The Unsatisfactory ThinPrep Pap Test

Missed Opportunity for Disease Detection?

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Abstract

Cervical cytology specimens classified as “unsatisfactory for interpretation” represent a potential source of undetected disease. This prospective study analyzed the potential benefits of a laboratory procedure to reprocess unsatisfactory ThinPrep Pap Tests (Cytyc, Boxborough, MA).

All unsatisfactory ThinPrep samples were reprocessed using a glacial acetic acid wash. The study period unsatisfactory rate was compared with that for the previous 12 months. The initial unsatisfactory rate was 1.3% (197/15,154). Of the unsatisfactory ThinPrep samples, 55.8% (110/197) had residual material for reprocessing. After reprocessing, 67.3% (74/110) were reclassified as “satisfactory” or “satisfactory but limited by,” and the final unsatisfactory rate was 0.8% (123/15,154), a 62% decrease. Compared with the previous 12-month rate of 0.9% (209/23,730), this was a 12% reduction. Seven (6.4%) of 110 initially classified as unsatisfactory contained an epithelial abnormality (atypical squamous cells of undetermined significance, 3; atypical glandular cells of undetermined significance, 2; low-grade squamous intraepithelial lesion, 1; squamous cell carcinoma, 1) on the reprocessed slide. Reprocessing of unsatisfactory ThinPrep slides yielded additional cellular abnormalities that otherwise would have been undetected. The present study confirms that reprocessing of unsatisfactory ThinPrep slides is a beneficial laboratory procedure.

The ThinPrep Pap Test (TP; Cytyc, Boxborough, MA) has demonstrated increased detection of both low- and high-grade squamous cervical lesions, compared with conventionally prepared (CP) cervical cytology samples.1,2 The increased TP sensitivity has been attributed to homogeneous sampling of the cervicovaginal specimen,3 enhanced cellular morphologic features, and reduction in technical artifacts that limit CP samples. The improvement in specimen adequacy also has been significant,2 most notably for the “satisfactory but limited by” (SBLB) categorization. However, improvements have not been achieved consistently for unsatisfactory rates, despite a suggestion from initial split-sample studies that unsatisfactory samples could be reduced by 60%.4

Longitudinal studies have shown that cervical cytology samples classified as unsatisfactory have an increased risk of being associated with disease, including carcinoma.5 These cases represent a potential source of significant abnormalities that may go undetected in routine cervical cytology screening.6-9 The unsatisfactory category emphasizes specimen unreliability for evaluation of epithelial lesions.

ThinPrep clinical trial split-sample data showed a 19% (1.6% CP sample vs 1.9% TP) increase in unsatisfactory cases and a 29% (27.8% CP sample vs 19.8% TP) reduction in samples reported as SBLB.10 Aggregate direct-to-vial studies showed no difference in unsatisfactory rates between TP and CP samples (mean, 0.8% vs 0.8%, respectively)2 and a 55% cumulative average reduction in the number of TP cervical samples reported as SBLB. A more recent report showed a statistical difference in improvement of both unsatisfactory and SBLB slides with the TP method.11

Since the introduction of the TP system at Associated Regional and University Pathologists (ARUP) Laboratories,
Salt Lake City, UT, we have noted an increased number of specimens classified as unsatisfactory compared with CP samples. This was particularly noticeable when the specimen vial (and subsequent slide produced) contained an abundance of blood. A recent report described a method to reprocess unsatisfactory TP specimens with a dilute glacial acetic acid wash before filtration.12

This prospective study was designed to analyze the potential benefits of a laboratory procedure to reprocess unsatisfactory TP samples. The results measured were identification of additional abnormal cases and effect on overall specimen adequacy.

Materials and Methods

All TP gynecologic samples received at ARUP Laboratories from April 15 to September 1, 2000, constituted the cases for our study. All TP slides were prepared using a ThinPrep 2000 automated slide processor (Cytyc) according to the manufacturer’s recommendations and stained by a modified Papanicolaou method. Following screening according to the Bethesda System for cytologic diagnosis and specimen adequacy, any TP sample determined to be unsatisfactory by a screening cytotechnologist was sent to a cytopathologist (C.J.M., J.S.B., or E.V.G.) for microscopic confirmation.

