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The utility of upper urinary tract urine cytology before and after application of the Paris System.

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Precis: The Paris System introduced diagnostic criteria for urine cytology to improve reproducibility among pathologists. However, modifications to diagnostic criteria, particularly in the upper urinary tract may enhance its diagnostic accuracy.

ABSTRACT

Background: The Paris System (TPS) introduced diagnostic criteria for urine cytology to improve reproducibility among pathologists. Thus far, most cytology studies have investigated application of TPS on lower urinary tract specimens. Also, it is unclear which cytologic features are most predictive of malignancy, particularly in the upper urinary tract. We evaluate concordance rates of preoperative upper urinary tract cytology specimens before and after application of TPS criteria with surgical resections and assess cytologic features associated with malignancy.

Design: 54 resections with high-grade and low-grade urothelial carcinoma (HGUC, LGUC) from 2000-2016 with available preoperative cytology (n=61) were identified. Cytology was re-reviewed to evaluate cytologic features and provide diagnoses before and after TPS implementation.

Results: The most common cytologic features associated with HGUC were N:C ratios ≥0.7 (88%), hyperchromasia (83%), coarse chromatin distribution (67%), and nuclear pleomorphism in cell clusters (65%). Application of TPS criteria resulted in 10 diagnostic downgrades and 6 diagnostic upgrades. After TPS criteria were applied, the sensitivity of a positive diagnosis decreased from 29% to 19%. The morphologic feature most consistently associated with a downgrade from positive to suspicious was a lack of marked nuclear contour irregularities in atypical urothelial cells.

Conclusion: Using strict TPS criteria in upper urinary tract cytology specimens may decrease the frequency of positive diagnoses with a concurrent increase in suspicious diagnoses. These findings may indicate that different morphologic features, particularly markedly irregular nuclear contours, may have different predictive values for HGUC in upper urinary tract cytology specimens compared to those from the lower urinary tract.

INTRODUCTION

Primary cancers of the urinary tract represent the ninth most common site of cancer worldwide [1]. Upper tract urothelial cancers (UTUC) are relatively rare, accounting for approximately 5-10% of all urothelial cell carcinomas and 5-7% of renal neoplasms [2]. Compared to urothelial carcinomas of the lower urinary tract, UTUC are less common, but also confer a worse prognosis, leading to an estimated 15,930 deaths in the United States in 2018 [3]. Additionally, the propensity of urothelial carcinoma for recurrence and multifocality results in increased surveillance and monitoring to guide patient management. As such, urothelial carcinoma results in the highest lifetime financial cost for a patient of any cancer [4].

While voided urine cytology (VUC) has been shown to be a convenient method of diagnosing high-grade urothelial carcinoma (HGUC) in the lower urinary tract , diagnosing upper urinary tract HGUCs by VUC is limited by poor cellular preservation and mechanical distortion [5-6]. Additionally, the increased technical difficulty in visualizing and accessing potential tumors in the upper urinary tract in comparison to those arising in the lower urinary tract also makes acquisition of diagnostic material by biopsy more difficult [7]. These factors highlight the utility of upper urinary tract washing cytology specimens, as there is a wider area of urothelium evaluated during an upper tract washing compared to a focused biopsy. Furthermore, cytologic atypia in upper urinary tract HGUC are more prominently featured in upper tract cytology washes in comparison to VUC due to better cellular preservation.

However, the utility of urine cytology has historically been limited by the variable usage of indeterminate categories such as "atypical" and "suspicious," which in turn leads to confusion as to

what management options should be pursued by the clinicians. As such, there have previously been several attempts to standardize diagnoses in urine cytology by various classification schema [8]. Currently, the most formal and widely utilized classification system has been the recently published Paris System for Urine Cytology.

