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ORIGINAL ARTICLE

Cervicovaginal Papanicolaou tests in transgender men: Cytomorphologic alterations, interpretation considerations, and clinical implications

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Abstract

Background: The transgender population faces unique psychosocial and physical obstacles to cervical cancer screening. Additionally, most individuals undergo masculinizing testosterone hormone therapy, and the physiologic changes can cause cytomorphologic alterations that may mimic lesions. Although the literature on cervicovaginal cytology is growing in this patient population, it is still limited.

Methods: The pathology information system was queried for all Papanicolaou (Pap) tests from transgender men from January 2013 to February 2023. The original diagnostic categories were catalogued. Cases were reviewed to evaluate the cytomorphologic alterations. Clinical data were also sought, including whether the sample was self-collected. Two comparison groups were established: one was a postpartum atrophic group and the other was an all-comer group.

Results: A total of 51 cases from 43 individuals were identified, with a mean age of 31 years. Approximately a third of cases (18 of 51; 35%) were self-collected. The abnormal rate was low, with 5.9% of cases rendered atypical squamous cells of undetermined significance on original review and no lesions identified. The Pap unsatisfactory rate according to original reports was 3.9%. This increased to 13.7% when the cases were rereviewed, which was significantly higher than the all-comer comparison group. The unsatisfactory rate did not correlate with self-collection. Atrophy was a prevalent cytomorphologic alteration, with the vast majority of cases (92%) showing at least mild atrophy. Small blue cells and transitional cell metaplasia were seen in many cases (53% and 43%, respectively).

Conclusions: There are clinical and morphologic considerations that are distinct to the transgender patient population. Laboratory personnel and diagnosticians need to be aware of these in order to optimize patient care.

KEYWORDS
cancer screening, cervical cancer, cytology, female-to-male, Pap test, transgender

INTRODUCTION

According to national population estimates by the Williams Institute at the University of California Los Angeles, over 1.6 million individuals, or approximately 0.6% of those aged 13 years and older, identify as transgender or gender diverse in the United States.[1] Of adults identifying as transgender, 38.5% reported they are transgender women, 35.9% reported they are transgender men, and 25.6% reported they are gender nonconforming.[1] "Transgender" is an umbrella term for those whose gender identity, gender expression, or behavior is discordant to the sex that they were assigned at birth. "Gender" is a social construct that refers to roles, behaviors, and attributes society considers masculine or feminine, whereas "sex" is a medical term that refers to one's biological status as either male or female based on chromosomes, hormone prevalence, and external and internal anatomy at birth.[2,3]

Gender-affirming surgeries (also known as confirmation surgeries) are surgical interventions that help individuals transition to their self-identified gender. For transgender men (those assigned female at birth but identifying as a man) these procedures may include mammoplasty ("top" surgeries) as well as hysterectomy, oophorectomy, and phalloplasty/scrotoplasty ("bottom" surgeries). This is usually preceded by masculinizing testosterone hormone therapy (also known as gender-affirming hormone therapy). However, for each transgender individual the development of self-identity is a unique experience, and not all will pursue gender-affirming surgeries. Some may elect to only undergo masculinizing testosterone hormone therapy, whereas others may elect for no medical interventions.

According to the 2015 US Transgender Survey, although transgender men are more likely to have had any kind of gender-affirming surgery relative to transgender women (42% vs. 28%) or nonbinary individuals (9%), only approximately 14% underwent hysterectomy procedures.[4] On the basis of the American College of Obstetricians and Gynecologists' Committees on Gynecologic Practice and Health Care for Underserved Women recommendations, any anatomical structure present that warrants screening should be screened regardless of gender identity when considering preventive medical care.[5] Therefore, patients who have cervical tissue should undergo cervical cancer screening according to age-related guidelines.

Despite the recommendations, studies have shown disparities in the rates of cervical cancer screening in transgender men relative to cisgender women.[6,7] This is due to a wide array of misconceptions and barriers to health care access and screening in this population. First, despite studies showing that gender expression does not affect the rate of human papillomavirus (HPV) infection and HPV-related disease, with transgender men and nonbinary patients with cervixes having similar rates relative to cisgender patients, there may be misconceptions about the rate of dysplasia in this group that can result in reduced screening.[7] Additionally, for transgender patients there can be severe psychological discomfort associated with these evaluations. Performance of a Papanicolaou (Pap) test may trigger gender dysphoria due to distress caused by the disconnect between biological sex and gender identity that can make the experience challenging emotionally and psychologically.[7,8] This can be a major deterrent for cervical cancer screening in this patient population. Other reasons for not seeking care include fear of discrimination, prior negative experiences with or distrust of health care providers (due to either a perceived lack of provider knowledge or sensitivity regarding transgender health issues), prior trauma and/or post-traumatic stress disorder (such as due to intimate partner violence, childhood abuse, or gender-based discrimination), issues with insurance coverage (which may be incurred if medical records are registered as male), and lack of trans inclusivity (such as clinics with stereotypical feminine aesthetics).[4,7-14] In addition to the psychosocial barriers to cervical cancer screening, the examination itself may be physically painful for transgender men who are on exogenous hormone therapy because testosterone induces atrophic changes in vaginal and cervical tissues.

