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Precancerous cervical lesions and HPV genotypes identified in previously unsatisfactory cervical smear tests after inexpensive glacial acetic acid processing

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Abstract

Objective—To determine the effectiveness of using glacial acetic acid (GAA) to convert unsatisfactory bloody ThinPrep (TP) cervical smear test to satisfactory, and identify associated missed diagnoses and high-risk HPV (hrHPV) genotypes.

Methods—In a retrospective descriptive cross-sectional analysis, all TP tests performed in Mississippi, USA, 2012–2016, were evaluated for unsatisfactory results owing to blood. Tests that were converted to satisfactory by GAA treatment, and corresponding anomalies and HPV genotypes were identified.

Results—Among 106 384 TP tests, there were 1460 (1.37%) unsatisfactory results, of which 1442 (98.77%) were converted to satisfactory after GAA treatment. Laboratory preprocessing with GAA increased costs minimally. Precancerous lesions were detected in 166 (11.51%) of 1442 GAA-treated samples, of which 12 (7.2%) were high-grade lesions, 110 (66.3%) were atypical squamous cells of undetermined significance, and 63 (57.3%) tested positive for hrHPV. Of 60 genotyped samples, 39 (65%) had non-HPV16 and non-HPV18. Including mixed infections, 48 (80%) contained less-common hrHPV types, reflecting an unexpected distribution in bloody specimens.

Conclusions—GAA pretreatment of bloody TP tests would reduce the incidence of unsatisfactory results and missed high-grade lesions, and prevent the cost of repeat tests and delayed treatment. Clinicians without access to GAA should consider HPV testing.

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AUTHOR CONTRIBUTIONS

CR and KRG contributed to study conception and design; data collection, analysis, and interpretation; and manuscript writing and revision. JCR and MWS contributed to data collection, analysis, and interpretation; and manuscript writing and revision. LZ contributed to data collection, analysis, and interpretation of data; and manuscript revision. RA and SSR contributed to study conception and design; and manuscript revisions.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

Keywords

ASC-US; Bloody cervical smear specimens; Cervical cancer; Cervical smear test; Glacial acetic acid; High-risk HPV; HPV genotypes; Unsatisfactory cervical smear specimens

1 | INTRODUCTION

Reducing the number of uninterpretable cervical smear tests that are unsatisfactory for evaluation would aid efforts to prevent cancer through earlier detection and would avoid costly repeat testing. Cost-effective laboratory methods, such as adding glacial acetic acid (GAA) during slide processing of ThinPrep (TP) (Hologic, Marlborough, MA, USA) cervical smear test specimens, can improve the detection of early disease in bloody samples.^{1–12} GAA improves the visibility and cellularity of epithelial cells by lysing the erythrocytes in the blood that clings to the filter during slide processing.¹ Using GAA converts previously uninterpretable slides from unsatisfactory to satisfactory for evaluation^{1–12} (Fig. 1). Repeat patient visits for unsatisfactory cervical cancer screens are largely avoidable by using this simple laboratory method to identify and pretreat bloody TP specimens with GAA. In turn, reducing patient follow-up visits for repeat cervical smear tests would save both time and resources for patients and providers—an advantage that is particularly crucial in underserved regions.

Several controllable factors result in samples being interpreted as unsatisfactory. One of the more common factors is excessive blood in the sample. Large amounts of blood may result from several sources, including women with high-grade precancerous lesions or cancer, who are more apt to bleed with minor manipulation during the examination as compared with women with a normal cervix.¹³ GAA is an inexpensive acid with a stronger capacity to lyse the obscuring red blood cells relative to the preservative and lysing solution in TP cervical smear test vials. Using GAA may facilitate an immediate interpretation of bloody specimens and reduce the need for patients to repeat cervical smear tests labeled unsatisfactory.

The aims of the present study were to demonstrate to clinicians how a simple, inexpensive laboratory procedure converts bloody TP cervical smear tests to satisfactory tests, to identify anomalous high-grade cervical smear and biopsy results in the resulting GAA samples, and to report the distribution of high-risk HPV (hrHPV) genotypes in bloody samples.

2 | MATERIALS AND METHODS

The present retrospective cross-sectional analysis assessed the records of all cervical smear tests with GAA treatment carried out in Mississippi, USA, from January 1, 2012, to December 31, 2016. Approval from the Institutional Review Board of the University of Mississippi Medical Center, Jackson, Mississippi, USA was acquired and waiver of consent was obtained.

The study data were collected from three databases: Mississippi State Department of Health; CoPath, a commercially available anatomic pathology laboratory information system; and

Epic, a commercially available electronic system. The 2014 Bethesda System was used to classify the cytologic interpretation.¹⁴

Records from all statewide health department clinics in Mississippi that were initially considered to be unsatisfactory for evaluation owing to excessive blood, and then treated by GAA were identified. The sample included all consecutive electronic records of cervical smear tests that contained codes for GAA and “unsatisfactory for evaluation.” Following a protocol established in 2008, a note of GAA in the record indicated that a cervical smear test specimen had been unsatisfactory for evaluation owing to obscuring blood, and had therefore been treated with GAA, reprocessed, and re-subjected to morphologic interpretation.

