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Does Liquid-Based Technology Really Improve Detection of Cervical Neoplasia?

A Prospective, Randomized Trial Comparing the ThinPrep Pap Test with the Conventional Pap Test, Including Follow-up of HSIL Cases

Jörg H. Obwegeser, M.D., M.I.A.C, Exec. M.B.A., and Susanne Brack, C.M.I.A.C.

OBJECTIVE: To compare the sensitivity, specificity and specimen adequacy of the ThinPrep Pap Test (TP) with the conventional Pap Test (CV) in a low-risk population with subsequent follow-up of HSIL cases.

STUDY DESIGN: A prospective, randomized, controlled design was chosen to compare the TP with CV. Cytologic diagnosis and specimen adequacy were evaluated and compared with histology data in high grade squamous intraepithelial lesion (HSIL) cases. Fifteen gynecologists in private practice, all trained in colposcopy, participated in the trial. Cytologic diagnosis, specimen adequacy and follow-up of the cytologic HSIL cases were compared in the two groups. In total, 1,999 patients were included, 997 in the TP group and 1,002 in the CV group. Randomization assignments were designated on cytology case report forms, which were placed in sealed envelopes. Each envelope had a sequential randomization number on the outside to allow tracking and authentication of randomization assignments.

The most important reason for improved specimen adequacy and improved detection rates of squamous intraepithelial lesions…in liquid-based technology [seems to be] better sampling.

RESULTS: Comparison of results between CVs and TPs revealed no statistically significant differences in all diagnostic categories, ranging from "within normal limits" to HSIL. Specimen adequacy, however, was superior with CVs (P<.001). The cytologic diagnosis of HSIL *correlated with the histologic diagnosis in 91% of the TP group and* 100% *of the CV group.*

From the Cytology Laboratory of Jörg H. Obwegeser, M.D., M.I.A.C., Exec. M.B.A., Zürich, Switzerland.

Dr. Obwegeser is Gynecologist, Cytologist and Clinical Director. Ms. Brack is Chief Cytotechnologist.

Address reprint requests to: Jörg H. Obwegeser, M.D., M.I.A.C., Exec. M.B.A., Gfennstrasse 39, CH-8603 Schwerzenbach, Switzerland (zylabob@swissonline.ch).

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CONCLUSION: Because there was no statistically significant difference in sensitivity and specificity of the two techniques, improved detection of cervical abnormalities and better specimen adequacy might not be a consequence of utilizing liquid-based preparations but of a better sampling technique. Removing mucus and cellular debris from the cervical surface with a cellulose swab before sampling cells with a proper sampling device results in the same sensitivity and specimen adequacy and is much less expensive than the liquid-based technique. (Acta Cytol 2001:45:709-714)

Keywords: Papanicolaou smear, cervical smears, cervix neoplasms, mass screening, laboratory techniques and procedures, ThinPrep Pap Test.

In the last few years, major improvements in detecting precursors of cervical cancer and improved specimen adequacy due to the use of liquid-based preparation technologies as compared to the conventional technique have been reported. [1-13] A higher rate of low grade intraepithelial lesions (LSILs) was detected in all studies, but it is not the goal of cervical cancer screening to detect a lesion that is likely to regress. Higher rates of high grade intraepithelial lesions (HSILs) have also been reported in some of these studies. [1,2,4,7,12] However, the collection device used for the conventional Pap smear (CV) was not always reported[1,6,10] or was at least not optimal for a Pap test.[8] This makes it difficult to compare the two techniques. The clinical trial leading to U.S. Food and Drug Administration approval of the ThinPrep Pap Test (TP) (Cytyc Corp., Boxborough, Massachusetts, U.S.A.)[5] demonstrated that in academic hospital centers the higher detection rate of LSIL+ (all lesions >LSIL) and the decrease in atypical squamous cells of undetermined significance/atypical glandular cells of undetermined significance (ASCUS/AGUS) was not statistically significant. An improvement in detection rates was evident for commercial screening laboratories.