The combination of physical alterations caused by hormonal therapy in addition to other factors including patient and/or provider discomfort leads to several important considerations in this patient population. For one, transgender patients may prefer to self-collect samples because it may trigger less emotional distress and gender dissonance.[7,15] The value of self-collection is that it may address some of the concerns preventing individuals from pursuing screening. However, self-collected cervical cytology testing is not currently approved by the Food and Drug Administration, with several important preanalytic, analytic, and postanalytic considerations for both laboratory personnel and clinicians.[16] Another consideration regardless of sampling technique is that there may be a high unsatisfactory sampling rate in this population due to many contributing factors. Sample adequacy is a significant issue. Studies on cisgender women have shown an association between inadequate Pap tests and an increased likelihood of developing cancer, and studies on transgender men have found them to have a lower likelihood of following up within the recommended time frame after an inadequate sample.[8,17-19] An additional consideration for diagnosticians is that testosterone therapy can cause morphologic alterations that may make cytologic evaluation challenging.

To date, the literature on cervicovaginal cytology samples from transgender men is growing but still limited. Cytotechnologists and cytopathologists need to be aware of not only the cytomorphologic alterations that are distinctive to this unique patient population but also of both the challenges to interpretation and the implications of our interpretations in order to better serve our patients. Herein, we explore our institutional experience with cervicovaginal samples from transgender men with an emphasis on diagnostic pitfalls and clinical considerations.

MATERIALS AND METHODS

After institutional review board approval was obtained, the pathology information system of the Massachusetts General Hospital (Boston, Massachusetts), a large tertiary care medical center, was retrospectively searched from January 1, 2013 to February 1, 2023,

for all Pap tests acquired from female-to-male transgender patients. A natural language query that searches case data including the provided clinical history was used for the identification of the cases (CoPathPlus, Sunquest, Tucson, Arizona). Search terms included "transgender," "transsexual," "gender dysphoria," "testosterone," and "androgen." A total of 51 cases were identified over the slightly more than 10-year period. An age-similar atrophic comparison group was identified by using the same natural language query function to search for postpartum patients during the same 10-year period, which yielded 127 cases. An additional all-comer control group was established by collecting data from all Pap tests received in our institution in the year 2022, which totaled 23,326 Pap tests.

The original diagnostic interpretation categories for all cases were catalogued by review of the cytology reports in CoPath. At our institution, diagnostic categorization of cervicovaginal cytology follows the Bethesda System for Reporting Cervical Cytology guidelines.[20] The cytotechnologist interpretation as well as the cytopathologist interpretation were documented. Notably, all Pap tests are processed as SurePath liquid-based preparations (Becton Dickinson, Franklin Lakes, New Jersey). HPV testing results, if performed, were also documented for each case. Patient demographics were gathered from CoPath, which included patient age as well as documented gender at the time of sample collection. The pathology information system as well as the electronic medical records (Epic) were further searched in order to identify whether there were any subsequent histology specimens, either biopsies after an abnormal Pap test or subsequent surgical specimens as part of gender-affirming surgery. Additional data were derived from the electronic medical record, which included whether the sample was self-collected.

The cytology slides were also retrieved and reviewed by a cytopathologist (V.F.T.). The degree of atrophy, if present, was ascertained for each case and categorized as mild, moderate, and severe depending on the constellation of features, including the cellular maturation pattern (with note of the proportion of superficial, intermediate, parabasal, and basal cells). The overall cellularity was assessed, including reassessment of sample adequacy blinded to the original reporting. A threshold of 5000 well-visualized and well-preserved squamous or squamous metaplastic cells was used as the adequacy criteria. The cases were also assessed by using a minimum of 2000 cells. Other cytomorphologic features that were specifically evaluated and documented for each case included the presence of small blue cells (with two categories, one based on prior studies that defined them as groups of cells with minimal cytoplasm and the other based on the Bethesda System that defined them as small groups of cells with stripped and molded nuclei), transitional cell metaplasia, inflammation, and reparative changes. The presence or absence of lactobacilli was also recorded.

Data analysis for this study was performed by using Microsoft Excel and RStudio Software (version 2021.09.0). Pearson χ^2 and Fisher exact tests, as appropriate, were used to compare data between groups. Results were considered statistically significant if the p value was less than .05.

RESULTS

There were 51 Pap tests performed from 43 female-to-male transgender patients during the study period. Patient age ranged from 21 to 72 years with a mean of 30.6 years and median of 29 years. The majority of patients, 40 of 43 (93.0%), were on testosterone treatment at the time of sample acquisition. Of the remainder, two (4.7%) had been on testosterone treatment but were no longer on it and one (2.3%) had never been on testosterone therapy. The majority of patients (29 of 43; 67.4%) had gender-affirming top surgery, which consisted of either bilateral mastectomy or other chest reconstructive surgery such as a reduction procedure. No patients had a history of hysterectomy at the time of Pap test acquisition.

The number of Pap tests received from transgender patients per year markedly increased over time, with approximately a quarter of the total cases being collected in the year 2021 and slightly less than half of the total cases being collected in 2022; only slightly over a quarter of the total cases predated 2021 (Figure 1). Approximately a third of the cases were self-collected (18 of 51; 35.3%). Most of the samples were labeled as cervical specimens (41 of 51; 80.4%), whereas the remainder were labeled as vaginal (10 of 51; 19.6%); all of the vaginal specimens were self-collected samples. When the gender listed in CoPath was searched for each case, two thirds were noted as "female" (34 of 51; 66.7%) and one third were noted as "male" (17 of 51; 33.3%).

