 1 summarizes the results of the sample deposition studies. Using either Ayer's spatula or cervix brush, the number of total isolated cells measurable on the slide by the image cytometer was about 4 fold higher by liquid based preparation followed by cytocentrifuge deposition of the cells on the microscopy slides in comparison to the conventional preparation (10,800 cells vs. 2300). Similarly, the number of measurable epithelial cells was about 3 fold higher (6700 vs. 1400 epithelial cells) by the new preparation. This preparation method demonstrated also several other advantages: (1) a reduced number of inadequate slides; (2) 50 fold increased density of cells, coupled with fewer overlapping nuclei and lumps; (3) more even distribution of the cells on the slide; and (4) a significant reduction (2.5 fold) of the scanning time of the slide. Figure 3 shows a typical cell distribution of conventional and liquid based preparations. In conventional deposition of smears, there were no significant differences in the total number of cells and epithelial cells between cervix brush and Ayer's spatula prepared smears with the possible exception of a greater number of endocervical cells by cervix brush.

3.3. Comparison of the results of manual cytopathology with DNA assisted cytology

Table 2 shows the results for the 876 women examined by colposcopy and biopsied. Pathology diagno-

Table 1
Comparative evaluation of two methods of cell preparation of cervical smears

Characteristics	Conventional method		Liquid-base method
	Spatula	Brush	Brush
Total cell numbers/slide	2263 ± 357	2378 ± 510	$10{,}843 \pm 896^*$
Number of epithelial cells/slide	1368 ± 103	1486 ± 149	$6758 \pm 287^*$
Area of cell distribution (mm^2)/slide	450–600	500–700	39
Number of slides presenting endocervical cells	32	46	44
Number of slides with evenly dispersed cells	43	47	50
Number of slides presenting cell clumps	6	5	1
Number of unsatisfactory slides	3	2	0
Screening time by cytometer (minutes)	103 ± 3	98 ± 4	$43 \pm 1^*$

$N = 50$. Values are mean $\pm$ SEM. *Liquid based method is significantly different from conventional method at $p < 0.01$.

Table 2
Comparative results of histopathology, conventional manual cytology and DNA assisted cytology of 876 biopsy cases

Pathology	Manual cytology					DNA assisted cytology		
	Normal	ASCUS	LSIL	HSIL	Cancer	Negative	Positive (aneuploidy >5c)	
							1–2 cells	3 or more cells
Normal/benign (459)	37	342	79	1		427	23	9
CIN1 (325)	36	207	82	0		248	62	15
CIN2 (36)	8	10	16	2		18	8	10
CIN3 or CIS (25)		6	8	11		7	3	15
Invasive cancer (31)	2	7	12	2	8	4	2	25