The ARUP Laboratories criterion is that a TP slide is considered unsatisfactory when the area of the 20-mm cell deposit contains less than 40% of well-preserved and well-visualized squamous epithelial cells (per ThinPrep 2000 package insert). An unsatisfactory designation does not include specimens that are rejected. Also, any TP specimen with abnormal cells identified on screening is, by definition, not characterized as unsatisfactory. During this study period, our criteria for final sign-out also began to incorporate use of numeric criteria for specimen adequacy (5,000 well-visualized and well-preserved squamous cells) as recommended by the proposed 2001 Bethesda System (http://bethesda2001.cancer.gov/postwrkshp_recs.html). For TP samples, more than 3 or 4 squamous epithelial cells per ×40 field has been suggested.

For this study, the adequacy of each slide prepared from the vial was determined separately and not cumulatively. For unsatisfactory cases, the cytology report noted whether blood, mucus, or inflammation contributed to an unsatisfactory sample, along with a comment about scant squamous cellularity. Some slides with cell clustering, atrophy, or cytolyis were technically difficult to count and were left to the judgment of the pathologist.

All slides characterized as unsatisfactory that had residual sample available were retrieved and reprocessed. All remaining sample was transferred to a 50-mL conical centrifuge tube and spun at 1,500 rpm (405g) for 10 minutes. After discarding the supernatant, a 30-mL solution of CytoLyt–glacial acetic acid (9:1) (Cytyc) was added to the cell pellet. This mixture was mixed for 5 seconds and centrifuged again at 1,500 rpm for 10 minutes. After the supernatant was decanted, the entire cell pellet was added to 30 mL of PreservCyt solution (Cytyc) and reprocessed using the ThinPrep 2000 Processor and a new TransCyt filter (Cytyc). Following rescreening by a senior cytotechnologist, the slides were submitted to a cytopathologist (J.S.B., E.V.G., or C.J.M.) for microscopic review and final report verification.

The TP unsatisfactory rates before and after sample treatment were compared. The unsatisfactory rate for the study period was compared with that for the previous 12 months using chi-square statistical analysis. The presence or absence of an epithelial abnormality was noted for cases that were reclassified as satisfactory or SBLB. Following the study period, these additional abnormal cases identified were reviewed to determine whether screening errors might have occurred during microscopic examination of the initial unsatisfactory slide. An attempt was made to obtain histologic follow-up for all additional abnormal cases identified.

Finally, to understand why bloody TP samples produced scanty cellular slides, we made cell blocks from the TP filter membrane. These were removed from the disposable plastic TransCyt cylinder of satisfactory and unsatisfactory TP cases. The filter was folded into quarters and embedded edgewise in a standard tissue cassette. Routine 5-µm paraffin sections were cut and stained with H&E.

Results

During the study period, ARUP Laboratories accessioned 15,154 TP and 14,654 CP gynecologic samples from a wide range of practitioners and clinical settings throughout the United States. The percentage of TP cases determined to be unsatisfactory following initial screening was 1.3% (197/15,154). A summary of TP and CP specimen adequacy is given in Table 1. Of the initially unsatisfactory TP cases, 55.8% (110/197) had adequate residual material in the specimen vial for reprocessing. After reprocessing, 67.3% (74/110) of the slides were reclassified as satisfactory (69% [51/74]) or SBLB (31% [23/74]). The final unsatisfactory rate, after reprocessing, was 0.8% (123/14,654). This was a 62% reduction in the unsatisfactory rate compared with the original unsatisfactory rate without reprocessing (1.3% vs 0.8%; P < .001). The clinical features of the reprocessed unsatisfactory cases are given in Table 2. Of note, 21.8% (24/110) of the specimens were obtained during the first half of the menstrual cycle.