All slides were available for review and assessment of the cytomorphologic features. When reviewed, only four cases (7.8%) did not show discernable features of atrophy. The vast majority of cases (47 of 51; 92.2%) showed atrophy to any degree: 14 of the total cases (27.5%) appeared mild, 27 (52.9%) appeared moderate, and 6 (11.8%) appeared severe. Interestingly, no cases showed discernable multinucleated histiocytes or granular debris, which can be seen in atrophy, although some cases showed mononuclear histiocytes. Clusters of cells with scant cytoplasm (termed "small blue cells" by prior studies) were seen in 52.9% of cases (27 of 51) (Figure 2A,B) and transitional cell metaplasia was seen in 43.1% (22 of 51) (Figure 2C, D). Small clusters of naked molded nuclei (termed small blue cells by the Bethesda System) were identified in 15.7% of cases (8 of 51) (none of which were found in cases that did not also have the clusters of cells with scant cytoplasm) (Figure 2E,F). A total of 20 cases (39.2%) showed increased neutrophils in the background, with 11 cases (21.6%) showing reactive-reparative changes. Only one of these cases showed fungal organisms morphologically consistent with *Candida* species, with no other cases demonstrating discernable infectious agents. Additionally, in only a third of cases (17 of 51; 33.3%) were lactobacilli appreciated. There were no cases with bacterial vaginosis.

On review of the cytology reports, only two of the 51 total cases (3.9%) were categorized as unsatisfactory for evaluation (Table 1). One of the cases was noted as unsatisfactory because of insufficient squamous component, whereas the other was noted as unsatisfactory for obscuring inflammation. The vast majority of the remainder of the cases received a diagnosis of negative for intraepithelial lesion

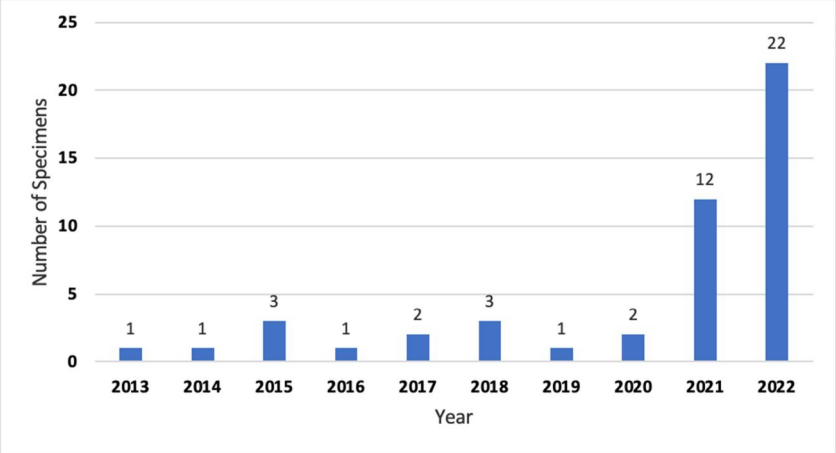

FIGURE 1 Distribution of the number of Papanicolaou tests from female-to-male transgender patients over a 10-year period.

or malignancy (NILM) or NILM (reactive) (46 of 51; 90.2%). Only three cases (5.9%) received an abnormal interpretation, which were all atypical squamous cells of undetermined significance (ASCUS). Two of the three abnormal cases were self-collected Pap tests. There were no cases of atypical squamous cells cannot rule out high-grade lesion (ASCH), atypical glandular cells, low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), or malignancy. The majority of cases were seen only by a cytotechnologist (45 of 51; 88.2%); only six cases (11.8%) were seen by a cytopathologist. The cases finalized by a cytopathologist included two that were screened as NILM (reactive) and finalized as NILM (reactive), three screened as ASCUS and finalized as ASCUS, and one screened as LSIL and finalized as NILM (reactive). A total of 39 cases (76.5%) underwent HPV testing, five of which were positive (5 of 39; 12.8%). Only one of these was found to be HPV 16 positive whereas the remainder were non-16, non-18. Additionally, only one of these corresponded to an ASCUS Pap, whereas the remainder were interpreted as NILM or NILM (reactive).

On blinded review of the slides, five cases that had been categorized as NILM should have been categorized as unsatisfactory when using the 5000-cell adequacy threshold. This would have led to a true unsatisfactory rate of 13.7% (7 of 51). Of the seven total unsatisfactory cases, only one (14.3%) was a self-collected sample, which equated to 5.6% of the self-collected Pap tests (1 of 18). The remaining six unsatisfactory cases were not self-collected, which equated to 18.2% of clinician-acquired Pap tests (6 of 33). When using 2000 cells as a minimum threshold for cellular adequacy, only two cases (3.9%) would have been considered unsatisfactory. Notably, this was more challenging to determine relative to using a 5000-cell threshold because of the presence of background inflammation, rare interspersed cellular clusters, and cells that mimicked squamous cells including histiocytes, rounded endocervical cells, and tubal metaplastic cells. An additional point on rereview of the cases is that, in retrospect, a NILM diagnosis may have been a more appropriate interpretation for one of the cases that had been originally interpreted as ASCUS: all of the marked groups looked consistent with atrophic changes and transitional cell metaplasia, and there did not appear to be any discernable cytologic atypia in the background unmarked groups (Figure 3).

The diagnostic categorization rates for the transgender group (both original diagnoses and blinded reviews) are shown relative to the postpartum and all-comer comparison groups in Table 1. The postpartum group had no unsatisfactory cases (0 of 127), which did not differ significantly from both the original diagnosis and review diagnosis transgender groups. The all-comer group had only 0.5% (116 of 23,326) unsatisfactory cases, which was significantly lower than both the original diagnosis ($p < .001$) and review diagnosis ($p < .00001$) transgender groups. The abnormal rate for the postpartum group was 17.3% (22 of 127), which did not differ significantly from that of the original transgender group (3 of 51; 5.9%; $p = .08$) but did differ from the rereview transgender group, which was found to be significantly lower than the postpartum group (2 of 51; 3.9%; $p = .03$). The abnormal rate for the all-comer group was 10.0% (2328 of 23,326), which did not differ significantly from either transgender group. The high–risk human papillomavirus (HR-HPV) positivity rate did not differ significantly between the transgender and postpartum groups (12.8% vs. 19.1%; $p = .5$) as well as the transgender and all-comer groups (12.8% vs. 8.1%; $p = .9$).

