False-Negative Rate of Papanicolaou Testing: A National Survey from the Thai Society of Cytology

Supinda Koonmee[a]   Andrey Bychkov[b]   Somruetai Shuangshoti[d]
Kanyaprin Bhummichitra[f]   Wanwisa Himakhun[c]   Anant Karalak[e]
Samreung Rangdaeng[g]

[a]Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, [b]Department of Pathology, Faculty of Medicine, Chulalongkorn University, [c]Department of Pathology, Faculty of Medicine, Thammasat University, and [d]Institute of Pathology, Department of Medical Services and [e]National Cancer Institute, Ministry of Public Health, Bangkok, [f]Division of Pathology, Suratthani Central Hospital, Suratthani, and [g]Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

Abstract

Objective: To evaluate the performance of Papanicolaou smear screening in Thailand at the national level, and to propose recommendations for continuing quality control. **Study Design:** This study was conducted by The Thai Society of Cytology and involved 124 laboratories in 76 provinces during 2010–2014. Random sampling suggested recalling of 10% of slides defined as negative at routine screenings (10% random rescreening [R10] model) directly from the reading unit. **Results:** Out of 330,075 smears covered by the rescreening project throughout its 5-year duration, the rates of abnormal, unsatisfactory, and normal results were 0.63, 1.82, and 97.55%, respectively. Abnormal findings were largely represented by ASC-US (54%) and L-SIL (21%). The average false-negative rate (FNR) measured at the level of L-SIL and higher was 13.8%. **Conclusion:** The national project was developed to address the accuracy of cervical cancer screening and to promote internal quality assurance based on the R10, on-site surveys, and education. The major output parameters of this study (FNR and number and distribution of abnormal cases on rescreening) improved significantly in the main phase of the project (2012–2014), after revising substantial logistics issues encountered during the first 2 years of this study. This project provided objective measurable evidence related to the quality of cytology-based cervical cancer screening in Thailand.

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Correspondence to: Dr. Samreung Rangdaeng
Department of Pathology
Faculty of Medicine, Chiang Mai University
110 Intavaroros Street, Chiang Mai 50200 (Thailand)
E-Mail samramed@yahoo.com

Introduction

Cervical cancer was the most common female cancer in Thailand until 1997, when breast cancer came to occupy the top rank due to intense imaging screening [1]. The most recent statistics rank cervical cancer as the second most common female cancer in Thailand, with 6,426 new cases per year, and the second leading cause of cancer deaths in women aged 15–44 years [2, 3]. The age-standardized incidence rate during the period from 2010 to 2012 was 14.4 per 100,000 women [4].

It has been clearly established that cervical cancer incidence and mortality can be reduced dramatically by implementation of appropriate screening measures based on the Papanicolaou (Pap) smear test [5]. In Thailand, there were no organized programs directed at screening for cervical cancer until 2002. For the most part, screening has been unsystematic or provided to women on demand [1, 6]. In 2002, the National Cancer Institute of Thailand, together with the Ministry of Public Health and the National Health Security Office of Thailand, implemented the National Cervical Cancer Screening Program (NCCSP) aimed at providing screenings at least once every 5 years for the entire population of women aged 35, 40, 45, 50, 55, and 60 years [1, 7, 8].

During the first phase (2005–2009), measures to increase the capacity for obtaining and interpreting Pap smears were put in place. By the end of that phase, the coverage of the target population had substantially improved from 25% (before 2005) to 79% in 2009 [8]. In the second phase (2010–2014), more diverse tasks were addressed, including evaluation of cytological diagnosis performance and establishment of a procedure for continuing quality control.

Different strategies of cervical cytology quality control include random rescreening, a full review, rapid prescreening, double screening, and others [9–12]. The most widely used approach is a 10% random rescreening (R10), which is a mandatory internal quality control tool according to US CLIA '88 regulations [13]. This method has several limitations, and it is less accurate than 100% rapid rescreening or rapid prescreening [14–16]. However, the R10 model is one of only a few methods that can be easily adopted for large-scale projects using remote expertise. The R10 model is aimed at identifying abnormal cells in negative Pap smears, and it provides the false-negative rate (FNR) as its output.

In Thailand, most cytological laboratories operate in low-resource settings and are faced with a lack of personnel and a heavy workload. Collection of cytological samples is performed by trained personnel at all levels of health care providers from subdistrict to provincial hospitals, which include physicians, nurses, and other health personnel at district health stations [8]. Alcohol-fixed slides are sent to cytology laboratories at provincial hospitals where cytotechnologists perform a primary screening. Most laboratories at provincial hospital levels do not have an on-site pathologist. All negative slides are signed out by qualified cytotechnologists. Only abnormal cases are sent to pathologists in other laboratories in the network for hierarchy reviews. Internal control of cervical smears is not systematic and it is often inconsistent among hospitals, despite being part of the list of basic requirements for laboratory licensing. The nationwide cervical cancer screening project provided an excellent opportunity to focus on the issue of quality assurance in cervical cytology. It was assumed that a large-scale external quality control project may be beneficial in several ways, such as auditing current practice in cervical cytopathology across the country, assessing reference FNR, instigating local efforts to maintain internal control, and detecting cytological abnormalities that had previously been overlooked.

