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Imaging, Diagnosis, Prognosis

DNA Ploidy Cytometry Testing for Cervical Cancer Screening in China (DNACIC Trial): a Prospective Randomized, Controlled Trial

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Abstract

Purpose: This randomized, controlled trial was designed to determine whether the DNA cytometry testing is superior to the conventional cytologic testing for mass cervical cancer screening.

Experimental Design: After approval by the institutional ethics review boards from three separate screening centers, a total of 23,993 Chinese women ages 20 to 65 years were randomly assigned into one of the two groups: a DNA cytometry testing group (11,999 women) and a cytologic testing group (11,994 women). Each woman underwent the other testing after first attending the assigned screening test. Women with positive results after assigned testing additionally underwent colposcopy and human papillomaviruses detections, and those with cervical precancerous or cancerous lesions received appropriate treatment. Sensitivity and specificity estimates were adjusted for verification bias. Analyses were by intention to treat and per protocol ways.

Results: In the cytometric DNA testing group, cervical cancer was diagnosed in 40 subjects, compared with 24 subjects in the cytologic testing group [hazard ratio for the detection of advanced cancer in the DNA cytometry testing group, 0.42; 95% confidence interval (CI), 0.27-0.60]. The sensitivity of the DNA cytometry testing for cervical cancer was 91.7% (95% CI, 64.3-95.8), whereas the sensitivity of cytologic testing was 44.5% (95% CI, 25.2-61.3; $P = 0.008$). The specificity was 54.1% (95% CI, 31.6-69.0) for DNA cytometry testing and 70.6% (95% CI, 46.8-82.5; $P = 0.003$) for cytologic testing. The sensitivity of both tests used together was 100%, and the specificity was 91.8%. A total of 187 subjects reported mild to severe adverse events after treatment with positive results in 319 women.

Conclusions: Our results highlight the benefit of the DNA cytometry testing strategy in mass cervical cancer screening with greater sensitivity and positive predicted value than the conventional cytologic testing in developing settings. (Clin Cancer Res 2009;15(20):6438–45)

Cervical cancer remains the leading cause of cancer death among women in low-resource countries. Approximately 320,000 of cervical cancer cases are diagnosed in the developing world every year, of which takes over 80% of all the cases of cervical cancer that are diagnosed annually worldwide (1). As thus, how the cervical cancer is sieved through effective methods during mass population screening is an essential question for global researchers investigating the effectiveness

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

H. Tong and R. Shen contributed equally to this work.

Members of the MACREG are listed in the Appendix.

Trial registration: DNA cytometry for cervical cancer screening in China. ClinicalTrials.gov ID: NCT00902551. UTL: http://clinicaltrials.gov/ct2/show/NCT00902551.

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Translational Relevance

Cervical cancer remains the leading malignancy among women in developing countries. Although several testing methods were used as the screening tools for detecting cancerous and precancerous cervical lesions, numerous studies have reported high false-negative rates for these procedures. Our study highlights that DNA cytometry testing alone as a mass screening procedure possesses potential benefits in detecting cervical cancer and its precursors with significantly high sensitivity and positive predicted value than conventional cytologic testing. Besides, adjunctive of human papillomavirus detection to DNA cyotmetry testing produces the greatest sensitivity and specificity in selecting women with a high risk for developing histologic lesions.

and accuracy of currently available means—particularly for predicting the emergence of the cancer after a single round of screening.

Cervical cytologic testing with the Bethesda System has proved to be one of the most successful examples of cancer screening over the simple Papanicolaou (Pap) smear test and has resulted in significant decreases in incidence and mortality from cervical cancer (2). Besides, numerous studies recommended that human papillomavirus (HPV) testing is an appropriate method when screening women in low-resource settings as a primary approach (3–6). Nonetheless, cervical cytology has recently been rethought and set aside for its low sensitivity because frequent retesting is required after a cytologic testing report (7). In addition, controversial concerns focused on the cost-effectiveness arose when HPV testing was used as the primary means of cervical cancer screening, especially in regions with scarce resources and fragile infrastructures (8–11).