The Paris System (TPS) introduced diagnostic morphologic criteria for urine cytology to improve reproducibility among pathologists and potentially help establish management guidelines. Thus far, many cytology studies have focused on the application of TPS on urine specimens from the lower urinary tract. Additionally, there is still uncertainty as to which cytologic features are most predictive of malignancy, particularly in upper tract urine cytology. This study evaluates the concordance rates of preoperative upper tract urine cytology specimens before and after application of TPS criteria with surgical resection specimens as well as cytologic features most predictive of malignancy.

MATERIALS AND METHODS

With Institutional Review Board approval, 114 patients with a diagnosis of upper urinary tract urothelial carcinoma who had undergone radical nephroureterectomy (RNU) or ureterectomy (UT) between 2000 and 2016 were retrospectively identified from the surgical pathology archive at Michigan Medicine. Of these, 59 patients had both surgical pathology and pre-operative urine cytology specimens (ThinPrep®) available for review for a total of 61 resections and 111 cytology specimens. Patients with non-diagnostic cytologic specimens, no residual disease on surgical resection, and/or only voided or urinary bladder washing urine cytology (no upper tract urine cytology) were excluded from the cohort.

Also, cases in which the pre-operative urine cytology specimens were collected from the contralateral side from the ultimate surgical resection or were collected over a year from the date of surgical resection were excluded from the cohort. This left a total of 53 patients with 54 resections and 72 cytology specimens. In this cohort, there were 7 patients who had more than one urine cytology specimen collected concurrently from the same site. 4 patients had 2 concurrent samples, 2 patients had 3 concurrent samples, and 1 patient had 4 concurrent samples. In these instances, the urine cytology with the most severe diagnosis for each of these patients was used for analysis. These final parameters left a total of 53 patients with 54 resections and 61 urine cytologic specimens for evaluation.

The cytology materials were reviewed by a cytopathologist (M.L.) who was blinded to patient demographics and clinicopathologic data. The cytopathologist evaluated each cytology specimen and provided diagnoses before and after application of diagnostic criteria put forth by TPS. In the initial evaluation of urine specimens before application of TPS criteria, the diagnostic categories of "no malignant cells identified," "atypical urothelial cells," "suspicious for urothelial carcinoma," and "positive for urothelial carcinoma" were utilized. After initial evaluation, diagnostic criteria of TPS were applied to categorize lesions as either "negative for high-grade urothelial carcinoma," "atypical urothelial cells," "suspicious for high-grade urothelial carcinoma," "positive for high-grade urothelial carcinoma," or "low-grade urothelial neoplasm". Cytologic features including increased nuclear to cytoplasmic (N:C) ratios, nuclear hyperchromasia, irregular nuclear contours, coarse chromatin distribution, and nuclear pleomorphism as well as the presence of mitotic figures, urothelial cells with atypical degeneration, and bizarre single cells were recorded for each specimen. The frequency of changed diagnoses before and after application of TPS diagnostic criteria, concordance with subsequent

surgical resection specimens (which was considered to be the gold standard for each patient), and the presence of distinct cytologic features in patients with subsequent resections were evaluated. Slides from the surgical resections were reviewed by three study pathologists (C.T.S., S.L.S. and R.M.) to confirm the pathologic features including the diagnosis, grade, and stage.

Associations between the cytologic diagnoses, features and resection grade were examined using the χ2 test or Fisher's exact test, as indicated; a p-value of <0.05 was considered to be statistically significant.

RESULTS

Demographic data from the patient cohort is summarized in Table 1. Of the 53 patients, 44 were male (83%). Patient age ranged from 47-94 years at the time of surgical resection. 13 of the patients had either received either neoadjuvant chemotherapy (8%) or adjuvant therapy (17%). Disease progression was noted as clinical recurrence in 20 patients (38%) and 10 (19%) patients had definitive metastasis (4 patients with urothelial carcinoma involving 1 metastatic site, 3 patients with 2 metastatic sites, 1 patient with 3, 1 patient with 4, and 1 patient with 5). The most common metastatic site was lung (6 cases), followed by liver (5 cases), bone (4 cases), kidney (2 cases), soft tissue (2 cases), axillary lymph nodes (2 cases), and prostate (1 case). The time to last clinical follow-up ranged from 7 to 3635 days.