A comparison of our laboratory data (conventional Pap smear over a period of 20 months) with data from two studies from Geneva[6,11] (comparing the liquid-based with the conventional technique) led us to think that there might be other reasons underlying improvements in specimen adequacy and higher detection rate for cervical cancer precursors than the use of a liquid-based preparation technique, as noted before by other authors.[8,14] To prove this point, we initiated a prospective, randomized study comparing TP with CV.

Materials and Methods

Fifteen gynecologists in private practice participated in the study. All are trained in colposcopy and utilize the CV as a primary cervical cancer screening sampling technique with a Szalay Cyto-Spatula (CSM Graf, Steinach, Switzerland) (Figure 1). This spatula collects cells from the endocervical canal as well as from the cervical surface simultaneously. We visited all 15 participating gynecologists before initiating the study to instruct them in the TP collection method according to the clinician reference guide from Cytyc Corp.

Patient Enrollment

All patients visiting the 15 private practices for a Pap smear between mid-July 1998 and the end of September 1998 were randomly assigned to either the TP or CV group. In total, 1,999 patients were included, 997 in the TP group and 1,002 in the CV group. Randomization was based on methods discussed by Fleiss.[15] Randomization assignments were designated on cytology case report forms, which were placed in sealed envelopes. Each envelope had a sequential randomization number on the outside to allow tracking and authentication of randomization assignments. Patients with a previous abnormal Pap smear were included in the study:
48 in the TP group (14 LSIL, 10 HSIL, 22 ASCUS/AGUS, 2 unsatisfactory) and 50 in the CV group (24 LSIL, 5 HSIL, 20 ASCUS/AGUS, 1 unsatisfactory). The screened populations were comparable in the two groups (Figure 2).

Figure 1 Szalay Cyto-Spatula. The spatula collects cells from the endocervical canal as well as from the cervical surface simultaneously. There are three different shapes.

