RESEARCH ARTICLE

Performance of Siriraj Liquid-Based Cytology: a Single Center Report Concerning over 100,000 Samples

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Abstract

Background: To evaluate the performance of Siriraj liquid-based cytology (LBC) for cervical neoplasia screening after increasing use of this technology. Materials and Methods: Cytological reports of 103,057 Siriraj-LBC specimens obtained in 2007-2009 were compared with those of 23,676 specimens obtained in 2006. Results: Comparing with the year 2006, the 2007-2009 patients were slightly older (43.4±12.yr *vs* 42.7±12.2 yr, p <0.001), and their specimens had much lower proportion of unsatisfactory slides (OR=0.06, 95%CI 0.04-0.09) with comparable detection rates (3.96% *vs* 3.70%, p=0.052) but different proportions of various cytological abnormalities (p<0.001). The 2007-2009 Siriraj-LBC had a negative predictive value (NPV) for cervical intraepithelial neoplasia 2+ (CIN2+) of 97.6% and an overall positive predictive value (PPV) of 43.9%. The PPV for CIN2+ varied with types of abnormal cytology, from 13.7% to 93.8% in atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells cannot exclude HSIL (ASC-H), high-grade squamous intraepithelial lesion (HSIL), atypical glandular cells (AGC), to squamous cell carcinoma (SCC), respectively. The PPVs for CIN2+ in ASCUS and LSIL were comparable, but the PPV for CIN1 was higher for LSIL than for ASCUS (41.63% *vs* 16.32%). Conclusions: Siriraj-LBC has demonstrated a stable detection rate and NPV for CIN2+ of >95% since the first year of use. The comparable PPVs for CIN2+ of ASCUS and LSIL suggests that these two conditions may undergo similar management; other cytological abnormalities need immediate evaluation.

Keywords: Cervix - liquid-based cytology - Pap smear - squamous intraepithelial lesion

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Introduction

Cervical cancer is the second most common cancer of women in developing countries, including Thailand (WHO, 2012). Cervical cytology is routinely used as a screening tool for cervical cancer and its preinvasive lesions. Papanicolaou's smear, the conventional cervical cytology technique introduced in 1940, had sensitivity for detection of any cytological abnormality of 68% and specificity of 79% (Abulafia et al., 2003). Liquid-based cytology (LBC), since the introduction in mid-1990s, has been proven to have higher performance than the conventional cytology did (Dupree et al., 1998; Papillo et al., 1998; Diaz-Rosario and Kabawat, 1999; Fremont-Smith et al., 2004; Canda et al., 2009). The advantages of LBC over conventional cervical cytology include the decrease in rate of unsatisfied smears and the increase in detection rate of cytological abnormalities (Dupree et al., 1998; Papillo et al., 1998; Diaz-Rosario and Kabawat, 1999; Fremont-Smith et al., 2004; Canda et al., 2009; Nandini et al., 2012). Nowadays, a number of different LBC techniques are in use worldwide. ThinPrep® and SurePath™ , the prototypes and the most commonly used LBC, have been approved by the Food and Drug Administration for cervical cancer screening in the USA (Lee et al., 1997; Bishop et al., 1998). However, the cost of the original LBC tests is deemed high for the routine use in many developing countries, including Thailand.

In 2005, our group developed an alcohol-based preservative solution, Siriraj liquid-based solution, and applied a modified Saccomanno's technique (Bales, 2006) for cell preparation. We named this technology as the "Siriraj liquid-based cytology" or "Siriraj-LBC". Our LBC does not require any expensive equipment; therefore it costs much less than the commercial ones do. In our preliminary study, using the "split-sample" method, we found that the screening performance of Siriraj-LBC was superior to that of conventional cytology

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(Laiwejpithaya et al., 2008). As a result, the Siriraj-LBC has totally replaced the conventional cytology for cervical cancer screening in our institute since 2006. In 2009, we reported the performance of our first year application of Siriraj-LBC in 25,510 specimens. Using the "direct-to-vial" method and historical comparison with conventional Pap smear, we found that the Siriraj-LBC increased detection rate of abnormal cytology, improved specimen adequacy, and enhanced NPV without compromising PPV (Laiwejpithaya et al., 2009). Despites, the detection rate of Siriraj-LBC in our first year experience was still lower than that of other reports (Dupree et al., 1998; Papillo et al., 1998; Diaz-Rosario and Kabawat, 1999; Fremont-Smith et al., 2004). In the present report, we collected more data of the years 2007 to 2009 and compared with the data of the year 2006 in order to determine the performance of Siriraj-LBC after longer experience of LBC technology with larger sample size.