Of the 54 surgical resections evaluated, 23 were from the right (43%) and 31 were from the left side (57%). The majority of these resections were diagnosed as HGUC (n= 41, 76%), while low-grade urothelial carcinoma (LGUC) was diagnosed in the remaining cases (n= 13, 24%). The resection specimen data is summarized in Table 2.

A total of 61 ipsilateral urine specimens collected 5-323 days prior to resection were evaluated before and after application of diagnostic criteria put forth by TPS. Table 3 summarizes cytologic specimen collection data. Application of TPS criteria resulted in a change of diagnosis in 16 (26%) cases. Of these, 16 cases, 10 (63%) were diagnostic downgrades while the remaining 6 (37%) resulted in diagnostic upgrades. Of the 6 upgrades, 3 (50%) represented a change from atypical to suspicious categories, 2 (33%) represented a change from negative to atypical categories, and 1 (17%) represented a change from suspicious to positive. Of the 10 downgrades, the most common change was from positive to suspicious (6, 60%). The remaining 4 downgrades were split between downgrades from suspicious to atypical and atypical to negative. Table 4 summarizes the results of applying TPS criteria to the upper tract urine specimens on diagnostic categories.

The frequency of cytologic features associated with subsequent LGUC and HGUC on surgical resection were evaluated. The presence of coarse chromatin distribution among atypical urothelial cells (Figures 1A-1C) and bizarre single cells were seen more commonly in HGUC in comparison to LGUC; these findings were statistically significant. In contrast, nuclear to cytoplasmic ratios, nuclear hyperchromasia, nuclear contour irregularities, pleomorphism, as well as the presence of mitoses and/or atypical degeneration were not statistically significant features in differentiating between low and high-grade urothelial carcinomas (Table 5).

The concordance rate between cytologic diagnoses and the subsequent surgical resection was evaluated.   The sensitivity of a positive diagnosis in upper urinary tract cytology in the detection of HGUC prior to the application of TPS criteria was 29% and decreased to 19% after application of TPS criteria. However, the sensitivities in detecting HGUC are more similar after suspicious and atypical

categories were included into the "positive" cohort (sensitivities of 60% when including the suspicious category into the "positive" cohort before and after application of TPS criteria, sensitivities of 88% and 85% after including both suspicious and atypical categories in the "positive cohort" before and after application of TPS criteria, respectively). For LGUC, the sensitivity of upper urinary tract urine cytology is much lower than that for HGUC, which is expected given that the primary focus of urine cytology stated by the TPS is the detection of HGUC. Evaluation of upper urinary tract cytology both before and after application of TPS shows higher sensitivity in the detection of HGUC in comparison to LGUC. The sensitivities of upper urinary tract cytology before and after application of TPS are shown for each diagnostic category in Table 6.

Of the 6 cases that were upgraded after application of TPS, all displayed N:C ratios ≥ 0.7 and nuclear hyperchromasia. The one case that was upgraded from suspicious to positive showed all key diagnostic features of HGUC highlighted in TPS criteria (N:C ratio ≥ 0.7, nuclear hyperchromasia, and coarse chromatin distribution) in cells with relatively small nuclei (Figures 2A-2B). Of the 10 cases that were downgraded with application of TPS, 8 cases (80%) showed N:C ratios ≥ 0.7, nuclear hyperchromasia, and pleomorphism. In our cohort, 6 of the 10 downgraded cases (60%) represented changes from positive to suspicious diagnostic categories. In these cases, the cells displayed N:C ratios ≥ 0.7, nuclear hyperchromasia, and coarse chromatin distribution, but lacked irregular nuclear contours (Figures 3A-3E) seen in other cases diagnosed as positive for HGUC (Figures 4A-B). All 10 (100%) of the cases that were downgraded were HGUC on resection, compared to the 4 of 6 (66%) cases that were upgraded after application of TPS criteria.