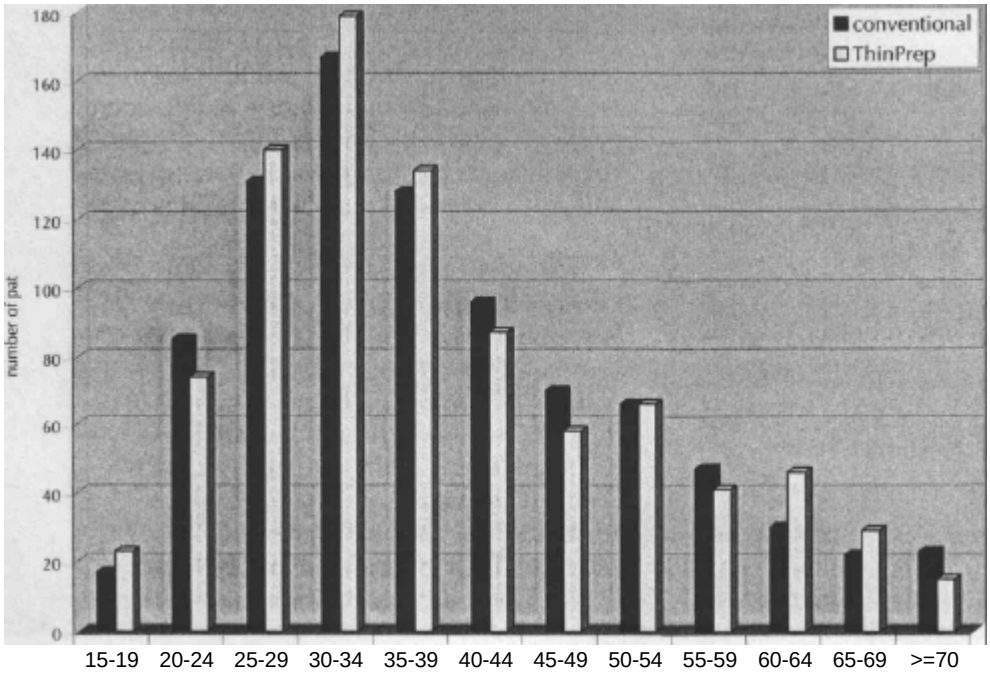

Figure 2 Age distribution of patients

Specimen Collection and Preparation, Slide Evaluation and Reporting

All Pap smears (TP and CV) were collected after mucus and debris had been removed from the cervical surface with a cellulose swab (standard practice recommended by the laboratory). All specimens were then collected under colposcopic guidance. The collection device used in the TP group was the Cervex-Brush (Rovers Medical Devices, B.V., Oss, the Netherlands) or Oribrush (Orifice Medical A.B., Ystad, Sweden) for endocervical cell collection combined with a plastic spatula for the cervical surface. The device was rinsed immediately after use in a vial of PreservCyt Solution (Cytyc), and a slide was prepared in the laboratory using the ThinPrep 2000 Processor (Cytyc) according to the operator's manual. In the CV group, the Szalay Cyto-Spatula (CSM Graf & Co., Steinach, Switzerland) (Figure 1) was used (standard practice). The Szalay spatula method has been described as superior to other cervical sampling methods.[16] Slides were fixed immediately in a 96% alcohol solution. TP and CV slides were stained with the laboratory's routine Papanicolaou staining.

Table I *Concurrent Results: TP vs. CV*

	CV (1,002 Patients)		TP (997 Patients)		
Result	**n**	**%**	**n**	**%**	**P value**
WNL	931	92.9	924	92.7	NS
LSIL	37	3.7	47	4.7	NS
HSIL	19	1.8	16	1.6	NS
Carcinoma	1	0.1	0	0.0	NS
ASCUS/AGUS	14	1.4	10	1.0	NS
LSIL+		5.6		6.3	NS
ASCUS/AGUS+		7.0		7.3	NS
SBLB	25	2.5	55	5.5	<0.001
US	0	0	14	1.4	<0.001

WNL=within normal limits; LSIL=mild dysplasia.including HPV changes; HSIL=CIN 2 and 3 and CIS; US = unsatisfactory; LSIL+=CIN 1 and more severe; ASCUS/AGUS+= all abnormal cells.

Table II *Specimen Adequacy*

	SBLB		Unsatisfactory	
Factor	**TP**	**CV**	**TP**	**CV**
Scant cellularity	16	3	14	0
Obscuring blood	3	1	8[a]	
No endocervical cells	30	14		
Obscuring inflammation	5	3		
Cytolysis	1	4		
Total	55 (5.5%)	25 (2.5%)	22 (1.4%)	0 (0%)

For the assessment of adequacy, Bethesda System criteria were used, but it was a visual estimate, and cell counts were not performed [a]Eight of 14 also had obscuring blood.

Tabelle III *Follow-up of HSIL Cases*

Follow-up	TP (n=16)		CV (n=19)	
Histology available	n=11/16	69%	n=12/19	63%
HSIL	10/11	91%	12/12	100%
LSIL	0/11	0%	0/12	0%
No SIL	1/11	9%	0/12	0%
Cytology + lost to follow-up	5/16	31%	7/19	37%
WNL	2/16	13%	2/19	11%
LSIL	1/16	6%	1/19	5%
HSIL	0/16	0%	2/19	11%
ASCUS/AGUS	1/16	6%	1/19	5%
Lost to follow- up	1/16	6%	1/19	5%

HSIL = high grade intraepithelial lesion (cervical intraepithelial neoplasia 2 and more severe, including 1 carcinoma); LSIL = mild dysplasia, including HPV changes; no SIL=nonintraepithelial lesion found; lost to follow-up = women who did not answer after recall. All histologic diagnoses were done on conization or hysterectomy specimen.

TP slides were evaluated by an experienced cytotechnologist who had successfully completed a training program offered by Cytyc and received a primary training certification. CV smears were evaluated by three other cytotechnologists with extensive experience in reading CV smears. Slides that contained abnormal cells or cells with uncertain significance were referred to the medical director of the laboratory.

Statistical Analysis

The proportion of the two patient populations that were abnormal were compared using the two-sample test for binomial proportions.[17] A P value of <=.05 was used as a criterion for statistical significance.