In follow-up, one patient who had a NILM Pap test with a positive non-16 HPV test had a subsequent cervical biopsy that was benign. The patient with NILM cytology and a positive HPV 16 test received a follow-up Pap test that was NILM. The remaining two NILM cases with positive non-16 HPV testing received follow-up Pap tests and were NILM on follow-up. Of the three ASCUS cases, two received follow-up Pap testing; one case that was HPV negative was found to be NILM on follow-up, whereas the one that had been HPV positive received a second ASCUS interpretation on follow-up. The third ASCUS case, which was HPV negative, did not receive follow-up Pap testing (as of 1 year after diagnosis). Of the two unsatisfactory cases, one received a follow-up Pap test 3 months later that was NILM; the second patient did not undergo repeat sampling. A total of five patients (9.8%) had gender-affirming surgery with total

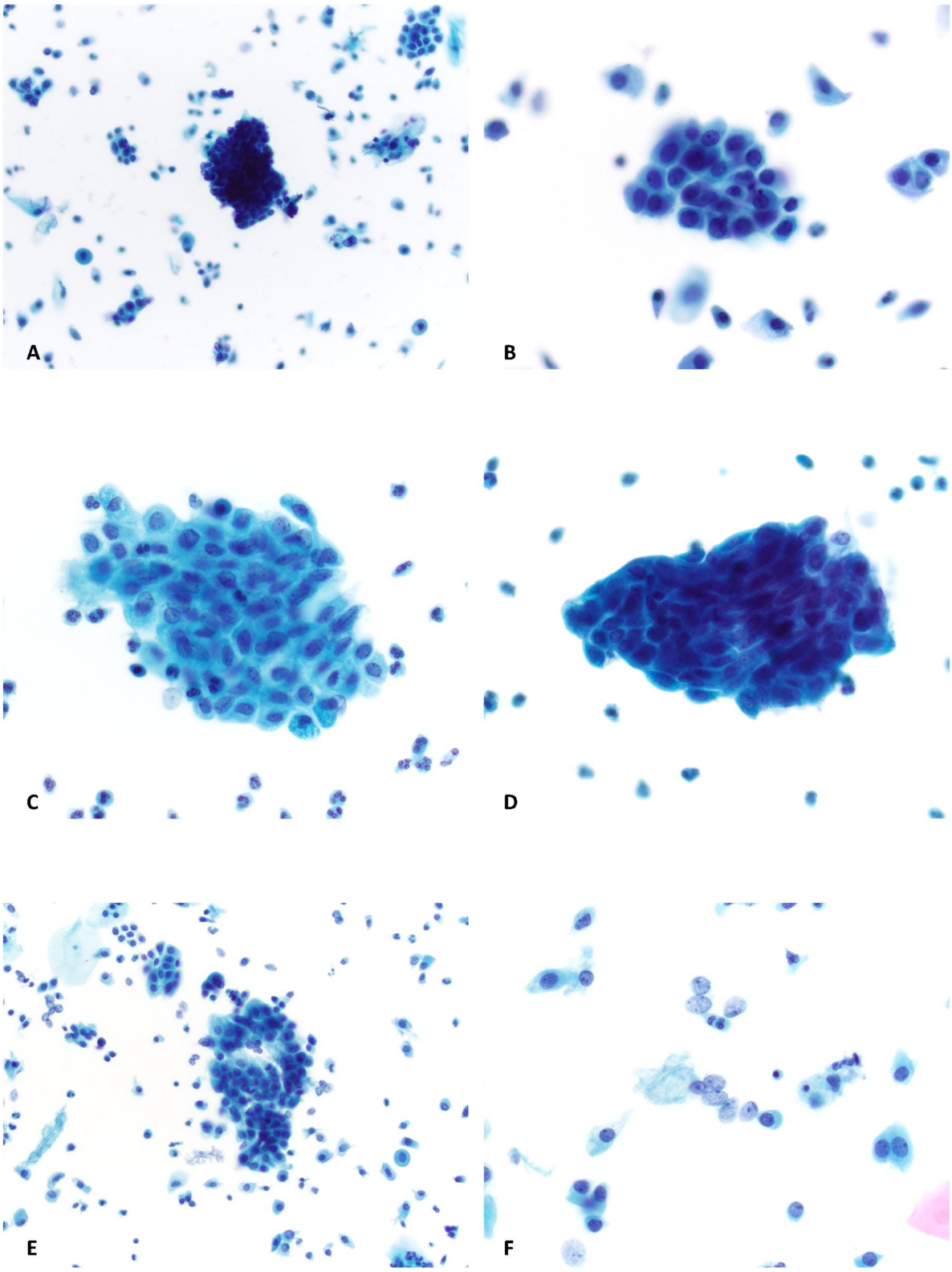

FIGURE 2 (A,B) Groups of small cells that demonstrate scant cytoplasm. These may be confused with endometrial cells when in clusters such as these. (C,D) Examples of transitional cell metaplasia, which has distinctive nuclear grooves. (E,F) Small blue cells as defined by the Bethesda System, which are small clusters of stripped and molded nuclei. These represent stripped parabasal/basal cells.

TABLE 1 Cytology diagnostic categorization rates of transgender patients and of comparison groups.