DISCUSSION

Prior to TPS, the institutional diagnostic rate of atypical urine cytology ranged from 2 to 31% with 8.3 – 37.5% of these cases associated with subsequent histologic diagnosis of high-grade urothelial carcinoma [9-11]. Taking note of the inconsistent utilization of the "atypical" category in urine cytology and its variable association with subsequent urothelial carcinoma diagnoses on biopsies/resections, the authors of TPS established the goal of creating a diagnostically more reproducible and, hopefully, more clinically meaningful diagnostic categories.

The utilization of TPS has yielded variable results, most of which are favorable, after implementation across institutions. Some institutions have noted that the most significant change seen from pre to post-Paris System evaluation lies in the diagnostic rate of the indeterminate category of 'atypical' and, to a lesser degree, the 'suspicious' category. While many institutions have displayed a decreased atypical diagnostic rate after implementing TPS diagnostic criteria [9, 12-13], few have reported no significant changes in the rate of atypical urine cytology [14] and rare others have reported an increased atypical rate [15]. Of note, many of the studies that showed a decrease in diagnostic rates of "atypical" urine cytology also reported an increased association of these "atypical" cases with subsequent histologic diagnoses of high-grade urothelial carcinoma. Additionally, several studies have noted the improvement in correlation of urinary cytology when using TPS classification with subsequent biopsy diagnoses [12,14,16-17]. However, while many of these studies have focused on the evaluation of urine cytology specimens derived from voided urines or bladder washings evaluating the lower urinary tract, there have few studies with a predominant focus on upper urinary tract cytology.

In our current study focusing on upper urinary tract cytology, the most common cytologic features associated with HGUC on subsequent resections were N:C ratios ≥0.7 (88% of urine cytology cases from patients with subsequent HGUC on resection), hyperchromasia (83%), coarse chromatin distribution (67%), and nuclear pleomorphism within cell clusters (65%). Application of TPS diagnostic criteria resulted in a slightly increased use of the suspicious diagnostic category (26% to 36%) with no significant change in the diagnostic rate of the atypical diagnostic category. Zheng *et al*., also evaluated the effect of utilizing TPS in 125 upper urinary tract urine cytology specimens from 74 patients who had subsequent biopsies or resections within 2 months of upper urinary tract urine collection [18]. In this study, the diagnostic rates in this cohort for non-diagnostic, negative for high-grade urothelial carcinoma, atypical urothelial cells, low-grade urothelial neoplasm, suspicious for high-grade urothelial carcinoma, and positive for high-grade urothelial carcinoma were 1.6%, 39.2%, 17.6%, 8.8%, 16.8%, and 16%, respectively. However, the study did not evaluate the diagnostic rates of these categories prior to implementation of TPS diagnostic criteria. Zheng *et al*., also reported higher sensitivity for the detection of HGUC when cytology was diagnosed as positive for HGUC than our study (45% vs. 19%). However, similar to our study, the sensitivity significantly increased when both suspicious and positive were considered "positive" (78.6% vs. 60%,) and even more so when the atypical diagnostic category is also considered as part of the positive cohort (92.9% vs. 85%). In a more recent study by McIntire *et al.,* implementation of TPS criteria also resulted in an improved correlation of upper urinary tract cytology specimens with subsequent biopsies (from 34% to 63%) and a decreased use in of the atypical diagnostic category (from 29% to 13%) [19]. However, in contrast to our current study, the pre-TPS original diagnoses

were gathered from the prior pathology reports and were not re-reviewed and distinct cytologic features of the urine specimens were not evaluated.