Previous studies reported that liquid-based, thin-layer cytology is more accurate than conventional cervical cytology and has a potential to optimize the effectiveness of primary cervical cancer screening (12–14). Nevertheless, although the frequency of false-negative reports has decreased after using the liquid-based cytologic testing, the sensitivity of cervical cancer screening still can be improved with the development of new approaches such as DNA ploidy image cytometry based on the same specimen (15, 16). It is well known that cervical lesions in which cells have an aneuploid DNA profile are more likely to persist or progress than those with diploid or polyploid profiles (17–20). Lorenzato and colleagues (21) reported that flow cytometric DNA ploidy measurement on conventional cervical smears positive for HPV could help detect women at high risk of cervical cancer. To our knowledge, there is limited data on DNA cytometry testing as a stand-alone screening test

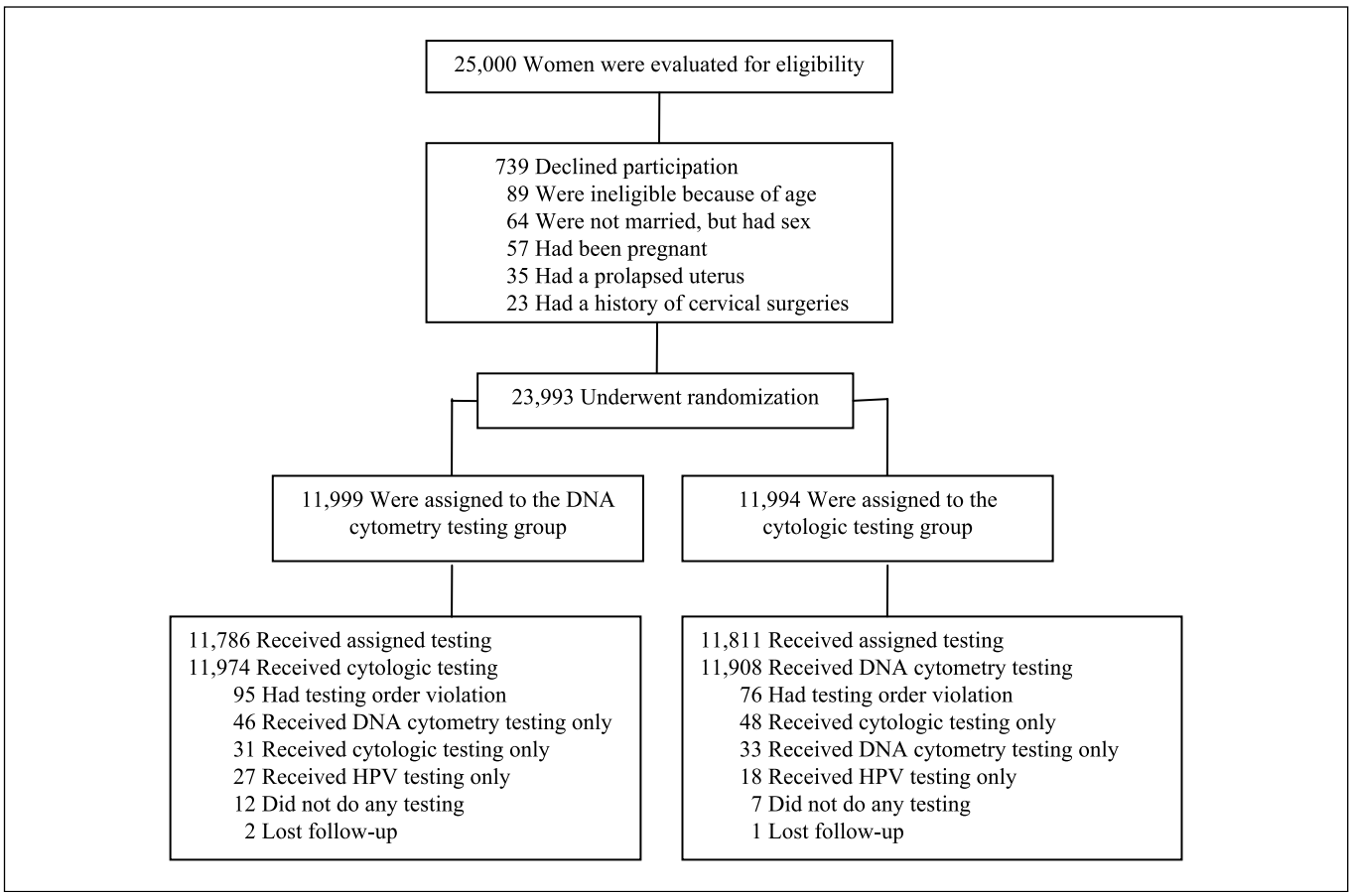

Fig. 1. Trial profiles of enrollment, screening, and randomization.