Follow-up of HSIL Cases

HSIL cases had follow-up for 12-15 months with either histology or cytology. All histology specimens were evaluated by pathologists independent of our cytology laboratory on conization or hysterectomy specimens, not on biopsies. Follow-up of the LSIL and ASCUS/AGUS cases in our trial is in process.

Results

During a period of 10 weeks (July 21, 1998-September 30, 1998), 997 TPs and 1,002 CVs were compared for cytologic diagnosis and specimen adequacy. Cytologic diagnoses of LSIL, HSIL, ASCUS/AGUS, LSIL+ and ASCUS/AGUS+ were not significantly different between the two groups (Table I). Specimen adequacy (significant but limited by [SBLB]) in the TP group (5.5%) was significantly higher as compared to that in the CV group (2.5%). The 1.4% unsatisfactory rate in the TP group was caused mostly by scant cellularity and was significantly higher when compared with none in the CV group (Table II). All these unsatisfactory cases were rescreened and confirmed by a cytotechnologist at Cytyc.

Cytologic diagnoses of HSIL were correlated with the available diagnosis on histologic specimens in 91% of the TP group and 100% of the CV group. Two patients with HSIL lesions in the CV group refused surgical therapy. Three patients were lost to follow-up (Table III).

Discussion

Our data differ from those in most other studies that compared direct-to-vial, liquid-based preparation techniques with

Table IV *Comparison Data from Three Swiss Laboratories*

	Weintraub(1997)[6] (n=31,457)		Vassilakos(1998)[11] (n= 48,058)		Obwegeser (n= 172,315)
Parameter	**TP (%)**	**CV (%)**	**CytoRich (%)**	**CV (%)**	**CV (%)[d]**
Specimen adequacy					
SBLB	10.0	24.7[a]	12.1	72.2	6.2
No endocervical cells	9.8	13.1[b]	9.2	33.8	3.6
Unsatisfactory	0.26	0.36[c]	0.4	1.8	0.3
Cytologic diagnosis					
HSIL	0.7	0.3	0.6	0.3	0.6
LSIL	2.1	1.8	3.0	0.8	1.7
LSIL+	3.9	3.2	3.6	1.1	2.3
ASCUS/AGUS	2.7	1.6	1.6	3.7	1.8
ASCUS/AGUS+	6.6	4.8	5.2	4.8	4.1

HSIL = high grade intraepithelial lesion (CIN 2 and more severe); LSIL = mild dysplasia and HPV changes; LSIL+=CIN 1 and more severe; ASCUS/AGUS+= all abnormal cells.
[a-c]Average of three years (1995-1997).[a]Range from 24.5% to 30.9%, [b]range from 11.8% to 14.4%, [c]range from 0.14% to 0.70%. [d]Unpublished data.

Tabelle V *Results of Rescreening 50% of TP Slides*

	Specimen adequacy							
	Laboratory I (U.S.)[a]				Laboratory II (investigator)[b]			
Cytologic diagnosis	Satisfactory	SBLB	US	Total	Satisfactory	SBLB	US	Total
Within normal limit	310 (62.4%)	119 (23.9%)		429 (86.3%)	437 (87.9%)	25 (5.1%)		462 (93.0%)
LSIL	9 (1.8%)	2 (0.4%)		11 (2.2%)	20 (4.0%)	1 (0.2%)		21 (4.2%)
HSIL	9 (1.8%)	1 (0.2%)		10 (2.0%)	6 (1.2%)			6 (1.2%)
ASCUS/AGUS	26 (5.3%)	7 (1.4%)		33 (6.6%)	4 (0.8%)			4 (0.8%)
Unsatisfactory			14 (2.8%)	14 (2.8%)			4 (0.8%)	4 (0.8%)
Total	354 (71.2%)	129 (26.0%)	14 (2.8%)	497 (100%)	467 (93.9%)	26 (5.3%)	4 (0.8%)	497 (100%)
LSIL+	18 (3.6%)	3 (0.6%)		21 (4.2%)	26 (5.3%)	1 (0.2%)		27 (5.4%)
ASCUS/Agus+	44 (8.8%)	11 (2.2%)		54 (10.9%)	30 (6.1%)	1 0.2%)		31 (6.3%)