	Unsat	NILM	ASCUS	ASCH	AGC	LSIL	HSIL	AIS	Cancer	Any abnormality	HPV+ rate
Transgender group (original diagnosis), % (n/N)	3.9 (2/51)	90.2 (46/51)	5.9 (3/51)	0 (0/51)	0 (0/51)	0 (0/51)	0 (0/51)	0 (0/51)	0 (0/51)	5.9 (3/51)	12.8 (5/39)
Transgender group (blinded rereview), % (n/N)	13.7 (7/51)	82.4 (42/51)	3.9 (2/51)	0 (0/51)	0 (0/51)	0 (0/51)	0 (0/51)	0 (0/51)	0 (0/51)	3.9 (2/51)	
Postpartum group, % (n/N)	0 (0/127)	82.7 (105/127)	12.6 (16/127)	0 (0/127)	1.6 (2/127)	3.1 (4/127)	0 (0/127)	0 (0/127)	0 (0/127)	17.3 (22/127)	19.1 (21/110)
All-comer group, % (n/N)	0.5 (116/23,326)	89.5 (20,882/23,326)	5.3 (1243/23,326)	0.2 (50/23,326)	0.3 (75/23,326)	3.7 (865/23,326)	0.3 (66/23,326)	0.01 (2/23,326)	0.1 (27/23,326)	10.0 (2328/23,326)	8.1 (1656/20,396)

Abbreviations: AGC, atypical glandular cells; AIS, adenocarcinoma in situ; ASCH, atypical squamous cells cannot rule out high-grade lesion; ASCUS, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; Unsat, unsatisfactory.

hysterectomy, all of whom were patients with prior negative cervicovaginal cytology samples and negative HPV testing.

The findings of this study relative to those of prior cytomorphologic studies[21-26] are summarized in Table 2.

DISCUSSION

In 1938, George Papanicolaou with colleagues Ripley and Shorr described atrophic changes in the vaginal smear of a young woman being treated with androgen therapy for dysmenorrhea and menorrhagia.[27] Despite this early observation, the literature on morphologic findings from patients undergoing androgen therapy remained relatively limited until recent years. In a 1986 study, Miller et al. reviewed the histologic findings in hysterectomies from 32 women who desired sexual reassignment and were treated with large doses of androgen for a year or more before surgery.[28] They observed that there were varying degrees of atrophy in the majority of their cases. Several of their cases with severe atrophy were initially mistaken as cervical dysplasia, given the presence of small basophilic cells with a lack of normal maturation before workup with ancillary studies. Additional notable histologic findings were observed in subsequent studies, including transitional cell metaplasia, which was noted in over 60% of cases from a 2019 study.[29] A more recent study also noted a significantly higher prevalence of several findings in gender-affirming hysterectomies relative to benign hysterectomies from cisgender women, including NKX3.1-positive basal keratinocytes (86% vs. 1.8%) and surface prostatic metaplasia (43% vs. 3.5%), which could be focal to florid, in addition to transitional cell metaplasia (80% vs. 3.5%) and small basophilic cells (67% vs. 7%).[30] These studies highlighted the importance of pathologists being aware of the effects of prolonged use of androgens because of the potential to mistake severe atrophic changes for squamous dysplasia.

The literature on cervicovaginal cytology specimens from transgender patients is limited but growing. Although the literature to date on Pap tests from transgender patients has been divided on the rate of abnormal interpretations relative to cisgender cohorts,[21-26] in the current study there was no significant difference in the abnormal rate within the transgender group and either the postpartum atrophic comparison group or the all-comer comparison group on the basis of original diagnosis. Fewer than 6% of cases received an atypical diagnosis (which was fewer than 4% after rereview and was actually significantly lower relative to the postpartum group) and no lesions were identified, either prospectively, retrospectively, or on follow-up. The literature has also been divided on the unsatisfactory rate in this patient population. In the current study, when a 5000-cell adequacy threshold was observed, a significant number of cases were found to be unsatisfactory relative to either comparison group. On cytomorphologic review, prevalent findings included atrophy, small blue cells with scant-to-absent cytoplasm, and transitional cell metaplasia.

The major cytomorphologic features of Pap tests from transgender patients are those of atrophy, which, depending on the study,