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Table 1. Demographic characteristics of the subjects

Variables	DNA cytometry testing	Cytologic testing	*P*
Subjects (*n*)			
All	11,999	11,994	
Range	1,861-4,879	1,792-4,786	
Age (y)			0.97
Mean (±SD)	41 ± 6.6	40 ± 7.1	
IQR	34-52	33-55	
Formal education, *n* (%)	3,816 (31.8)	3,886 (32.4)	0.32
Working environment, *n* (%)			
Rural area	5,004 (41.7)	5,097 (42.5)	0.21
Urban area			
Mental labor	2,220 (18.5)	2,219 (18.5)	0.99
Physical labor	4,775 (39.8)	4,678 (39.0)	0.21
Smoking status *n* (%)*			
Never	11,111 (92.6)	11,094 (92.5)	0.76
Rarely	816 (6.8)	816 (6.8)	0.99
Frequently	72 (0.6)	84 (0.7)	0.33
Currently marriage status, *n* (%)			
Yes	11,567 (96.4)	11,598 (96.7)	0.20
No	432 (3.6)	396 (3.3)	0.20
No. of successful pregnancies			1.0
Mean (±SD)	1 ± 1	1 ± 1	
IQR	0-3	0-3	
Condom use†			
Never	1,872 (15.6)	1,919 (16.0)	0.39
Rarely	6,983 (58.2)	6,885 (57.4)	0.21
Frequently (always)	3,144 (26.2)	3,190 (26.6)	0.48
Duration of oral contraceptive use, y			
Never	4,884 (40.7)	4,942 (41.2)	0.43
≤5	4,644 (38.7)	4,630 (38.6)	0.87
>5	2,471 (20.6)	2,422 (20.2)	0.44
History of abortion, *n* (%)	3,948 (32.9)	3,910 (32.6)	0.62
Age of the first sex			0.94
Mean (±SD)	21 ± 3	21 ± 4	
IQR	19-27	18-27	

NOTE: Plus-minus values are means ± SD. IQR means interquartile range.
*Smoking status indicates how many cigarettes the subjects have smoked in the past 3 y. Never, no cigarette was consumed; rarely, <300 cigarettes per year were smoked; frequently, ≥300 cigarettes per year were consumed.
†Condom use indicates the frequency of condom used every month. Never, condom used never; rarely, the number of condom used was fewer than five; frequently, the number of condom used was over five.

for large-scale cervical cancer screening in developing countries.

In January 2007, we initiated a prospective randomized controlled trial to assess the effectiveness of flow cytometric DNA image ploidy analysis at the first round large-scale population screening for cervical cancer compared with conventional cytologic measurement in China.

Materials and Methods

Participants and ethics. All study-participating sites obtained ethical approval from institutional ethics review boards before recruiting patients. All participants signed an informed consent and a full explanation was given about techniques of DNA image cytometry, cytologic testing, colposcopy, and HPV detection. Recruitment for DNA Ploidy Cytometry Testing for Cervical Cancer Screening in China took place between January 2007 and June 2009. Invited women were eligible if they were undergoing large-scale population physical screening, were ages between 20 and 65 y, and fulfilled the following criteria: currently or had been married, not pregnant, had an intact uterus with no prolapse, had no history of cervical cancer, and living in China. Participants were excluded from the study if they were not willing to participate or finish the study at any time, or had a history of cervical surgeries (Fig. 1). The data were collected at three University-affiliated tertiary teaching hospitals in China. Hospital teaching status was ascertained from the Council of Teaching Hospitals of Chinese Medical Colleges.

Study procedures. After obtaining written informed consent, women participating screening were randomly assigned into two observational groups by means of a computer-generated, random-number list using block randomization stratified by center: one DNA cytometry testing group and one cytologic testing. The block size is unknown to study center personnel. Participants, health-care providers, and outcome adjudicators were masked to allocation but data analysts were not. Pathologists were masked with the knowledge of the patient's status as a participant or her other testing results, and the physicians who performed colposcopy testing and follow-up were blinded to group allocation.