During the 12 months before the study, an unsatisfactory classification was seen more frequently with TP samples...
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(0.9%; 209/23,730) compared with CP samples (0.5%; 232/46,367). During the study period, the CP samples (1.0%; 143/14,654) were unsatisfactory more often than the TP samples (0.8%; 123/15,154). The unsatisfactory rate was reduced by 12% during the study period compared with the previous 12 months.

The main reason for classifying a TP sample as unsatisfactory was scant squamous cellularity in the presence (69.1% [76/110]) or absence (12.7% [14/110]) of abundant fresh blood. Other causes included obscuring inflammation (11.8% [13/110]) or mucus (6.4% [7/110]). The CytoLyt–glacial acetic acid wash was used for all 110 study samples, regardless of the reason for the classification of unsatisfactory. By using the acid wash, TP specimen adequacy was changed in 67.3% of the reprocessed TP study samples (74/110). The most significant improvement was seen in the adequacy of bloody samples Image 1 and Image 2.

Following reprocessing, an interpretation of unsatisfactory persisted in 32.7% of the cases (36/110). Samples demonstrating only scant squamous cellularity, without abundant fresh blood before reprocessing, remained unsatisfactory in 57% of cases (8/14).

We noted the final interpretation on cases reclassified as satisfactory or SBLB Table 3. Once reprocessed, 6.4% of unsatisfactory cases (7/110) contained epithelial abnormalities. For comparison, the laboratory TP abnormal rate for atypical squamous cells of undetermined significance (ASCUS) or

| Table 1 |
| Unsatisfactory Rates for Cervical Cytology Samples |
| 12-mo Period Before Study | Study Period |
| TP (n = 23,730) | CP (n = 46,367) | TP (n = 15,154) | CP (n = 14,654) |
| Unsatisfactory for interpretation | 209.0 | 232.0 | 123.0 | 143.0 |
| Rate (%) | 0.9 | 0.5 | 0.8 | 1.0 |

CP, conventional Papanicolaou smear; TP, ThinPrep Pap Test (for proprietary information, see the text).

| Table 2 |
| Clinical Features of Patients With Reprocessed Unsatisfactory ThinPrep* Cervical Samples |
| Feature | No. (%) |
| Premenopausal | 60 (54.5) |
| Postmenopausal | 11 (10.0) |
| Pregnant | 6 (5.5) |
| Postpartum | 9 (8.2) |
| Specimen obtained during first half of menstrual cycle | 24 (21.8) |
| Total cases | 110 (100) |

* For proprietary information, see the text.

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A, ThinPrep slide initially interpreted as unsatisfactory owing to scant squamous cellularity and obscuring blood. There are areas of patchy cells lost, especially in the center of the cell disk. This slide also demonstrates the “halo effect” with accumulation of cellular material at the periphery of the cell deposit. B, The corresponding reprocessed slide was reclassified as satisfactory for interpretation.
above during the study period was 9.1%. On microscopic re-review, no screening errors were noted on the initial unsatisfactory slide. These abnormal cases included diagnoses of ASCUS (3 cases), atypical glandular cells of undetermined significance (AGUS; 2 cases), low-grade squamous intraepithelial lesion (LSIL; 1 case), and carcinoma (1 case). Histologic follow-up was available for 5 of 7 abnormal slides. Of greatest clinical significance, a TP slide from a 33-year-old woman with abnormal bleeding initially showed a few degenerated epithelial cells and blood. On reprocessing, this specimen was shown to contain squamous cell carcinoma. Unfortunately, no follow-up was available. For 4 of 5 atypical cases, histologic follow-up was available, and all corresponding biopsy specimens showed no evidence of disease. Histologic follow-up revealed cervical intraepithelial neoplasia 1-2 in the case classified as LSIL.

The paraffin section from a satisfactory TP filter membrane was compared with a TP interpreted as unsatisfactory, due to scant squamous cellularity and the presence of blood. The satisfactory specimen filter showed numerous small pores, free of debris or occupation by noncellular elements. There also were numerous squamous cells captured on the filter surface of the membrane. In contrast, the unsatisfactory specimen filter showed that many of the small pores were plugged with amorphous debris and covered with a thick layer of debris. No epithelial cells seemed to be captured on the slide surface of the filter.