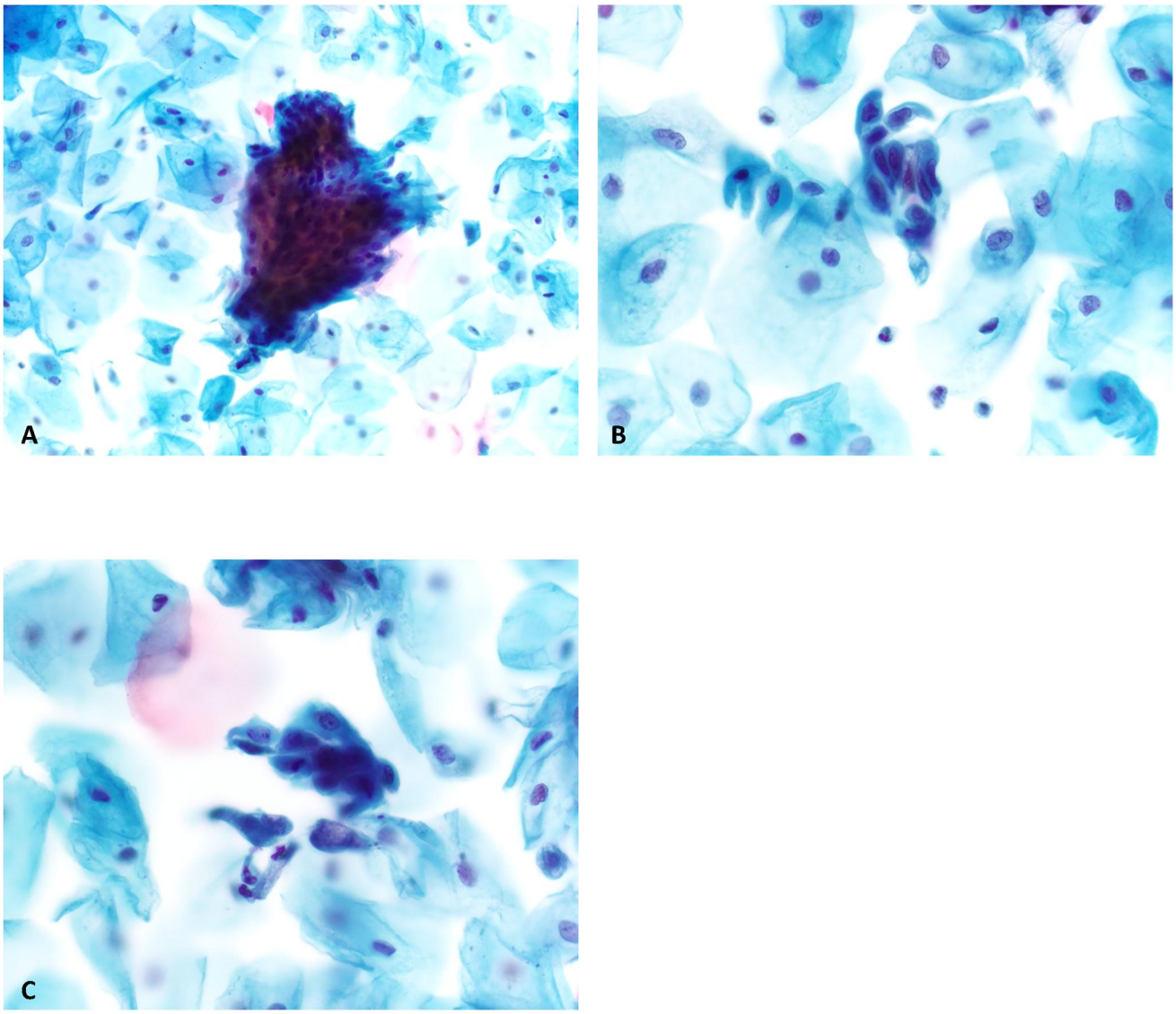

FIGURE 3 (A–C) Representative photographs from one of the cases originally designated as atypical squamous cells of undetermined significance. Several of the marked groups are shown, which most likely represent benign atrophic changes including transitional cell metaplasia.

has been found to involve anywhere between 62% and 93% of patient samples.[21-25] In the current study, the vast majority of cases (92%) showed at least mild atrophy. Atrophy is a physiologic response resulting from decreased estrogen stimulation, which leads to thinned immature cervicovaginal squamous epithelium consisting of parabasal and basal cells. It is seen with various low-estrogen states that include menopause and the postpartum period but also with bilateral oophorectomy or with the use of exogenous hormones or drugs. Cervicovaginal cytology specimens with atrophic changes are particularly challenging for a number of reasons, which include the variability in appearance depending on the degree of atrophy present as well as associated background metaplastic and inflammatory changes.[31-33] Intermediate, parabasal, and basal cells will be present in varying proportions. Parabasal-type cells can demonstrate nuclear enlargement and increased nuclear-to-cytoplasmic ratios. Generally, the nuclear contours are smooth with finely granular and uniformly dispersed chromatin, although mild hyperchromasia can be seen. Different cellular patterns are possible, including sheets with some crowding or dispersed single cells. In cases of extreme atrophy, abundant inflammatory infiltrate and/or a basophilic granular background resembling tumor diathesis can be seen.[20] Mitoses usually are not seen. The atrophic changes seen in cervicovaginal cytology samples from transgender patients can usually be distinguished from high-grade squamous lesions, although they may cause diagnostic difficulty in a subset of cases. Distinguishing features of high-grade squamous lesions to be aware of include that the cellular groups in dysplastic lesions tend to show more cellular crowding relative to those with benign atrophy as well as a loss of cellular polarity within the groups. Additionally, atypical nuclear features can generally be seen, which include nuclear contour irregularity, hyperchromasia, and chromatin coarseness.

Transitional cell metaplasia is another relatively common finding in cervicovaginal cytology specimens from transgender patients.[22,24]

TABLE 2 Summary of studies with corresponding HPV findings.

	Present study (original review)	Adkins et al.[21]	Williams et al.[22]	Plummer et al.[23]	Lin et al.[24]	Davis et al.[26]	Moatamed et al.[25] (original review)
Patients, No.	43	11	14	71	61	89	111
Cases, No.	51	24	17	77	65	89	122
Mean age, years	31	N/A	42.5	28	28	31.3	N/A
Pap categorization, % (*n*/*N*)							
Unsat	3.9 (2/51)[a]	13 (3/24)	5.9 (1/17)	23.4 (18/77)	16 (10/65)	0 (0/89)	9.8 (12/122)
NILM	90.2 (46/51)	58 (14/24)	82 (14/17)	68.8 (53/77)	74 (48/65)	94.4 (84/89)	84.4 (103/122)
ASCUS	5.9 (3/51)	13 (3/24)	5.9 (1/17)	5.2 (4/77)	6 (4/65)	0 (0/89)	3.3 (4/122)
ASCH	0 (0/51)	13 (3/24)	0 (0/17)	0 (0/77)	0 (0/65)	0 (0/89)	1.6 (2/122)
LSIL	0 (0/51)	0 (0/24)	5.9 (1/17)	1.3 (1/77)	1 (1/65)	4.5 (4/89)	0.8 (1/122)
HSIL	0 (0/51)	4 (1/24)	0 (0/17)	0 (0/77)	3 (2/65)	1.1 (1/89)	0 (0/122)
AGC	0 (0/51)	0 (0/24)	0 (0/17)	1.3 (1/77)	0 (0/65)	0 (0/89)	0 (0/122)
Cancer	0 (0/51)	0 (0/24)	0 (0/17)	0 (0/77)	0 (0/65)	0 (0/89)	0 (0/122)
Abnormal total, % (*n*/*N*)	5.9 (3/51)	29.2 (7/24)	11.8 (2/17)	7.8 (6/77)	10.8 (7/65)	5.6 (5/89)	5.7 (7/122)[b]
Transgender unsat rate versus comparison group	NS	Higher	NS	Higher	Higher	NS	Higher
Transgender abnormal rate versus comparison group	NS	Higher	NS	NS	Higher (HSIL)	NS	NS
HPV performed, % (*n*/*N*)	76.5 (39/51)	50 (12/24)	29.4 (5/17)	35 (27/77)	49 (32/65)	56.2 (50/89)	59.8 (73/122)
HPV+ rate, % (*n*/*N*)	12.8 (5/39)	33 (4/12)	20 (1/5)	18.5 (5/27)	19 (6/32)	8 (4/50)	16.4 (12/73)
Transgender HPV+ rate versus control cohort	NS	Higher	N/A	NS	N/A	NS	N/A