Given the ethical consideration, we included both tests in each group. In the DNA cytometry testing group, the women received a DNA cytometric test first, whereas in the cytologic testing group, the women received a cytologic test first; the two tests were done sequentially at the same visit. In each group, the first test was called the index test, of which enabled us to analyze each index test as if it had been done alone. Different cytotechnologists performed DNA cytometry or cytologic testing separately, and they did not know the result of each

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Table 2. Rates of screening, colposcopy, HPV, inflammation, CIN, and cancer

Variables in age group	DNA cytometry testing	Cytologic testing	*P*
Rate of screening*			0.026
<30 y	2,013/2,612 (77.1)	2,230/2,705 (82.4)	
30-39 y	9,845/10,946 (89.9)	10,066/11,104 (90.6)	
40-49 y	5,541/5,647 (98.1)	5,197/5,379 (96.6)	
50-59 y	3,219/3,478 (98.5)	3,216/3,455 (93.1)	
≥60 y	853/1,011 (84.4)	984/1,142 (86.2)	
Subtotal	21,471/23,694 (90.6)	21,693/23,785 (91.2)	
Rate of positive screening			<0.0001
<30 y	319/2,013 (15.8)	197/2,230 (8.8)	
30-39 y	2,364/9,845 (24.0)	1,165/10,066 (11.6)	
40-49 y	1,861/5,541 (33.6)	951/5,197 (18.3)	
50-59 y	1,502/3,219 (46.7)	599/3,216 (18.6)	
≥60 y	268/853 (31.4)	208/984 (21.1)	
Subtotal	5,946/21,471 (27.7)	3,120/21,693 (14.4)	
Rate of positive colposcopy			<0.0001
<30 y	231/319 (72.4)	167/197 (84.7)	
30-39 y	2,152/2,364 (91.0)	1,082/1,165 (92.8)	
40-49 y	1,661/1,861 (89.2)	897/951 (94.3)	
50-59 y	899/1,502 (59.8)	587/599 (97.9)	
≥60 y	224/268 (83.6)	182/208 (87.5)	
Subtotal	5,167/5,946 (86.8)	2,915/3,120 (93.4)	
Rate of positive HPV			0.003
<30 y	22/319 (6.8)	6/197 (3.0)	
30-39 y	357/2,364 (15.1)	118/1,165 (10.1)	
40-49 y	206/1,861 (11.1)	99/951 (10.4)	
50-59 y	117/1,502 (7.7)	82/599 (13.6)	
≥60 y	10/268 (3.7)	4/208 (1.9)	
Subtotal	712/5,946 (11.9)	309/3,120 (9.9)	
Rate of inflammation			<0.0001
<30 y	31/2,013 (1.5)	12/2,230 (0.5)	
30-39 y	156/9,845 (1.6)	76/10,066 (0.7)	
40-49 y	84/5,541 (1.5)	32/5,197 (0.6)	
50-59 y	47/3,219 (1.4)	17/3,216 (0.5)	
≥60 y	10/853 (1.2)	5/984 (0.5)	
Subtotal	328/21,471 (1.5)	142/21,693 (0.6)	
Rate of CIN grade 1			0.0006
<30 y	7/2,013 (0.3)	3/2,230 (0.1)	
30-39 y	31/9,845 (0.3)	17/10,066 (0.2)	
40-49 y	21/5,541 (0.4)	14/5,197 (0.3)	
50-59 y	16/3,219 (0.5)	8/3,216 (0.2)	
≥60 y	8/853 (0.9)	3/984 (0.3)	
Subtotal	83/21,471 (0.4)	45/21,693 (0.2)	
Rate of CIN grade 2 or 3			0.0007
<30 y	8/2,031 (0.4)	3/2,230 (0.1)	
30-39 y	56/9,845 (0.6)	42/10,066 (0.4)	
40-49 y	50/5,541 (0.9)	31/5,197 (0.6)	
50-59 y	35/3,219 (1.1)	28/3,216 (0.8)	
≥60 y	19/853 (2.2)	9/984 (0.9)	
Subtotal	168/21,471 (0.7)	113/21,693 (0.5)	
Rate of cancer			0.04
<30 y	2/2,031 (0.09)	1/2,230 (0.04)	
30-39 y	11/9,845 (0.1)	8/10,066 (0.07)	
40-49 y	12/5,541 (0.2)	8/5,197 (0.2)	
50-59 y	11/3,219 (0.3)	5/3,216 (0.2)	
≥60 y	4/853 (0.5)	2/984 (0.2)	
Subtotal	40/21,471 (0.2)	24/21,693 (0.1)	

*Rate of screening refers to the subjects who underwent screening compared with those who were invited to screening.

other. Besides, colposcopy testing or HPV detection was used to positive-screening women in each group.

Demographic characteristics. The following data were collected as demographic characteristics of the subjects: age, smoking status, education level, working environment, marriage status, history of abortion, successful pregnancies, condom use, time duration of oral contraceptive use, and age of first sexual intercourse.