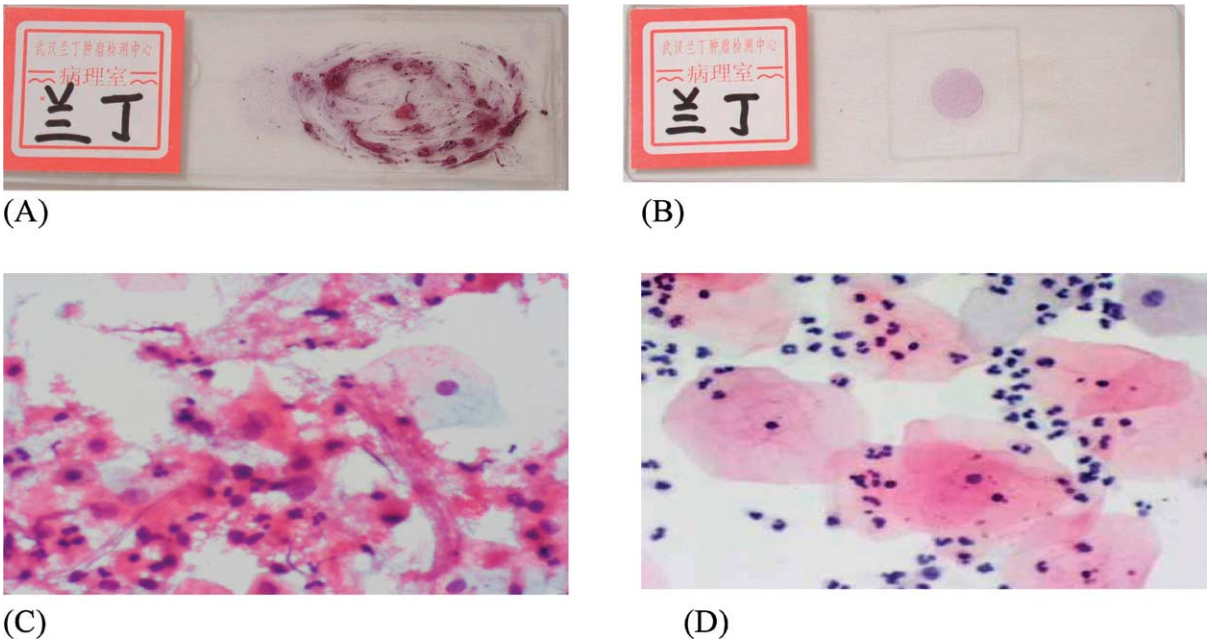

Fig. 3. Comparison of conventional Pap smear to liquid-based preparation. (A) Conventional smear slide. (B) Liquid-based preparation slide. (C) Overlapping and unevenly distributed cells of the convention slide in (A) above (Papanicolaou staining, ×200). (D) Monolayer epithelial cells evenly dispersed of the liquid-based preparation slide in B above (Papanicolaou staining, ×200).

Table 3
Comparative detection yields of manual cytology versus DNA assisted cytology for various colposcopy referral rules

Criterion for colposcopy referral	Number of slides examined by cytotechnologists	Number of colposcopies required	Number of CIN II+ that would be detected (rate per 10,000 cases %)	Sensitivity* for CIN II+ detection (%)	Specificity* for CIN I− detection
Cytology HSIL+	9905	24	23 (23.2)	25.0 ± 5.2	99.9 ± 0.1
Cytology LSIL+	9905	221	59 (59.6)	64.1 ± 6.2	79.3 ± 1.6
DNA ploidy 3 or more >5c cells	700	74	50 (50.5)	54.3 ± 7.0	96.9 ± 0.6
DNA ploidy 1 or more >5c cells	700	172	63 (63.6)	68.5 ± 5.9	86.1 ± 1.3

HSIL+means high grade SIL, CIS or cancer; LSIL+means low grade SIL, high grade SIL, CIS or cancer; CIN II+means cervical intraepithelial neoplasm grade II, grade III, CIS or cancer; CIN I − means cervical intraepithelial neoplasm grade I, normal or benign atypia.
*Sensitivity and specificity are defined here for detection of CIN II+ based only on the 876 cases subjected to biopsy. This is a highly biased selection of cases comprised only of those cases suspicious for disease. Presumably, the majority of the remaining 9029 cases were correctly called negative by both cytology and DNA ploidy, so the true test specificity is expected to be much higher than indicated. Similarly, the experimental design will not discover all CIN II+ cases in the 9905 subjects, so the sensitivity indicated is an upper limit.

sis by two histopathlogists was used as the "truth" for sensitivity and specificity calculations. There were 31 cases of invasive cancer, 25 cases of CIN3/CIS, and 36 CIN2 cases diagnosed for a total of 92 lesions of clinical significance, meaning that in most countries they would be managed by clinical intervention (removal of the lesion). If manual cytology were used for screening these cases, it would have called 16 HSIL lesions and 8 cancers, for the total of 24 cases to be referred to colposcopy. Of these 2 would have been found with CIN2 lesions, 11 with CIN3/CIS lesions and 10 with invasive cancer, for the overall sensitivity of 25.0 ± 5.2% and specificity of 99.9 ± 0.1%, as indicated in Table 3. In some countries LSIL lesions too are sufficient to direct the women to colposcopy, and in such case a total of 221 women would be referred to colposcopy of which

18 would have been found with CIN2 lesions, 19 with CIN3/CIS lesions and 22 cancers for the sensitivity of 64.1 ± 6.2% and specificity of 79.3 ± 1.6%.

For DNA assisted cytology, one could choose two or more different criteria to call a case potentially positive (or suspicious) and to direct the woman to colposcopy. At this stage of the technology development we considered only a single criterion, namely that there must be at least 3 cells (out of approximately 6000 cells) with the DNA amount greater than 5c. Using this single criterion, a total of 74 women would have been directed to colposcopy of whom 10 would be found with CIN2, 15 with CIN3/CIS and 25 with invasive cancer. In this case for clinically significant lesions the sensitivity and specificity would be 54.3 ± 7.0% and 96.9 ± 0.6%, respectively. It may be of interest to see the results if even a single cell criterion (i.e. the sample that contains at least 1 cell with DNA amount exceeding 5c would be sufficient to call the sample "positive") would be applied to send the women to colposcopy. In such case 172 women would have been directed to colposcopy and a total of 63 clinically significant cases would have been found: 18 CIN2, 18 CIN3/CIS, and 27 cancer for a sensitivity of 68.5 ± 5.9% and specificity of 86.1 ± 1.3%.