Materials and Methods

The study was carried out at the Gynecologic Cytology Unit, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University. Data were retrieved from the database of the Gynecologic Cytology Unit. The study protocol was approved by the Siriraj Institutional Review Board.

Eligible study population

The specimens for cervical cytology were obtained from patients who had pelvic examination at the outpatient department of Siriraj Hospital from 2007 to 2009. We excluded the cases whose cervical cytology specimens might be contaminated by abnormal cells from endometrial or vaginal epithelia as a result causing false positive result at a very high rate. For example, the cases whose cervical cytology specimens showed abnormality, but histopathology showed normal cervical mucosa would be excluded if their endometrial or vaginal mucosa showed neoplasia.

Cervical cytology specimens

Cervical cytology specimens were collected by residents or faculty members of the Department of Obstetrics and Gynecology. Specimens were collected from posterior fornix, portio vaginalis and endocervix using a special plastic spatula (SL spatula) as described in our previous report (Laiwejpithaya et al., 2009). Immediately after cell collection, the spatula was manually broken at the scores locating at four cm from both ends of the spatula; the broken ends were put into a 30-mL plastic container filled with 10 mL of Siriraj liquid-based solution. The specimens were kept at room temperature until processing. Most of the specimens were processed on the day of collection. Siriraj-LBC slides were prepared by experienced technicians as described in our previous report (Laiwejpithaya et al., 2009). The Siriraj-LBC slides were screened by a team of cytoscreeners and cytotechnologists. The abnormal slides were reviewed by a cytopathologist who made the final cytological diagnosis. The processing and examination of the cytology specimens were performed at the Gynecologic Cytology Unit, Department of Obstetrics and Gynecology.

Cervical tissue specimens

Cervical tissues specimens were obtained by the following operative procedures: colposcopic directed cervical biopsy, loop electrosurgical excision procedure (LEEP), cold-knife conization, or hysterectomy. All specimens were subjected to paraffin section and routine hematoxylin/eosin staining. The histopathological diagnosis was made by gynecological pathologists. The processing and examination of the histopathology specimens were performed at the Department of Pathology.

Classification of cervical cytology and histopathology

Cervical cytology was classified according to the classification of 2001 Bethesda system (Solomon et al., 2002), as the followings: *i*) negative for intraepithelial lesion and/or malignancy or NILM; *ii*) atypical squamous cells of undetermined significance or ASCUS, *iii*) atypical squamous cells cannot exclude HSIL or ASC-H, *iv*) low-grade squamous intraepithelial lesion or LSIL, *v*) high-grade squamous intraepithelial lesion or HSIL, *vi*) atypical glandular cells or AGC, *vii*) adenocarcinoma in situ or AIS, *viii*) adenocarcinoma, *ix*) squamous cell carcinoma or SCC, and *x*) other malignancy. The cervical cytology other than NILM was considered abnormal.

Cervical histopathology was classified as normal, cervical intraepithelial neoplasia (CIN)1, CIN2, CIN3, and invasive lesions (Wright et al., 2002). Normal cervical histology was considered when the cervical mucosa was clear from any neoplastic lesion, ignoring the histopathological result of endometrium or vaginal epithelium.

Performance of Siriraj-LBC

The performance of Siriraj-LBC was evaluated from detection rate of abnormal cervical cytology, and predictive values. The detection rate was the percentage of abnormal cervical cytology specimens in all cervical cytology specimens of the same study period. The predictive values were calculated using cervical histopathology as the gold standard. The data for calculating negative predictive value (NPV) were obtained from the patients who had normal results of prehysterectomy screening cervical cytology. The data for calculating positive predictive value (PPV) were obtained from the patients who had abnormal results of cervical cytology and underwent operative procedures to obtain cervical tissue specimens.