DNA image cytometry testing. Flow cytometric DNA ploidy measurement was used as reported elsewhere (15). The DNA cytometric histograms were classified into two groups: normal and suspect. The normal one corresponded to class one, i.e., diploid with low proliferation fraction according to Auer's classification (22) and to the polyploid (diploid + tetraploid) histograms without any cells exceeding 5c. All other histogram types, i.e., aneuploid, polyploid, or diploid with more

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than two cells exceeding 5c, and multiploid profiles (more than one aneuploid peak), were regarded as suspect (see representative images in Supplementary Fig. S1A-E; Detailed methods were seen in Supplementary Materials and Methods).

Cytologic testing. A total of 23,785 women underwent a cervical scrape with a Cervexbrush (Rovers Medical Devices) at the first examination. Smears were classified according to the 2001 Bethesda System terminology (23) for reporting cervical diagnosis: within normal limits, with atypical squamous cells of undetermined significance, with atypical squamous cells suggestive of high-grade squamous intraepithelial lesion, or suggestive of low-grade squamous intraepithelial lesion and high-grade squamous intraepithelial lesion, or carcinoma (see representative images in Supplementary Fig. S1F-J; Detailed methods were seen in Supplementary Materials and Methods).

Colposcopy testing. Participants were referred for colposcopy testing as reported previously (6) if they had a positive DNA cytometry or cytologic testing. Colposcopy testing results were presented as following classification: inflammation, cervical intraepithelial neoplasia (CIN) 1, CIN 2 or 3, and cancer. Patients were treated if a warranted result received in a biopsy. A loop electrosurgical excision procedure (LEEP) or cold-knife conization could be done. When ablative treatment was done, confirmatory biopsies were done at the treatment visit (detailed methods were seen in Supplementary Materials and Methods).

HPV testing. Cervical specimens were tested for the presence of HPV DNA by a previously described PCR protocol amplifying a highly conserved 450-bp segment in the L1 viral gene (flanked by primers MY09/11; refs. 16, 24; Detailed methods were seen in Supplementary Materials and Methods).

Patient follow-up. We undertook a 1-y follow-up for all participants. All patients with cytologic abnormalities (from atypical squamous cells of undetermined significance to high-grade squamous intraepithelial lesion) were systematically recalled for colposcopy during subsequent weeks. Punch biopsy specimens were taken from the areas colposcopically suggestive of squamous intraepithelial lesion (SIL). Study personnel followed-up patients by phone at 1 year after first round cervical cancer screening.

Trial outcomes. The primary outcome of the DNA Ploidy Cytometry Testing for Cervical Cancer Screening in China trial is the cervical cancer rate detected using DNA cytometry testing or cytologic testing in a population-based physical screening in China. Secondary outcomes include incidence of inflammation, CIN 1 to 3, and death (recording the single rate of death in each group including those that undergone treatment of LEEP or cold-knife treatment). The specificity, sensitivity, and positive and negative predicted values are calculated for the two testing means. Finally, adverse events were recorded when and after LEEP or cold-knife treatment for inflammation and CIN, and hysterectomy or modified radical surgery and chemotherapy for invasive cervical cancer.

Outcome adjudication. A committee of physicians who are blinded to the group allocation adjudicated the aforementioned outcomes. We used the decisions from the Adjudication Committee for all statistical analyses involving these outcomes.

Statistical analysis. According to previously reported studies on the reduction in the cumulative rate of cancer death from cervical cancer (3) and the institutional early database, the mean difference in death decrease was 4 per 10,000, i.e., 2.40% in the DNA cytometry testing group and 2.36% in the cytologic testing group; we set the two-sided $\alpha = 0.05$, one-sided $\beta = 0.10$, and the power of test = 0.90. Therefore, a minimal sample size of 10,000 subjects per group was needed to detect the difference. We increased the sample size to 12,500 in each group to account for potential missing data and dropout during the study course. The 25% increase in sample size was mainly on the basis of the institutional database that round 19% [median; interquartile range (IQR), 16-25%] patients dropped out or their data were lost during studying period. Therefore, we increased the sample size to 12,500 per group following the upper limit 25%. In our study, the index test of each screening method was analyzed firstly, and then we combined the data together to detect the difference of the two screening means.