The purpose of the current study was to evaluate the performance of Pap smear screening in Thailand at the national level, and to propose recommendations for continuing quality control.

Materials and Methods

Study Setting

To promote confidence among health service users and enhance screening accuracy, the National Cancer Institute of Thailand in collaboration with the Thai Society of Cytology and the Royal College of Pathologists of Thailand organized the Cervical Screening External Quality Assurance Project. The project was funded by the National Health Security Office of Thailand and it was conducted over a 5-year period, with the initial phase in 2010–2011 and the main phase in 2012–2014. Although the R10 model of evaluating negative cervical smears was selected for its affordability, the final budget only included approval for a limited number of slides to be submitted for rescreening. As such, 100,075 Pap smears were rescreened in the initial phase, followed by 230,000 in the main phase.

All 76 Thai provinces were involved in the project. A total of 124 government and private laboratories (out of the 249 that were registered as slide reading service providers for the NCCSP) participated voluntarily and contributed to the project in various ways over the course of 5 years (Table 1). Local laboratories varied widely in terms of the daily workload, staff experience, and equipment. The Thai Society of Cytology has a network of 4 operating regional centers, responsible for consultation services in the northern, northeastern, central, and southern regions of the country. Local

Table 1. Study participants

Region	2010	2011	2012	2013	2014
Northern					
Laboratories	15	17	22	23	17
Slides submitted for rescreening	16,000	10,900	27,000	29,000	7,538
Northeastern					
Laboratories	16	19	31	17	24
Slides submitted for rescreening	9,351	9,554	27,000	26,000	7,500
Southern					
Laboratories	26	30	31	25	23
Slides submitted for rescreening	10,666	10,396	19,000	20,000	7,500
Central and eastern					
Laboratories	15	44	40	38	23
Slides submitted for rescreening	13,988	19,220	27,000	25,000	7,462
All regions					
Laboratories	72	110	124	103	87
Slides submitted for rescreening	50,005	50,070	100,000	100,000	30,000

Values are presented as numbers.

cytology laboratories were requested to submit a set of slides with negative Pap smears to the regional center before scheduled annual deadlines. Random sampling suggested recalling of 10% of slides determined to be negative at the time of routine screening directly from the reading unit. Each laboratory that participated was advised to select slides with a definite final digit, e.g., 0 (10, 20, 30, etc.), and if the smear was diagnosed as negative it was included in the rescreening set. This study was approved by the Khon Kaen University Ethics Committee for Human Research (HE591343), to which the first author is affiliated.

Review Process

A Quality Assurance panel was established by the Thai Society of Cytology. Thirty-five pathologists and 158 cytotechnologists with well-regarded experience in the field of cervical cytopathology were recruited as expert reviewers and were responsible for the rescreening. Panel members were distributed among 4 regional centers, as per their primary affiliation. It is important to note that no reviewer evaluated slides from laboratories at which they participated in routine screening.

The rescreening procedure was conducted as follows: all of the slides submitted to the regional center were distributed evenly among the experts, with the first round of reading being conducted by 2 cytotechnologists independently. After that, all abnormal slides and a random 10% of the negative slides were reviewed by a pathologist. This kind of multistep approach ensured robust identification of abnormal smears and provided additional data on the concordance rate among experts. It should be noted that a majority of provincial cytology laboratories have no staff pathologist. Pap smears were assessed under a magnification of ×100 for adequacy and cytological abnormalities, and the results were recorded in a spreadsheet. The nomenclature used was the 2001 Bethesda System for Reporting Cervical Cytology [17]. There was no time limit for slide examination. The workload of each reviewing unit, which consisted of 1 pathologist and 2 cytotechnologists, was ap-

proximately 100 slides every 2 weeks. Cases in which there was disagreement between the findings of the cytotechnologists and those of the pathologists were resolved on site or at the regional center by consensus review using a multiheaded microscope. In cases in which there were abnormal findings, local laboratories were informed about how to adequately manage the patients in question.

Statistical Analysis

The FNR was defined as the proportion of estimated false-negative cases divided by the sum of false negatives and true positives, and it was expressed as a percentage [18]. The number of estimated false-negative cases was calculated at the L-SIL threshold by projecting the rate of abnormal cases onto the total number of normal and unsatisfactory cases at screening.

A χ^2 test with Yates's correction was applied to analyze differences between the initial and main phases of this study, and $p < 0.05$ was considered statistically significant.