Before the addition of GAA, a separate 4-mL untreated aliquot of the vial’s content was removed for reflex HPV testing of specimens with atypical squamous cells of undetermined significance (ASC-US). Before 2013, Hybrid Capture II (Qiagen, Gaithersburg, MD, USA), which does not provide genotype-specific information, was used for HPV testing.¹⁵ In 2013, the lab initiated the COBAS 4800 test (Roche Diagnostic, Pleasanton, CA, USA), a molecular DNA test that identifies hrHPV genotypes. COBAS gives a type-specific report for HPV16 and HPV18 and pools 12 (HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, HPV68) additional high risk types in a category termed “other”.¹⁶

The costs for materials and reagents to use GAA were estimated for a pretreatment protocol, as suggested by Haack et al.,³ and for the post-treatment method used in the present study, as suggested by Bentz et al.¹ In the pretreatment protocol, bloody TP cervical smear test vials are identified with the naked eye and GAA is added to the vial before the first slide is processed; in the post-treatment method, the specimen is reprocessed after an initial determination of unsatisfactory owing to obscuring blood, and a second slide is made (Fig. 2). The post-treatment method was used during the 5 years of the present retrospective analysis. The cost of materials was estimated from the actual costs incurred at the study institution for 2017.

The new diagnoses of cervical smear test specimens after GAA were identified and compared with the corresponding HPV test and biopsy results. An electronic data collection tool, developed for quality assurance evaluations, correlated the results of the records from the cervical smear tests, HPV tests, and the corresponding cervical biopsy histopathologic reports. The study findings were reported by simple descriptive statistics using SPSS version 24 (IBM, Armonk, NY, USA).

3 | RESULTS

During the 5-year study period, a total of 106 384 cervical smear tests were evaluated. Among these, 1460 (1.37%) samples were initially classified as unsatisfactory, of which 1442 (98.77%) were recovered sufficiently by using GAA to permit interpretation. The remaining 1.2% remained unsatisfactory owing to low cellularity. Of the 1442 samples

recovered after using GAA, 166 (11.5%) specimens had cervical anomalies in cervical smear tests (Fig. 3).

The demographics of the 166 women with anomalies included a mean age of 29.5 years (range 18–64 years). Ninety-five (57.2%) women were black, 57 (34.3%) were white, and 14 (8.4%) were other minorities. The majority of women (109/166, 65.7%) were uninsured, and 57 (34.3%) had Medicaid.

Among the 114 tests interpreted as ASC-US, reflex HPV testing was conducted for 110. Of these, 63 (57.3%) tested positive for hrHPV. Sixty samples were genotyped. Thirty-nine (65%) had test results positive for the pool of 12 “other” hrHPV genotypes, and not the most common types HPV16 and HPV18; an additional 9 (15%) contained multiple genotypes. Thus, 48 (80%) of all samples that tested positive for hrHPV samples contained “other” hrHPV types, reflecting an unexpected distribution of genotypes (Fig. 4).

Thirty-six (57.1%) of the 63 women with ASC-US and a positive hrHPV test result had a cervical biopsy at the study institution. Of 36 women with histologic follow-up, 15 (42%) had a high-grade lesion. Of these 15 women with a high grade lesion, 4 (11%) had moderate squamous dysplasia (cervical intraepithelial neoplasia 2; CIN2), 9 (25%) had severe dysplasia (CIN3), and 2 (6%) had carcinoma-in-situ (CIS). Among the 63 women with ASC-US and a positive hrHPV test result, 21 had mild squamous dysplasia (CIN1), six samples were inadequate, and two were negative. Nineteen (30.2%) of the 63 women did not have a biopsy or histologic record in the study database and were considered lost to follow-up.

The use of GAA for the study samples was estimated at only US \$0.49 per specimen, with 30 mL of GAA required per TP cervical smear test vial. A 9:1 solution of Cytolyte (Hologic) to GAA was required at a cost of \$1.15 per specimen. Thus, the additional laboratory costs for materials and reagents was \$1.64 per specimen. In post-treatment, GAA is added to the vial after the first slide has been made and diagnosed. For an unsatisfactory diagnosis, therefore, an additional slide must be processed, requiring twice the materials and labor. As a result, the costs associated with post-treatment nearly double.

4 | DISCUSSION

The study has shown that high-grade cervical cancer lesions, including carcinomas, might be identified after using GAA to convert unsatisfactory cervical smear tests to satisfactory. In addition, the bloody specimens were found to show a previously unrecognized and unusual distribution of less common hrHPV genotypes, warranting further investigation.