Analyses were done using GraphPad Prism version 5.0 (GraphPad Software, Inc.). Values are expressed as the mean, SD, IQR, or numbers. All our data assessment primarily was based on an intention-to-treat analysis. Meanwhile, a per protocol analysis was done, in which the subjects excluded, withdrawn, and lost follow-up were precluded. All categorical data were analyzed with a χ^2 test to indicate the trend. The difference in parametric data including the demographic

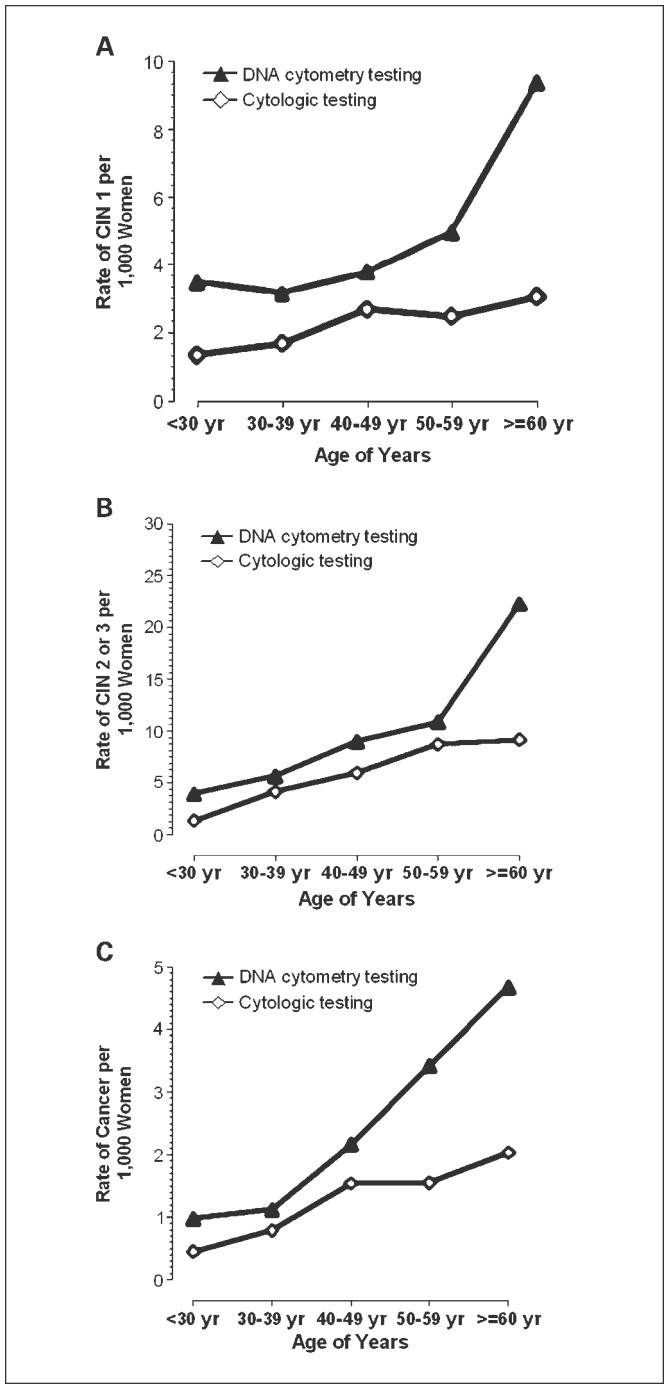

Fig. 2. Risks for CIN 1, CIN 2 to 3, and cervical cancer in different age groups.

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data and background characteristics were compared with Student's *t* test. Cumulative-event curves indicating different risks of cervical cancer at different age groups with HPV infection or not were estimated with the Kaplan-Meier method and the groups compared using the log-rank test. The statistical significance was accepted at the *P* value of ≤0.05.

Results

A total of 25,000 women were invited and assessed for cervical cancer screening. Figure 1 presents the patient's screening profiles and the reasons for 1,007 excluded subjects during enrolling period. Finally, 23,993 women were randomly assigned to the two groups and followed up. In the DNA cytometry testing group, 98.2% of the women received the assigned intervention, as did 98.5% of those in the cytologic testing group. Totally, three women were lost follow up because they were migrated before the completion of the study, but their data still used for intention-to-treat analyses.

Table 1 summarizes the demographic and background characteristics of the subjects. No significant difference was observed between the two groups.