Results

Among the 330,075 smears covered over the 5 years of this rescreening project, the rates of abnormal, unsatisfactory, and normal results were 0.63, 1.82, and 97.55%, respectively (Table 2). Abnormal findings were largely represented by ASC-US (54%) and L-SIL (21%), while H-SIL and other borderline lesions, including ASC-H, AGC, AGC endometrium, and AGC favor neoplastic lesions, were recorded less frequently. There were 22 cases of cancer found over the 5 years of rescreening. Squamous cell carcinoma, adenocarcinoma, and adenocarcinoma in

Table 2. Cytological findings on rescreening

Diagnosis	2010	2011	2012	2013	2014	Total	
						n	%
Normal	48,979	48,844	97,466	97,424	29,271	321,984	97.55
Unsatisfactory	592	814	2,067	1,997	542	6,012	1.82
Abnormal	434	412	467	579	187	2,079	0.63
ASC-US	240	224	243	315	109	1,131	54.00[c]
Other borderline[a]	74	67	80	59	29	309	15.00[c]
L-SIL	74	68	100	164	37	443	21.00[c]
H-SIL	42	45	43	34	10	174	9.00[c]
Cancer	4	8	1	7	2	22	1.00[c]
AIS	0	3	1	1	0	5	
Squamous cell carcinoma	3	2	0	3	1	9	
Adenocarcinoma	1	2	0	2	1	6	
Other[b]	0	1	0	1	0	2	

[a] ASC-H, AGC, AGC endometrium, AGC favor neoplastic. [b] Small-cell carcinoma, malignant mixed müllerian tumor. [c] Percent out of all abnormal cases.

situ were the most common cancers missed by the initial cervical screening. There was a substantial proportion of unsatisfactory results, likely induced by the study design (e.g., slow delivery of slides from local labs to the regional centers led to deterioration of the staining quality).

Results of the rescreening were projected onto the national cervical cancer screening statistics to calculate the FNR (Table 3). The average FNR measured at the level of L-SIL and higher was 13.8%, with substantial fluctuation from 8.99% in 2012 to 18.4% in 2010. The FNR in the initial phase of rescreening (2010–2011) was higher compared to that of the main phase (2012–2014, $p = 0.04$). Although there were significantly more abnormal smears detected in 2010–2011 than in 2012–2014 ($p < 0.01$), the number of clinically important abnormalities (L-SIL and higher) was higher in the main phase of rescreening ($p = 0.04$).

Discussion

Here we report the results of a national rescreening of cervical smears performed by the Thai Society of Cytology. This external quality control project reevaluated 330,075 Pap smears from all provinces in Thailand over a 5-year period and established a reference FNR in the local settings. Significant effort was expended to set up a logistics network, provide educational support, and im-

prove communication between regional and central laboratories.

The FNR of Pap smear interpretation can be expressed as a broad range rather than a precise number [15]. Naryshkin [19], for example, performed a literature review and found an FNR that ranged from 1.6 to 28%. A survey among US-based university hospital laboratories reported a 10–31% FNR [20]. A more recent examination by Lonnberg et al. [21] found an even wider range of 15–63%. As reducing the FNR to below 5–10% is thought to be extremely difficult [22], a 10–20% FNR is considered good or fair [15, 19]. A false-negative rate is best characterized as an estimate of the staff's average screening sensitivity in a given laboratory. Random rescreening of negative and inadequate smears is an essential procedure as part of internal quality control. However, random rescreening may also be employed as an external quality control measure. A recent study based on the clinical outcomes conducted by the national cervical cancer screening program in Finland found a 35% FNR at the cutoff of L-SIL or worse [21]. Our average FNR over 5 years was 13.8%, and there were 2 years in which the rate was lower than 10%. We regard these to be good numbers, considering the national scale of the study. This FNR can be used as a reference rate in future projects. In parallel to this project, an interlaboratory comparison program was also embarked on by distributing a set of 20 reference slides to each participating laboratory that was then provided with

Table 3. FNR based on the results of rescreening

	2010	2011	2012	2013	2014	2010–2014
Screened cases, n	2,316,790	1,899,709	1,494,194	1,241,009	992,574	7,944,276
Normal, borderline, and unsatisfactory cytology on screening, n	2,292,394	1,877,028	1,472,734	1,223,464	977,101	7,842,721
Abnormal cytology on screening[a], n	24,396	22,681	21,460	17,545	15,473	101,555
Abnormal cytology on screening[a], %	1.05	1.19	1.44	1.41	1.56	1.28
Rescreened cases, n	50,005	50,070	100,000	100,000	30,000	330,075
Rescreening coverage, %	2.16	2.64	6.69	8.06	3.02	4.21
Abnormal cytology on rescreening[a], n	120	121	144	205	49	639
Abnormal cytology on rescreening[a], %	0.24	0.24	0.14	0.21	0.16	0.19
False-negative cases (estimated), n	5,501	4,536	2,121	2,508	1,596	16,262
Total abnormal cytology (screening and rescreening), n	29,897	27,217	23,581	20,053	17,069	117,817
FNR, %	18.40	16.67	8.99	12.51	9.35	13.80

FNR, false-negative rate. [a] L-SIL, H-SIL, and cancers only.

feedback regarding their individual performance. There were 6 laboratories with perfect scores and 6 laboratories with suboptimal performance. In the latter cases, educational efforts and greater supervision were implemented. Our retrospective investigation into possible errors that led to the false-negative readings found that the most common causes were screening and slide-processing errors, while interpretation errors were infrequent.