Abbreviations: AGC, atypical glandular cells; ASCH, atypical squamous cells cannot rule out high-grade lesion; ASCUS, atypical squamous cells of undetermined significance; Higher, higher with statistical significance; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; N/A, not available; NILM, negative for intraepithelial lesion or malignancy; NS, not significant; Unsat, unsatisfactory.

[a]On rereview, seven total cases (13.7%) did not meet cellular criteria for adequacy. This would have been statistically significantly higher than either of the comparison groups.

[b]On rereview, three cases were downgraded from an atypical category to NILM, which resulted in an abnormal rate of only 3.3% (4/122), which was significantly lower than the authors' control group.

In general, transitional cell metaplasia is considered an infrequent but not rare finding in cytology and surgical specimens that is likely underrecognized. In the older literature, transitional cell metaplasia was predominantly described in peri- and postmenopausal women, the majority of whom were not on exogenous hormones.[34,35] However, even early reports noted its association with androgen and testosterone therapies, and it is now recognized as a common finding in histology specimens from patients undergoing exogenous testosterone therapy, being observed in up to 88.2% of Pap tests from transgender patients.[22,29,36] In this current study, it was found in 43% of cases. Transitional cell metaplasia, as the name implies, is a metaplastic process where the epithelium morphologically resembles urothelium; it is considered by some to be a morphological variant of atrophy or basal cell hyperplasia. Cytomorphologically, transitional cell metaplasia is characterized by three-dimensional groups of oval-shaped cells in a streaming arrangement with ovoid to spindled nuclei generally demonstrating fine, evenly distributed chromatin, inconspicuous-to-small nucleoli, mild nuclear membrane irregularities, and longitudinal grooves. As in conventional atrophy, the importance of transitional cell metaplasia, whether in the transgender population or the cisgender population, lies in its potential to be misdiagnosed as a lesion because the lack of maturation seen in transitional cell metaplasia can impart a resemblance to high-grade dysplasia. Features that help distinguish transitional cell metaplasia from high-grade dysplasia, aside from the characteristic longitudinal grooves, include the streaming appearance of the cellular groups, lower nuclear-to-cytoplasmic ratios relative to those seen in high-grade dysplasia, lack of hyperchromasia, and lack of significant mitotic activity.

Additional morphologic mimics beyond high-grade squamous dysplasia may exist and are important to note. The study by Williams et al. highlighted the prevalence of cases with small cells demonstrating scant-to-absent cytoplasm arranged in grape-like clusters that occurred in 82.4% of their cases and histologically correlated to severely atrophic parabasal cells clinging to areas of transitional cell metaplasia or atrophic epithelium.[22] In the current study, clusters of cells with scant cytoplasm were identified in slightly over half of the cases. Small clusters of naked nuclei with complete loss of cytoplasm and nuclear molding (referred to as small blue cells by the Bethesda System for Reporting Cervical Cytology) were identified in approximately 16% of cases. A key importance of these findings is that there is a potential to mistake these clusters of cells with scant or absent cytoplasm as endometrial cells. If occurring in older transgender patients (i.e., older than 45 years of age), this may lead to additional unnecessary procedures such as endometrial biopsies or curettages. Noting the history of testosterone therapy may prevent the overinterpretation of these groups as endometrial cells. Additionally, features that are helpful in identifying true endometrial cells include arrangement in tight ball-like clusters, nuclei at the edges of the cluster with a cup-shaped appearance, and/or karyorrhexis, if present.[20]

In addition, the presence of background inflammatory changes can also cause diagnostic challenges. In the current study, 39% of cases had background inflammation, with approximately 22% showing associated reactive-reparative changes. One case with reactive-reparative changes had *Candida* fungal organisms, but the other cases did not have discernable infections. These findings are similar to a prior study by Plummer et al. in which inflammation was noted in 41% of their 77 cases and reactive-reparative changes in 34%.[23] Reactive-reparative changes may include variable degrees of nuclear enlargement, mild hyperchromasia, occasional bi- or multinucleation, and cytoplasmic vacuolization or perinuclear halos (which lack peripheral thickening and are generally narrower than those seen with true koilocytes).[20] The presence of nucleoli can be a helpful clue that denotes the underlying reactive nature. However, reactive-reparative changes can at times be challenging to distinguish from true dysplasia. In a study by Adkins et al., the authors noted that cases with extensive inflammatory changes in the setting of atrophy were especially challenging to evaluate.[21]