**Discussion**

After the introduction of the TP for gynecologic cytology at ARUP Laboratories, we noted a higher rate of specimens characterized as unsatisfactory compared with the rate for CP samples. These slides often had grossly observable problems

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (y)</th>
<th>Final Diagnosis</th>
<th>Histologic Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46</td>
<td>ASCUS</td>
<td>Squamous metaplasia, reactive changes</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>LSIL</td>
<td>CIN 1–CIN 2</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>AGUS</td>
<td>Benign endocervical polyp</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>ASCUS</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>AGUS</td>
<td>Normal</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>ASCUS</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>Squamous cell carcinoma</td>
<td>NA</td>
</tr>
</tbody>
</table>

AGUS, atypical glandular cells of undetermined significance; ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; LSIL, low-grade squamous intraepithelial lesion; NA, not available.

* For proprietary information, see the text.
with the uniformity of the cell deposit. This resulted in the interpretation of unsatisfactory, often owing to a scant number of squamous epithelial cells, obscuring blood, or both on microscopic examination.

A variety of factors have limited the cytology laboratory’s ability to achieve the full potential of specimen adequacy benefits with liquid-based cytology. In particular, there have been no clearly defined specimen adequacy criteria provided from test manufacturers, the US Food and Drug Administration, or the cytology profession, including the Bethesda System. The proposed 2001 Bethesda System attempts to provide more specific criteria concerning determination of adequacy for liquid-based specimens.

We hypothesized that excessive blood in cervical samples may cause obstruction of the membrane filter used in the ThinPrep 2000 System, competing for the capture of epithelial cells, thus producing a scanty cellular slide. It has been shown that variations in use of the TP collection device seem to have a contributing role in TP specimen adequacy. The microscopic sections from the filtration membrane of the TransCyt filter seem to confirm these initial assumptions. Further study, with larger numbers of cases, is required.

The morphologic training material from the manufacturer states that “biologic variability of the patient sample has a direct effect on the cellular composition of the ThinPrep slide…. Too few epithelial cells may be recovered in such bloody samples.” The manual further states, “The ThinPrep processor virtually eliminates the most common technical limitations found on conventional Pap smears, including air-drying artifact, obscuring blood, inflammation, or thick areas partially or totally impairing interpretation.” We found that 21.8% of our bloody (and subsequently unsatisfactory) TP samples were obtained during the first half of the menstrual cycle. A potential cause of concern would be that these types of statements give the clinician an impression that satisfactory TP samples can be obtained during menses.
Low numbers of well-preserved epithelial cells available for interpretation can have a direct effect on the number of unsatisfactory diagnoses made by the laboratory. This result has far-reaching effects for the patient, clinician, and laboratory. An unsatisfactory report necessitates patient retesting, the result of which is added health care cost and patient inconvenience.

Cervical abnormalities, including malignant neoplasms, are known to be associated with unsatisfactory cytology specimens and could result in a false-negative report. The number of abnormal cases, including 1 case of cancer, identified in this study population would support this contention. To our knowledge, this is the first published report concerning unsatisfactory liquid-based cytology specimens. Additional confirmation of these results in the literature would lend support to making final recommendations concerning reprocessing of unsatisfactory TP samples.

The Food and Drug Administration–approved use of the TP does not recommend preparation of additional slides from the vial, and all of these cases should have been called “unsatisfactory.” A request for a repeated sample should occur. However, a recent study has shown that follow-up, in terms of a repeated smear, after an unsatisfactory report is very low. The cytology laboratory may have little influence in the follow-up of unsatisfactory Papanicolaou smears. But not reprocessing unsatisfactory TP specimens seems to be a missed opportunity for the laboratory to detect epithelial abnormalities. Also, reprocessing unsatisfactory TP specimens can have a favorable effect on clinician and patient satisfaction with cervical cancer screening by reducing the number of recalls and return office visits.