The major cytological categories that contributed to the FNR were ASC-US with other indeterminate abnormalities and L-SIL, with a minority of H-SIL and cancers. Both the ASC-US and the L-SIL thresholds are routinely used to evaluate laboratory and individual performance. We were only able to calculate FNR at the L-SIL cutoff because borderline categories are not entered into the national cancer registry. An L-SIL threshold is usually preferred over ASC-US since the degree of reproducibility for the interpretation of indeterminate categories is low [23].

There were substantial logistics issues during the initial period of the 5-year project, including delays, losses, and frequent admixture of abnormal smears into the rescreening set. As a result, there were more abnormal smears and a higher FNR in the initial phase of rescreening (2010–2011) compared to the main phase (2012–2014). Logistical matters were regularly addressed during annual meetings and related educational events arranged by the Thai Society of Cytology. Direct communication among regional centers and the administration of hospitals and local laboratories appeared to be effective measures in reducing organizational flaws. As a result, the major output parameters of the study (FNR, number of abnormal cases on rescreening, etc.) improved significantly in the main phase of the project. We speculate that the mean FNR recorded over the last 3 years of the study (10.3%) represents an accurate rate of false negatives in our local settings.

There were several limitations in this study that must be taken in account. No abnormal smears were included in the rescreening in order to potentially upgrade the diagnosis or evaluate the false-positive rate. In addition, no unsatisfactory smears were requested for rescreening. Both issues are design flaws, which can be considered for future studies. While the R10 model was selected as the prototype, the actual rescreening coverage was much lower (4.21% over 2010–2014), with a highly uneven distribution across the years. This limitation can be assumed to be inherent due to budget restrictions, which were not under direct purview of the Thai Society of Cytology. Another issue is that borderline lesions were combined with normals for FNR calculation, which is a limitation of the national cancer registry. Again, only about half of the registered laboratories (49.8%) had contributed slides for rescreening. Due to procedural limitations, those rescreening slides were from 6 months up to 1 year old before being subjected to the rescreening process. In addition, the hospitals or reading laboratories may be reluctant to issue amended reports. Thus, a more robust real-time system and effective follow-ups are needed to make the process more efficient and provide better care for the patients. In the future, the Thai Society of Cytology will embark on prereport rescreening for negative cases. Use of the R10 model as the sole method of quality control in cervical cytology has long been criticized [9, 15, 16]. Nevertheless, we believe that the R10 model is the only affordable option for such large-scale projects in low-resource settings.

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The overall quality of cervical cytology is affected by several factors in preanalytical and postanalytical steps, including: (1) biological variability; (2) collection of samples (site and sampling method); (3) laboratory procedure, including processing; (4) primary screening (manual or computer assisted); and (5) interpretation [24, 25]. It should be noted that our project involved only the primary screening phase, which is just a part of the overall quality assurance process. The Thai Society of Cytology plays a core role in quality assurance at the national level, including laboratory accreditation, external quality control programs and training, and certification and continuing education of cytotechnologists and pathologists. With limited human resources, the National Cancer Institute is undergoing a paradigm shift by judiciously evaluating primary HPV testing or cotesting for the next phase of the cervical cancer screening program. A similar model has been recently successfully implemented and reported in a middle-income country in Latin America [26].

Prospective initiatives from the Thai Society of Cytology are focused on a wider delegation of quality control responsibilities to the various regions and laboratories. Internal quality assurance based on the R10 and more advance models, on-site surveys, and education programs are especially being promoted. External quality control measures have already been implemented in parallel with this program to ensure adequate accreditation, certification, and proficiency testing.

Conclusions

This is the first report on the FNR of cervical smears from Thailand or elsewhere in Southeast Asia, and it is one of the largest-scaled projects in the field. Rescreening of 330,075 Pap smears submitted nationwide revealed a 13.8% FNR of cervical screening. Although some limitations were encountered, this project provided objective measurable evidence related to the quality of cytology-based cervical cancer screening in Thailand. It should be a stepping stone toward a more robust and timely system to promote quality assurance in cytological practice.

Disclosure Statement

The authors have no conflict of interests to disclose.

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