4. Discussion

Cervical cancer predominately afflicts relatively young, sexually active women. It was shown in several countries [2,8,14,24], that population based screening for early cervical cancer and pre-cancerous lesions such as severe dysplasia could prevent the incidence of invasive cervical cancer and with this the mortality due to this malignancy. The earliest example can be found in the program in British Columbia, Canada, where the BC Cancer Agency instituted population based screening of women aged 20 to 75 over 50 years ago. This program is thought to be responsible for the 60–75% reduction in both the age-adjusted incidence of and mortality from invasive cervical cancer since 1955. It is of interest to note that during the initial phase of 30 years (until 1984), the program was only focused on detecting cancer (the clinically most significant lesion), yet the lowest point of the invasive cancer incidence and mortality was already achieved by 1977.

The incidence of cervical cancer in China of the unscreened population may be very similar to that of the developing world. If that were the case, one would expect the prevalence of approximately 30–50 invasive cervical cancer cases per 100,000 women in the age group of 20–75 years. In China today, there are some pockets of excellent cervical screening programs in a few major cities that match the best in the world. However, even in these cities these programs cover only a very small fraction of the population that should be regularly checked for the presence of cervical neoplasia. This is in great part due to the shortage of the sufficient number of specialized technologists (cytotechnologists and cytopathologists) as well as other required infrastructure. China is committed to overcome this problem in the shortest possible time. Realistically, therefore, one needs to seek solutions that involve implementation of technology to assist in this goal.

In this study we examined how a simple and inexpensive liquid based preparation in combination with DNA amount measurements using a fully automated image cytometer could overcome this impediment. The tested hypothesis came from literature that suggested that the presence of cells with a significant increase of DNA amount in their nuclei is a strong indication for the presence of cervical cancer [3,7]. For example, Böcking [7] has suggested that the detection of aneuploidy does not depend on the percentage or rate of such cells in the sample, but that the threshold for assuming malignancy is the existence of $\geqslant 3$ nuclei with DNA amount greater than 5c. As indicated in Table 3, if we take this criterion in our study, the sensitivity of DNA assisted cytology was 54.3 ± 7.0% which is double that of the manual cytology using HSIL as the threshold for colposcopic investigation (25.0 ± 5.2%, albeit at a significant lower specificity 96.9 ± 0.6% and 99.9 ± 0.1%, respectively). Furthermore, when LSIL is used as the threshold for colposcopy investigation, this DNA assisted cytology sensitivity is statistically equivalent to that of manual cytology (54.3 ± 7.0% *vs*. 64.1 ± 6.2%), but has a substantially higher (and statistically significant) specificity (96.9 ± 0.6% and 79.3 ± 1.6%, respectively).

It could be argued that in this study the manual cytology was not at the level of a typical cytology laboratory given that there are no specialized gyne cytopathologists available in the Wuhan region of China. However, the detection rate of invasive cancers by manual cytology in this study is comparable to those reported in the literature. For example, Kok et al. [21] reported that only 23 cases from 71 invasive cancer patients were suspected as carcinoma by cytology, sensitivity of ∼33%. Similarly, van der Graaf et al. [34] reported that only about half of biopsy-documented invasive cancers

were appropriately recognized by cytology of cervical smears.

The DNA assisted cytology required approximately only one tenth of a cytotechnologists' time in comparison to the manual approach. Approximately only 7% of all cases in the study of nearly 10,000 cases had some objects in the DNA histogram that could have been cell nuclei with DNA amount greater then 5c. All of these cases had to be examined by a cytotechnologist to assure that these few objects of apparent >5c DNA content were indeed true, single cell nuclei and to weed out any artefacts such as dust, air bubbles, overlapping cells, etc. At the current algorithm of true cell nuclei recognition, it took a trained cytotechnologist less than 5 minutes per slide to "clean" such images from the image and cell data base. On 10,000 sample size, this required ∼700 cases to be examined at ∼10 slides per hour for the total of 70 hours of the cytotechnologist's time. In manual cytology, on average a cytotechnologist could screen ∼80 slides per day, i.e. 10 slides per hour, but in this case all 10,000 slides must be examined, for the total of 1000 hours of cytotechnologists' time. This represents a cytotechnologist resource savings of nearly 15 fold. It could be argued that manual cytology would save on the number of colposcopy and histopathology examinations if using HSIL and cancer as the criterion to direct women to these procedures. However, if one wants to match approximately the sensitivity of the DNA assisted cytology, then LSIL cases too would have to be directed to colposcopy for a disease confirmation yield of 59 (manual) *vs*. 50 (DNA assisted) cases. Again significant savings would be on the side of DNA assisted cytology (221 *vs*. 74 cases requiring colposcopy workup) for an approximate factor of 3.