Management of abnormal cervical cytology

The patients who had abnormal cervical cytology were managed according to the guideline of Siriraj Hospital. Briefly, the patients with cytology results of ASC-H, HSIL or more aggressive cytological results were referred for immediate colposcopy, whereas the patients with cytology results of ASCUS or LSIL were advised to have either colposcopy or a repeated cervical cytology testing in 6 months. If the repeated cytology were abnormal, the patients were referred for colposcopy.

Statistical analysis

Data were analyzed using SPSS for Windows, version 14.0. The data were presented in mean and standard deviation (SD), number (n) and percent (%) with or without 95% confidence interval (CI), or odds ratio (OR) and 95%CI, as appropriate. Continuous data were tested for normality of distribution using histogram, QQ plot, and Kolmogorov–Smirnov test. Data were analyzed using Student's t-test for normally distributed continuous data, and Chi-square test or Fisher's exact test for categorical data. All tests were 2-sided, and a p-value of <0.05 was considered statistically significant.

Table 1. Cases Excluded from Analysis (N=59)

Cervical cytology	Histopathology results (n)	
	Endometrial neoplasia	Vaginal neoplasia
ASCUS	12	0
ASC-H	5	1
HSIL	3	2
AGC	21	0
ADC*	14	1
Other malignancy	0	1

*One case had neoplasia at both endometrial and vaginal epithelia. Neoplasia included both preinvasive and invasive lesions. ADC=adenocarcinoma; AGC=atypical glandular cells; ASCUS=atypical squamous cells of undetermined significance; ASC-H=atypical squamous cells cannot exclude HSIL; HSIL=high-grade squamous intraepithelial lesion; LSIL=low-grade squamous intraepithelial lesion

Results

From 2007 to 2009, there were 103,116 cervical cytology specimens, 6,227 of which had histopathology results of cervical tissue. Fifty-nine pairs of cytology-histology specimens were excluded due to the probable contamination of cervical specimens by abnormal cells from endometrial and/or vaginal epithelia. The details of excluded specimens were shown in Table 1. Therefore, 103,057 cervical cytology and 6,168 cervical histopathology specimens were analyzed.

Table 2 shows characteristics of patients and cervical cytology. Compared with the 2006 population, the 2007-2009 ones were slightly older (43.44±12.37 yr vs. 42.66±12.21 yr, p <0.001); and their cervical cytology had much lower proportion of unsatisfied slides (OR=0.06, 95%CI 0.04-0.09) with comparable detection rate (3.96% *vs* 3.70%, p=0.052), but had significant difference in proportion of various abnormal cytology types (p <0.001), i.e., more ASCUS (OR=1.54, 95%CI 1.35-1.76), less HSIL (OR=0.65, 95%CI 0.55-0.76), and more AGC (OR=1.81, 95%CI 1.19-2.75).

Table 3 shows the performance of Siriraj-LBC for the prediction of cervical lesions. Combined data of the years 2007 to 2009 showed that Siriraj-LBC had an NPV for CIN2+ of 97.64% and an overall PPV of 43.85%. The PPV for CIN2+ varied with different types of abnormal

Table 2. Characteristics of Patients and Cervical Cytology of 2006* and 2007-2009

		2006 (N=25,510)	2007-2009 (N=103,057)	p-value or OR (95%CI)
Age, years		42.6±12.2	43.4±12.3	<0.001
Unsatisfied slides†		153 (0.6, 95%CI 0.5-0.7)	38 (0.04, 95%CI 0.03-0.05)	0.06 (0.04-0.09)
Overall detection rate		944 (3.7, 95%CI 3.4-3.9)	4,085 (3.9, 95%CI 3.8-4.1)	1.07 (1.0-1.2)
Types of abnormal cervical cytology	ASCUS	251 (0.9)	1,551 (1.5)	1.54 (1.35-1.76)
	ASC-H	117 (0.5)	402 (0.4)	0.85 (0.69-1.04)
	LSIL	278 (1.1)	1,154 (1.1)	1.03 (0.90-1.17)
	HSIL	213 (0.8)	564 (0.5)	0.65 (0.55-0.76)
	AGC	25 (0.1)	183 (0.2)	1.81 (1.19-2.75)
	SCC	31 (0.1)	94 (0.1)	0.84 (0.67-1.02)
	ADC	28 (0.1)	88 (0.1)	0.75 (0.52-1.08)