Interestingly, it has been noted that there is an alteration in vaginal flora in transgender individuals, with cervicovaginal samples from these patients showing decreased lactobacilli, which is usually a dominant member of a healthy vaginal microbiome during reproductive years. Winston McPherson et al. analyzed the molecular profiles of the bacterial flora corresponding to vaginal samples from a cohort of healthy transgender men on testosterone and found that the vaginal microbiome of transgender men differed from that of cisgender women, with transgender men being less likely to have *Lactobacillus* as the primary genus and having a significantly increased relative abundance of other bacterial species.[37] In a subsequent cytologic evaluation of cervicovaginal Pap tests from this patient population, Lin et al. noted lactobacilli to be substantially decreased in 89% of their cases, which correlated with both atrophy and the length of time receiving testosterone therapy.[24] In the current study, two thirds of cases (67%) did not show appreciable lactobacilli on cytologic review. In a mechanism similar to that seen in postmenopausal women, testosterone therapy decreases estrogen, which affects the availability of glycogen and thereby makes the vaginal environment less favorable for lactobacilli.[37,38] Changes in vaginal flora have been found to correlate with bacterial vaginosis, which has been shown to increase the risk of HIV transmission as well as HPV infection according to some studies,[39-41] but additional studies are needed to evaluate the significance in the transgender population. In this study as well as the one by Lin et al., there were no cases with evidence of bacterial vaginosis.

In the current study, the abnormal rate was 5.9%. In review of the literature, the abnormal cervicovaginal Pap test rate for the transgender population has varied from 5.6% up to 29.2%.[21-26] There are likely several factors contributing to this variation in abnormal rate. As reviewed in the preceding paragraphs, there are a number of alterations that may cause challenges with cytomorphologic interpretation, including the presence of atrophy and inflammatory changes. These may lead to placement in an abnormal category, including indeterminate categories such as ASCUS or ASCH. Another contributory factor may be that inherently the rate of lesions will vary from institution to institution, with the HR-HPV positivity rate serving as a surrogate for how high risk the patient population is. The HR-HPV positivity rate has ranged from 8% to 33% in prior studies, which correlates with the abnormality rate in these studies (Table 2). In the study by Lin et al., the authors combined their cases with those from prior studies in the literature at the time and found that HPV testing sensitivity in the combined data was 100%, specificity was 87%, positive predictive value was 36%, and negative predictive value was 100% for the detection of HSIL.[21-24] Therefore, performance and knowledge of HR-HPV status can be especially helpful in this patient population for both minimizing overcalls by overinterpreting alterations such as those related to atrophy as well as aiding in the detection or possibility of lesions given its high sensitivity.

Another important consideration in this patient population is the unsatisfactory rate of cervicovaginal specimens. In the current study, rereview showed that if strict cellular criteria as established by the Bethesda System were followed, almost 14% of cases would have been considered unsatisfactory for evaluation. The unsatisfactory rate has ranged from 0% up to 23.4%, with variation in whether the rate differed from overall institutional or matched cohorts.[21-26] In a large study by Peitzmeier et al., the investigators recorded the results of Pap tests from their large transgender population and found the inadequacy rate to be 10.8% (44 of 415).[8] Authors have speculated whether adequacy criteria for this patient population may be adjusted in a manner similar to other patient populations. According to the Bethesda System for Reporting Cervical Cytology, a minimum of an estimated 5000 well-visualized and well-preserved squamous and/or squamous metaplastic cells is needed in liquid-based preparations from women with a cervix. However, there may be

exceptions at the discretion of laboratories, particularly in women who have had chemotherapy or radiation therapy, who are postmenopausal with atrophic changes, or who are posthysterectomy, although samples with fewer than 2000 cells should be considered unsatisfactory in most circumstances.[20] Because of the psychosocial issues with acquiring Pap tests from these patients and the generally low rate of lesions, it may be prudent to use the same criteria as for cisgender women with atrophic changes. Consideration of the HR-HPV results will likely be an important contributor to the management of these patients. Interestingly, the performance of HR-HPV testing in this patient population ranged in studies from 29.4% up to 76.5% (in the current study). Although this may reflect the patient age in these studies, given the prior discussion points it may be helpful for HR-HPV testing to be performed more frequently in this patient population and outside of the established guidelines for cisgender patients in order to help guide the management of these patients. Primary HPV testing may also be relevant in this patient population for those who may prefer self-collection. An additional important yet likely underpowered finding of this study was that self-collection did not appear to affect the unsatisfactory rate, with only one of the seven unsatisfactory cases being a self-collected case. Additional larger studies into these areas would be of interest, particularly considering the potential clinical and laboratory implications.

In conclusion, as the transgender community continues to grow and there is increasing use of gender-affirming hormonal therapy, cytotechnologists and cytopathologists will increasingly encounter cytology samples from these patients. There are important considerations that laboratory personnel and diagnosticians must be aware of when receiving and evaluating cervicovaginal samples from transgender men. These include not only being aware of benign morphologic alterations that should not be overinterpreted as abnormalities but also being aware of the clinical implications. Categorization into unsatisfactory or atypical categories may have significantly different implications in the transgender population, given that transgender individuals face unique psychosocial and physical barriers to screening. Knowledge of the clinical implications in this patient population in addition to the morphologic alterations is imperative and will help ensure that we provide optimal patient care. Additional research will be important to help refine guidelines including with regard to specimen collection, processing, and adequacy criteria.

AUTHOR CONTRIBUTIONS

Vanda F. Torous: Project conceptualization, data curation, formal analysis, writing–original draft, and writing–review and editing.

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CONFLICT OF INTEREST STATEMENT

The author declares no conflicts of interest.

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