Overall, 11.5% of previously unsatisfactory cervical smear tests demonstrated a cytologic anomaly after the use of GAA. Typically, the average rate of anomalies in satisfactory specimens is 14%–16%. Among those women whose specimens converted to satisfactory and who had a cervical biopsy, 41.7% were found to have high-grade precancerous lesions. The majority of these women were black, a population that is unrepresented in many investigations. Of the 110 specimens with ASC-US interpretation and reflex HPV testing, more than half had positive test results for hrHPV, and only a minority were associated with

HPV16 or HPV18. Unexpectedly, 65% of the 110 HPV genotyped specimens had positive test results for only genotypes in the “other” category and 15% contained multiple genotypes; thus, 80% contained other genotypes. This finding differs from studies where HPV16 and HPV18 were the dominant genotypes among 63%–70% of women with cervical cancer.¹⁷ It should be noted that the interpretation of HPV tests is not altered by blood in the specimen; however, the present incidental HPV findings highlight the benefit of HPV testing. Clinicians who encounter bloody specimens and do not have access to a laboratory using GAA might consider HPV testing rather than a repeat cervical smear test.¹⁸

In contrast to the present evaluation, other studies of GAA have not considered histologic and HPV testing follow-up data. Similar to the present post-treatment method, Bentz et al.,¹ who first reported using GAA in 2002, applied GAA after the initial slide was processed and interpreted as unsatisfactory. In 2004, Rowe and Bentz² advocated a GAA pretreatment method. Subsequently, Haack et al.³ conducted a prospective study to establish a pretreatment protocol to identify hemorrhagic specimens requiring GAA treatment with the naked eye; only bloody specimens were selected for pretreatment, eliminating the cost of reprocessing the specimen (Fig. 2). Among 490 (94%) specimens that were converted to satisfactory by using GAA, 53 (11%) showed an epithelial anomaly, including five squamous cell carcinomas and two adenocarcinomas. The frequency of anomalies among the specimens recovered by Haack et al.³ is consistent with the present findings. Importantly, in all of these studies, GAA effectively converted bloody samples that could not be interpreted to satisfactory samples.

Cost is not a deterrent to using GAA. The present analysis showed that pretreatment with GAA is more cost-effective than post-treatment because it eliminates the expense of processing a second slide. Using GAA permits an immediate reinterpretation of specimens, thereby preventing delays in screening, and helps to ensure that women are not lost to follow-up. The cost of a woman returning for a repeat cervical smear test after an unsatisfactory result varies: self-pay women are responsible for the repeat test; some insurances may not cover a repeat sample; and private offices may need to absorb this cost. If the potential cost of preventing one cervical cancer case is compared with the cost of treating one, it can be seen that the increased cost of the solutions needed for the addition of GAA is minimal.

Furthermore, the use of a GAA after the determination of a visually bloody cervical smear test sample targets GAA treatment to only specimens with blood, thereby avoiding the costs of pretreating every specimen or reprocessing. Overall laboratory costs might be reduced or obviated by the technician visually identifying grossly bloody specimens and adding GAA to only those samples. Although the laboratory costs would increase, this minimal increment should be weighed against the overall healthcare expense of a repeat pelvic exam and cervical smear test, plus the compounding costs associated with the patient's lost time from work, childcare expenses, and the expense of treating one missed case of cancer. Some women might not return for several years owing to the expense and inconvenience. In a worst-case scenario, uninterpretable cervical smear tests deemed unsatisfactory that have missed high-grade lesions might progress to cancer. Thus, for society overall, the cost of

adding GAA, approximately \$1.64 per specimen, would represent a “value-added” step by pathology laboratories.

To our knowledge, all related studies of GAA have been reported only in pathology journals.^{1–12} Because not all laboratories offer this method, clinicians should inquire about the use of GAA in the laboratory responsible for interpreting their patients’ specimens.

The present study had some limitations. First, the costs were based on data from the study institute and might not be generalizable to other laboratories. Second, molecular testing using COBAS was not available in 2012. Last, the analyses were limited to descriptive statistics and should be expanded in future studies. Although not a limitation, it should be noted that specimen quality is a significant determinant of the ability to detect cytologic anomalies for satisfactory interpretation, and GAA can convert specimens obscured only by blood, not other contaminants.

In conclusion, the success rate for converting unsatisfactory specimens obscured with blood to satisfactory was almost 99%. Previously unrecognized high-grade cervical cancer lesions, including carcinomas, were identified after using GAA. In addition, bloody specimens seemed to show an unusual distribution of less common hrHPV genotypes. Further investigation of this distribution and its association with anomalous cervical smear test results, particularly in a representative sample of black women, is warranted.

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FIGURE 1.

Use of GAA to convert cervical smear tests with unsatisfactory results. Left, cervical smear specimen with gross amount of blood before GAA treatment. Right, cervical smear specimen with minimal amount of blood after treatment with GAA. Abbreviation: GAA, glacial acetic acid.

FIGURE 2.

Examples of cervical smear test vials with no blood, minimal blood, and gross blood for a visual identification procedure.

FIGURE 3.

Results of cervical smear test interpretation after the use of GAA to convert unsatisfactory tests to satisfactory. Abbreviations: ASC-H, atypical squamous cells cannot exclude high-grade SIL; ASC-US, atypical squamous cells of undetermined significance; GAA, glacial acetic acid; HG-SIL, high-grade squamous intraepithelial lesion; LG-SIL, low-grade squamous intraepithelial lesion.

FIGURE 4. Distribution of HPV genotypes including mixed genotypes among 60 samples with atypical squamous cells of undetermined significance morphology.