We have no doubt that this approach could be (and will be) greatly improved in the near future. For example: (1) many cells were excluded from the analyses due to two or more nuclei touching or slightly overlapping that could be readily improved by implementing new segmentation algorithms; (2) similarly no small clumps of cells were taken in consideration for the same reason; that could be overcome by better sample preparation techniques or better image processing algorithms; (3) in this study a very small scanned area of the cytocentrifuge deposition was used that contained on average only 6000 epithelial cells that can be readily increased to the size containing 20,000 to 30,000 epithelial cells; and (4) a variety of different criteria (not only counting cell nuclei containing DNA amount exceeding 5c) could be tested and then implemented, etc.

However, even without any of these improvements, our approach as used in this study would yield a significant reduction of mortality due to cervical cancer as well as a concomitant reduction of prevalence and incidence of invasive cervical cancer in the screened population. Our test uncovered an extremely high prevalence of cervical cancer in the Wuhan area. Even if we assume that (1) no women that did not come to colposcopy had an invasive cervical cancer (2) that punch biopsies and histopathology did not miss any cervical cancers (neither of these two assumptions are likely correct), the prevalence of the invasive cancer in the test population of 9905 women was over 300 cases per 100,000 women. This is an order of magnitude higher than that found in the British Columbia population in early 50's, prior to the introduction of population based screening for this malignancy. These results provide us with enormous incentive to introduce invitational screening program in Wuhan and other parts of China and to expand the use and study of the approach described in this paper as soon as possible.

Acknowledgements

The authors would like to thank Prof. Dong Yuan and Dr. Xiang Cheng Yan for their valuable discussions and their superb technical support.

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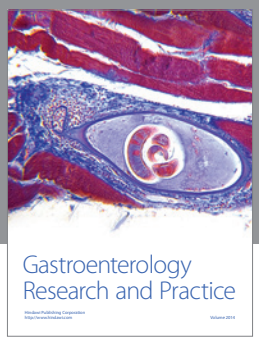

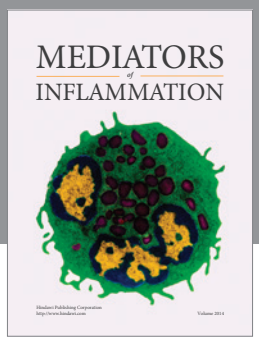

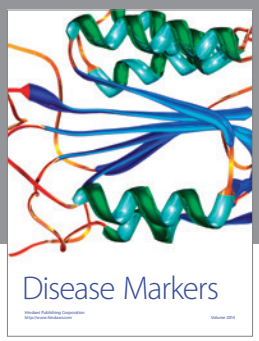

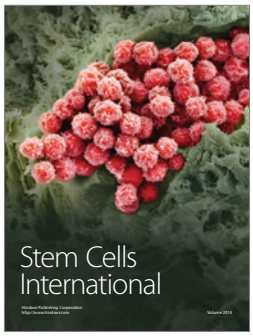

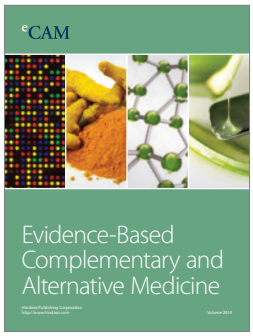

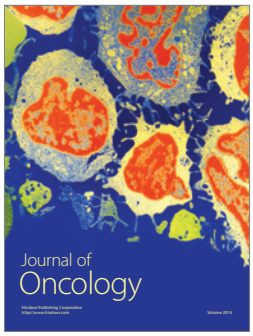

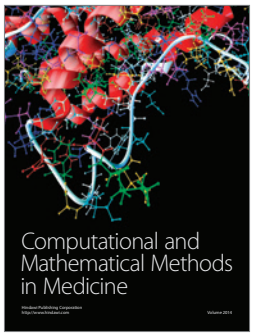

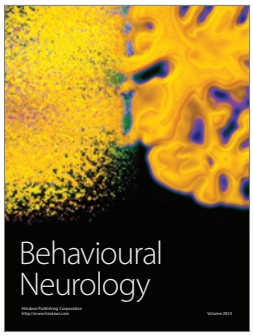

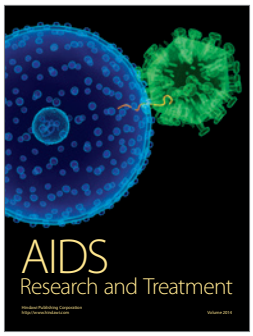

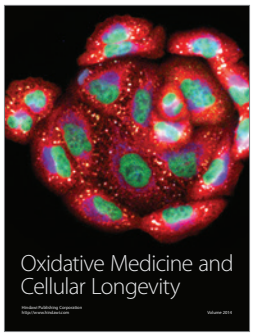

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