Data are mean±standard deviation (SD), n (%), or odds ratio (OR) and 95% confidence interval (CI). Data were compared using Student's t-test or Chi-square test. *Data of the year 2006 was published in Laiwejpithaya, 2009. †Unsatisfied slides were slides with inadequate amount of cells to interpret the result (scanty smear). ADC=adenocarcinoma; AGC=atypical glandular cells; ASCUS=atypical squamous cells of undetermined significance; ASC-H=atypical squamous cells cannot exclude HSIL; HSIL=high-grade squamous intraepithelial lesion; LSIL=low-grade squamous intraepithelial lesion; SCC=squamous cell carcinoma

Table 3. Performance of Siriraj Liquid-Based Cytology (Siriraj-LBC) for Prediction of Cervical Lesions in 6,168 Cervical Specimens During 2007-2009

Cervical cytology		N	Cervical histopathology results			
			Normal	CIN1	CIN2-3	Invasive
NILM		4,923	4,742 (96.3)	65 (1.3)	59 (1.2)	57 (1.2)
Overall abnormal cytology		1,245	490 (39.4)	209 (16.8)	345 (27.7)	201 (16.1)
Types of abnormal cytology*	ASCUS	190	133 (70.0)	31 (16.3)	20 (10.5)	6 (3.2)
	ASC-H	201	96 (47.8)	37 (18.4)	49 (24.4)	19 (9.4)
	LSIL	245	108 (44.1)	102 (41.6)	33 (13.5)	2 (0.8)
	HSIL	351	66 (18.8)	38 (10.8)	212 (60.4)	35 (10.0)
	AGC	109	66 (60.5)	0 (0.0)	11 (10.1)	32 (29.4)
	SCC	81	4 (5.0)	1 (1.2)	12 (14.8)	64 (79.0)
	ADC	62	13 (21.0)	0 (0.0)	8 (12.9)	41 (66.1)
	Other malignancy	6	4 (66.7)	0 (0.0)	0 (0.0)	2 (33.3)

*Data of various types of abnormal cytology were analyzed using Chi-square test; ADC=adenocarcinoma; AGC=atypical glandular cells; ASCUS=atypical squamous cells of undetermined significance; ASC-H=atypical squamous cells cannot exclude HSIL; CIN=cervical intraepithelial neoplasia; HSIL=high-grade squamous intraepithelial lesion; LSIL=low-grade squamous intraepithelial lesion; NILM=negative for intraepithelial lesion and malignancy; SCC=squamous cell carcinoma.

Table 4. Performance of Liquid-Based Cytology in Various Reports During 2006-2013

Author, year	Countries	Sample size	Detection rate (%, (95%CI))	Predictive values for CIN2+ (%, (95%CI)) PPV	NPV
Taylor et al., 2006	South Africa	3,114	16.4	9.4 (7.0–12.3)	99.2 (98.8-99.5)
Ronco et al., 2007	Italy	22,708	6.3	7.4	NR
Siebers et al., 2009	Netherlands	49,222	0.83 (0.75-0.91)	28.9 (26.3-31.7)	NR
Pan et al., 2013	China	25,830	17.4	38.3 (36.0-40.5)	99.3 (99.2-99.4)

*CI=confidence interval, CIN=cervical intraepithelial neoplasia, LBC=liquid-based cytology, NPV=negative predictive value, NR=not report, PPV=positive predictive value.

cytology, ranging from 13.69% in ASCUS, 14.29% in LSIL, 33.33% in other malignancy, 33.83% in ASC-H, 39.45% in AGC, 70.37% in HSIL, 79.03% in ADC, and 93.82% in SCC, respectively. The PPV for CIN2+ in ASCUS and LSIL were comparable (approximately 14%), but the PPV for CIN1 was higher in LSIL than in ASCUS (41.63% *vs* 16.32%, p<0.001).

Table 4 shows the performance of LBC in previous reports during 2006-2013. The Siriraj LBC in 2006-2009 with the detection rate of 3.91, the PPV for CIN2+ of 31.25%, and the NPV of 95.88% were in line with those of other reports.