[a]LcytoDx Laboratory, Peabody, Massachusetts,
[b]U.S.A. laboratory of the investigator.

the direct smear (CV) technique. The use of an adequate collection device, the Szalay spatula, by participating clinicians may explain the difference. We also insist on removing mucus and cellular debris from the cervical surface with a cellulose swab before cell sampling is done, even though there might be a loss of some degenerated abnormal cells. The colposcopically guided sampling procedure allows us to verify that the collection device has entered the cervical canal and sampled the whole circumference of the cervical surface. For that reason, full colposcopy is not necessary. If colposcopically guided sampling is not possible, as is the case in many countries, the cervix has to be well visualized with an adequate light source. In any case, the collection device must enter the cervical canal. If the cervical canal can not be identified clearly, no smear should be taken.

Our results suggest that the most important reason for improved specimen adequacy and improved detection rates of squamous intraepithelial lesions (SILs) in liquid-based technology is better sampling. The greatest advantage of liquid-based techniques is therefore to force the clinician to use an adequate collection device. The reason for better specimen adequacy and the higher detection rate, especially of LSILs, in the initial trials of TP systems based on split samples[2,5,9] may be that CVs, which were always taken first, contained a lot of mucus. Therefore, debris and inflammatory cells may obscure the well-preserved cells scraped from the cervical surface. With the CV, clinicians can utilize an inadequate collection device, such as a cotton swab or Ayre spatula, alone without a second device that harvests cells from the endocervix. Because it is less expensive, this option is often chosen. For this reason we offer collection devices that harvest cells from the endocervical canal and surface of the cervix (Szalay spatula or Cytobrush used in combination with an Ayre spatula) free of charge to our clinicians.

Another important step is removal of mucus and cellular debris before sampling. This step is partially replaced in the liquid-based technique, although 15% of specimens require special treatment, an additional washing procedure because of blood, mucus and cellular debris.[8] Therefore, the liquid-based technology solves only one problem in the whole sampling chain as compared to the direct smear method—namely, incomplete transfer of harvested cells to the slide. However, each sampling will still be a subsampling, independent of the preparation technique. Precursors of cervical cancer and invasive cervical cancer will be missed with the liquid-based technology as well as with the CV technology, independent of screening and interpretation errors.[8,18] A comparison of data from our laboratory with two studies from Geneva[6,11] (Table IV) demonstrates that also under routine conditions in a large number of Pap smears, CV shows the same detection rate of HSIL lesions as the liquid-based technology does and that specimen adequacy is the same or even better. Therefore, the liquid-based preparation technology can improve specimen adequacy only when clinicians ignore the basic rules of the sampling procedure. A paper by Vassilakos,[11] with an SBLB rate of 72% and satisfactory rate of only 26%, demonstrates this impressively.

Utilization of an adequate sampling device and removal of mucus and cell debris prior to collecting cells is simple and inexpensive and results in the same specimen adequacy and detection rates for SIL. It is the duty of the laboratory director to give feedback to clinicians and instruct them in taking a CV if clinicians' specimen adequacy is suboptimal.

This applies to the CV as well as to fine needle aspiration. If specimens are obtained by individuals not well versed in direct smear preparation, liquid-based preparation technology is preferable even if important details are lost.[19,20]

Addendum

Because our results differ from those of previously reported direct-to-vial studies, Cytyc asked for a rescreening of 50% of the TP specimens by an independent laboratory, chosen by the company, using the TP preparation technique as a primary cervical cancer screening technology in addition to the prior study design. The results of these rescreened slides are reported in Table V.

Of special interest was a 60% higher rate of HSILs in the reviewing laboratory. Also notable was a 8.25 times higher rate of ASCUS, a 5 times higher SBLB rate and a 3.5 times higher unsatisfactory rate in the reviewing laboratory.
In five cases an HSIL was diagnosed in the reviewing laboratory, whereas the author's laboratory diagnosed three LSILs and twice a normal smear. All five cases had follow-up with cytology. None of these could be confirmed by histology because all but one, which persisted as an LSIL case, had a negative smear after 15-30 months.

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