Several studies have demonstrated that routine preparation of additional slides from the TP PreservCyt vial adds no additional information. Hutchinson et al found that even though the number of cells on TP slides was less than on CP slides, the total number of cells available for evaluation in the TP vial was greater than that on the CP slides. These investigators concluded that the TP method is highly reproducible, captures the majority of samples collected, and provides a representative subsample on each slide prepared. A recent investigation showed that additional TP slides from the cellular residue of the PreservCyt vial are helpful for making a definitive diagnosis in difficult cervicovaginal specimens initially interpreted as ASCUS, AGUS, LSIL, or high-grade squamous intraepithelial lesion. This was particularly useful because of the uniformity of the TP preparation. Additional abnormal cells were almost always (95%) found on subsequent slides when they were identified on the initial slide. However, Hoerl et al noted that 97% of the positive TP cases had fewer than 25 abnormal cells on the slide. Their study showed that for 48% of the TP cases, the diagnosis was changed after preparation of additional slides and was useful for making a more definitive diagnosis. None of these studies evaluated unsatisfactory TP samples with additional slide preparation. Some authors have noted a decreased number of abnormal cells present in TP specimens compared with CP specimens. Rare abnormal cells in TP specimens can make the recognition and the accurate classification of dysplasia difficult. Additional slides from the residual TP vial, whether unsatisfactory or not, seem to provide additional material to help with difficult cases.

Papillo et al reported variations in the reprocessing procedure, shown to help eliminate obscuring mucus and inflammatory elements. We used just one procedure (acid wash) even for these types of unsatisfactory specimens. Our results suggest reprocessing using the various methods reported by Papillo et al, because our unsatisfactory samples that demonstrated little improvement included those with inflammation and mucus. Perhaps an even greater improvement in unsatisfactory rate and detection of additional abnormal cases could have been achieved with modifications of this procedure for specimens that included inflammation or mucus.

Performing the acid wash of unsatisfactory TP samples increased our cost per case by about 60%. Disposables used in processing (TransCyt filter, technician time, miscellaneous disposables) account for the majority of this increase. Several steps have been taken to help reduce this increased cost. The original TransCyt filter now is saved in the vial and reused when an additional slide is required. We also have validated a procedure in which the CytoLyt–glacial acetic acid wash is performed before initial processing of the TP sample. With visual standards for grossly bloody samples that require treatment, we believe we can eliminate a substantial amount of the cost associated with reprocessing.

Based on this pilot study, we concluded that this procedure should be incorporated into the routine practice of our laboratory. In addition to unsatisfactory specimens, we also have started to obtain an additional slide on diagnostically difficult cases, frequently with only a few abnormal cells. Results obtained since incorporating these procedures seem to confirm our initial observations and will be described in a subsequent report.

Reprocessing of unsatisfactory TP samples has resulted in the following: (1) a 67.3% improvement in reclassification of reprocessed TP samples, (2) a 62% reduction in the overall unsatisfactory rate for TP samples, and (3) detection of cellular abnormalities in 6.4% of unsatisfactory TP cases, which otherwise would have been undetected. The present study confirms that reprocessing of unsatisfactory TP slides is a beneficial laboratory procedure. We believe that these results justify the additional effort of reprocessing in selected cases. Reprocessing of unsatisfactory TP slides can help identify undetected disease and assist in more accurate classification of difficult...
cases. Cytology laboratories that process and examine TP gynecologic samples might consider adoption of such a procedure. Confirmation from other institutions is required for final recommendations. If laboratories consider the data concerning the poor follow-up of unsatisfactory Papanicolaou tests, reliance on follow-up testing to identify disease seems to be misplaced. We must stress that the results of this study do not support universal repreparation of slides from routine liquid-based gynecologic samples. However, this study confirms that unsatisfactory TP samples are an opportunity to identify lesions that otherwise would be missed during routine cytologic examinations. These data also should be used to inform those obtaining smears of the importance of follow-up in managing patients with unsatisfactory ThinPrep Pap Test specimens.

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