Discussion

The major concerns of LBC over the conventional cytology are the higher cost and the more false positive rate. The latter drawback leads to unnecessary investigations such as colposcopic examination, LEEP or cervical conization which may cause adverse consequences to the patients and their families.

The Siriraj-LBC was developed in the Siriraj Hospital, a tertiary care university hospital, to overcome the cost concern of LBC in Thailand. The Siriraj-LBC was proven to have higher performance than the conventional cytology and lower cost than the commercial LBCs (Laiwejpithaya et al., 2008; Laiwejpithaya et al., 2009). Although the Siriraj LBC in our first year experience had higher detection rate than the conventional cytology did, the detection rate of our LBC was lower than that of other LBCs were (Dupree et al., 1998; Papillo et al., 1998; Diaz-Rosario and Kabawat, 1999; Fremont-Smith et al., 2004). Despite of the increasing use of Siriraj-LBC, its detection rate was stable at approximately 4% over the past four years, suggesting the real detection rate of this technique in our population. With this detection rate, the NPV for all cervical neoplasia of 96.32% was very satisfied as compared with that of 96.24-96.33% in other reports (Beerman et al., 2009; Laiwejpithaya et al., 2009).

Compared with the 2006 data, the 2007-2009 data had dramatically lower proportion of unsatisfactory slides (0.04% *vs* 0.60%; OR 0.06, 95%CI 0.04-0.09), even though the population were slightly older. The approximately one year difference in age of reproductive period (43.44±12.37 *vs* 42.66±12.21 yr, p<0.001) did not affect the rate of unsatisfactory slide, as previous study showed that only the age of >55 years had adverse effect (Castle et al., 2010). Moreover, Siriraj-LBC in the later years had significant alteration in the proportion of various cytological abnormalities, despite of stable detection rate. The proportion of ASCUS significantly increased whereas that of LSIL was stable. The ASCUS-to-LSIL ratio of less than 2 indicated the quality of our LBC technique (Davey et al., 1994). Both ASCUS and LSIL had comparable PPV for CIN2+ of approximately 14%, suggesting that these two conditions could undergo the same management.

The proportion of AGC significantly increased. The AGC plus other types of abnormal glandular cells had PPV for CIN2+ of >50%, the majority of which were invasive diseases, suggesting that these conditions needed prompt evaluation. The proportions of other cytological abnormalities (ASC-H, HSIL and malignant cells) decreased, but only the reduction in proportion of HSIL was statistically significant. The similarity between HSIL and ASC might result in misinterpretation HSIL as ASCUS or ASC-H, leading to the decrease in one and the increase in the other.

We found an unpleasantly lower PPV for CIN 1+ of overall cytological abnormalities. Such PPV reduced from 83.03% in 2006 (data not shown) to 60.64% in the later years. Despites, the PPV for CIN2+ of overall cytological abnormalities were comparable between the two periods (32.57% in 2006 *vs* 43.85% in 2007-2009). Since CIN2+ needs immediate treatments, the overall cytological abnormalities detected by Siriraj-LBC with a PPV for CIN2+ of 43.85% provided a favorably low number-needed-to-treat of 2.3. The PPV for CIN2+ increased with types of abnormal cytology, from 13.69% to 93.82%, in ASCUS, LSIL, ASC-H, abnormal glandular cytology, HSIL, and SCC, respectively. These results were comparable with the results in the first year of Siriraj-LBC and in other studies (Dupree et al., 1998; Papillo et al., 1998; Diaz-Rosario and Kabawat, 1999; Fremont-Smith et al., 2004; Ruengkhachorn et al., 2012). Our 4-year data strongly indicated that Siriraj-LBC, a low cost LBC technology, was qualified for cervical cancer screening.

In conclusion, with the increasing use from 2006 to 2009, the Siriraj-LBC has a stable detection rate of approximately 4%. The NPV for CIN2+ is favorably higher than 95%. The alteration in the proportion of cervical cytology types detected during the later years of Siriraj-LBC needs further evaluation. The PPV for CIN2+ of ASCUS and LSIL were comparable (approximately 14%), suggesting that these two conditions may undergo similar management. Other cytological abnormalities need immediate further evaluation.

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