In our study, we reset the patients into five subgroups according to different ages. Table 2 expresses the combined number of screened women, the number and proportion of positive screen results, and the number of women detected with inflammation, CIN, and cervical cancer according to different ages. In fact, our data reflected the number of specimen underwent screening, i.e., participating woman had been screened by the two methods, but merely in difference in testing order. Of the 23,694 samples in the DNA cytometry testing group, 21,471 (90.6%) were detected and 5,946 (27.7%) had positive results. Of the 23,785 samples in the cytologic testing group, 21,693 (91.2%) were detected and 3,120 (14.4%) had positive results. Among these positive results, over 85% were positive tested via colposcopy. The positive rates of CIN grade 1 to 3 were higher in the DNA cytometry testing than the cytologic testing ($P = 0.0006$ and 0.0007 in CIN 1 and CIN 2 or 3, respectively). The incidence rates of cervical cancer in both groups are some 2 and 1 per 1,000 women ($P = 0.04$). Furthermore, the rates of CIN and cancer in both groups increased with the increase of age (Fig. 2).

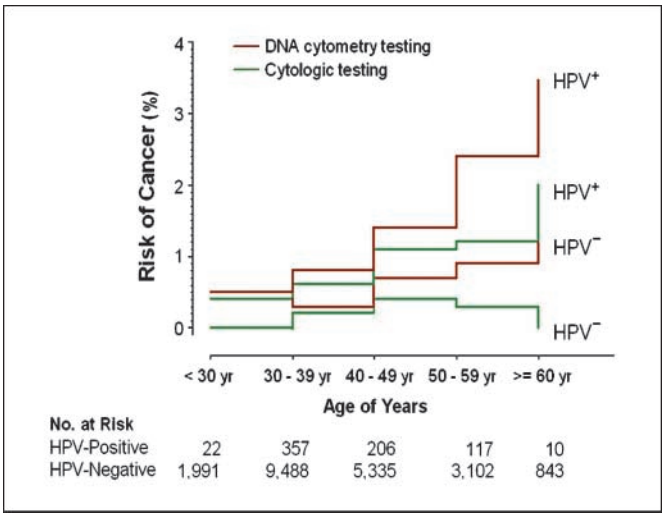

Fig. 3. Kaplan-Meier curves for the risks of cervical cancer with positive or negative HPV detection.

In addition, we detected HPV infection for those with positive results after first round screening, and found that 712 (11.9%) in the DNA cytometry testing group and 309 (9.9%) in the cytologic testing group were HPV positive ($P = 0.003$, Table 2). In addition, HPV-positive women had a higher risk of cancer, and this risk was increased with the increase of age (Fig. 3).

A total of 54 women found positive of cervical cancer after first round screening except for those with repeated positive in both testing methods. The proportions of cancer diagnosed after 3 months for those who with negative results were 52. The incidences of the stage I cancer were 41 and 22, and the stage II or higher cancer were 28 and 15 in the DNA cytometry testing and cytologic testing groups, respectively (see Supplementary Table S1). Totally, nine (8.2%) women died 6 months later after diagnosis of cervical cancer, of which six were from the DNA cytometry testing group and three were from the cytologic testing group. The hazard ratios for the detection of cervical cancer were 0.55 [95% confidence interval (CI), 0.48-0.76) and 0.42 (95% CI, 0.27-0.60) at the stage I and stage II or higher, respectively.

The per protocol analyses received similar results presented as the above ones as the intention-to-treat analyses.

Table 3 summarizes the specificity, sensitivity, and positive and negative predicted values for the two testing methods in cervical cancer identification. The DNA cytometry testing had a significant higher sensitivity and positive predicted value than the cytologic testing ($P = 0.008$ and 0.05, respectively), but the cytologic testing had a relatively higher specificity and negative predicted value than the DNA cytometry testing did ($P = 0.003$ and 0.027, respectively).

A total of 319 women underwent treatment after screening, and 187 reported experienced mild to severe adverse events including nausea and vomiting during postoperative pain management and chemotherapy (89 women), coagulation dysfunction after chemotherapy (16 women), pruritus during postoperative pain management (10 women), and uncontrolled bleeding after LEEP and cold-knife that resulted in hysterectomy (1 woman to each testing).

Discussion

The results of this randomized, controlled trial indicate that DNA cytometry testing as a stand-alone screening method has a higher positive rate in identifying cervical cancer precursors compared with the conventional cytologic testing in women with different ages. A single round screening with flow cytometric DNA ploidy measurement expressed much higher sensitivity, but a round 20% difference in specificity than the cytologic testing. Adjusted estimates for correcting the verification bias gave the absolute estimates, which reflected a potentially community-based mass screening strategy.

The primary purpose of a screening program is to detect a number of advanced cases of cancer in the early stages, in particular in places where knowledge of symptoms is low and access to health care is poor (25). As reviewed by Marcus et al. (26), in the 1990s, the Pap smear screening was so well established to public health that the lack of such screening was considered one of the prime risk factors for invasive

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Table 3. Sensitivity and specificity for DNA cytometry and cytologic testing methods in identifying cervical cancer

Variable	Crude estimates			Adjusted estimates*		
	DNA cytometry testing	Cytologic testing	*P*	DNA cytometry testing	Cytologic testing	*P*
Specificity, % (95% CI)	57.6 (38.1-77.5)	76.2 (55.9-88.6)	0.022	54.1 (31.6-69.0)	70.6 (46.8-82.5)	0.003
Sensitivity, % (95% CI)	80.9 (63.3-91.4)	40.0 (20.2-58.7)	0.006	91.7 (64.3-95.8)	44.5 (25.2-61.3)	0.008
Positive predicted value, % (95% CI)†	24.3 (11.2-36.8)	17.5 (7.0-28.6)	0.032	23.6 (9.5-30.8)	15.4 (6.1-25.6)	0.05
Negative predicted value, % (95% CI)	57.6 (24.3-70.6)	90.9 (76.5-97.5)	0.038	50.4 (16.6-67.1)	87.3 (66.4-94.7)	0.027

*The estimates are adjusted by verifying the bias.
†Positive predicted values denote the result of ≥3 DNA ploids for the DNA cytometry testing and atypical squamous cells of undetermined significance or worse for cytologic testing.

cervical cancer. Under this consideration, sometimes many women erroneously perceive taking Pap smears as a means to diagnose cervical cancer, so it is quite natural that some of them were missed out because of the negative results from the Pap smears. In addition, the unsatisfactory rate of Pap smear analysis was high due to a low quality ThinPrep slides in assessment (27). As thus, Pap smear as a mass screening method requires a more sensitive and effective way to be an alternative.

Numerous studies recommended that HPV detection should be adapted in cervical cancer prevention for widespread implementation (3–6, 28). However, HPV infection does not always develop into invasive cancer[8] (29, 30), and the cases diagnosed with cervical cancer do not always have HPV infection (31). To this end, although HPV testing is an effective way in cervical cancer screening, stand-alone still cannot be a once-for-all method in a primarily-initiated single round mass population screening (32, 33). In our study, we also detected the HPV infection for those with positive results after first round screening, and found that HPV-infectious women had a higher risk of incidence of cervical cancer than those with HPV-negative results. In addition, some one third of women with CIN or greater cervical lesions did not find any infection of HPV.

The persistence of aneuploidy of cells is a pivotal characteristic for cervical carcinoma development (34), of which reflects a situation of uncontrolled increase of DNA and loss of crucial information and plays an essential role in neoplastic transformation (35). The increased aneuploid DNA value with the increase in grades of cervical dysplasia has long been considered to be a specific prognostic marker of malignancy (34). In 2001, Melsheimer and colleagues (36) reported that flow cytometric analysis of DNA ploidy may be a potential means providing a strategic diagnostic tool for early detection of cervical cancer. Therefore, Singh et al. (37) proposed a conception of combining the DNA ploidy cytometry testing, which provides qualitative information and presence of aberrant aneuploid cells in cytologic specimen through flow cytometry by measuring the DNA content, and an HPV screening with reflex cytology would be an optimal method to detect progressive lesions with the greatest possible sensitivity and specificity. In our trial, we still did HPV detection after the assigned screening testing, and obtained concur findings with previous literatures that DNA cytometry plus HPV testing had a significant higher sensitivity and positive predicted value than the conventional cytology plus HPV testing.

We found the risk of cervical lesions ranging from CIN 1 to invasive cancer in per 1,000 women increased along with the increase of patient's age. Those over 60 years had a highest positive incidence of cervical abnormalities. In addition, we observed that the youngest woman with positive result of DNA cytometry testing was only age 21 years who had a CIN grade 3 lesion of her cervix, and was age 26 years who had a cervical cancer. Beside, the percentage of cervical lesions under the age of 30 years was similar to those of other different age groups. This indicates that women who have cervical diseases have a tendency to be younger than ever in China.

In summary, DNA cytometry testing alone as a mass screening procedure possesses potential benefits in detecting cervical cancer and its precursors with significantly high sensitivity and positive predicted value than conventional cytologic testing. Besides, adjunctive of HPV detection to DNA cyotmetry testing produces the greatest sensitivity and specificity in selecting women with a high risk for developing histologic lesions.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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[8] eMedTV: High-risk HPV. http://hpv.emedtv.com/hpv/high-risk-hpv.html. Last accessed: July 30, 2009.

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