As noted previously in our results, there was a diagnostic category change after application of TPS criteria in 16 (26%) cases, 10 of which represent diagnostic downgrades while the others represent diagnostic upgrades. The most common upgrade was seen in cases originally diagnosed as atypical and then called suspicious for HGUC after TPS criteria were applied (50%, 3 of 6 upgrades) while the most common downgrade was seen in cases originally diagnosed as positive for urothelial carcinoma and then called suspicious for HGUC after strict TPS criteria were applied (60%, 6 of 10 downgrades). Upon re-review of the cases resulting in diagnostic changes, we found that the one case resulting in an upgrade from suspicious for urothelial carcinoma to positive for HGUC after application of TPS criteria displayed atypical urothelial cells with N:C ratios ≥ 0.7, coarse chromatin distribution, nuclear hyperchromasia, and nuclear contour irregularity in cells; however, these features were seen in urothelial cells with relatively small nuclei compared to surrounding benign urothelial cells (Figures 2A-B). Additionally, the most consistent feature associated with a diagnostic downgrade from positive for urothelial carcinoma to suspicious for HGUC is the lack of marked nuclear contour irregularity in atypical urothelial cells (Figures 3A-3E), one of the key Paris System diagnostic features of malignant urothelial cells. This finding highlights how strict application of TPS criteria for upper urinary tract cytology specimens can decrease the frequency of positive diagnoses.

Our findings may indicate that different morphologic features, particularly markedly irregular nuclear contours, may have different predictive values for HGUC in upper urinary tract urine cytology specimens compared to those from the lower urinary tract. However, there are limitations to this

current study, including the retrospective nature of patient selection. This could provide a source of selection bias, as slides were reviewed knowing that subsequent resection specimens had either LGUC or HGUC. As such, mild cytologic features may be more carefully evaluated. Additionally, the cytology specimens were only re-reviewed by one cytopathologist during the study, which does not allow for a consensus opinion. Nonetheless, if similar findings are validated by other independent studies, future iterations of TPS may consider revision of diagnostic criteria, particularly for upper tract urine specimens.

Suh *et al*., evaluated 142 urinary tract washing specimens derived from the urinary bladder, ureter, and renal pelvis for cytologic features (including anisonucleosis, India ink nuclei, apoptotic bodies, increased N:C ratios, irregular nuclear membranes, coarse chromatin, hyperchromasia, pleomorphism, tumor diathesis) associated with HGUC. After regression analyses, anisonucleosis and India ink nuclei were reported to be independent predictors for HGUC. The authors then applied a modified TPS to include these two features, which resulted in an increase sensitivity in the detection of HGUC without a significant loss in specificity. Another study of 112 urinary tract cytology specimens from patients with a subsequent diagnosis of HGUC on tissue biopsy by Cowan *et al.* noted that strict application of TPS criteria (requiring the presence of a N:C ratio ≥ 0.7, nuclear hyperchromasia, markedly irregular nuclear contours, and coarse chromatin distribution as well as a minimum quantity of atypical urothelial cells with the aforementioned features) could potentially restrict a diagnosis of HGUC. While the study showed that N:C ratio ≥0.7 was the most stringent TPS diagnostic criteria (78%), it also noted that smooth nuclear contours in HGUC cells could be a potential pitfall in the evaluation of urine cytology specimens, as markedly irregular nuclear contours were identified in 83% of specimens [20].

However, both of these studies did not restrict their evaluation to upper urinary tract specimens or substratify their findings between lower and upper tract specimens. Nonetheless, the findings of these studies also suggest that perhaps more fluid application of TPS diagnostic criteria, in contrast to strict adherence to all its diagnostic criteria, as well as potential revisions of diagnostic criteria in future iterations of TPS may increase the diagnostic accuracy of urine cytology.

A potential revision of diagnostic criteria for urothelial carcinoma in both upper and lower urinary tract may affect not only diagnostic accuracy but also reproducibility. More studies are needed to establish the effect TPS has had on diagnostic accuracy and inter-observer variability, as well as to further evaluate key morphologic features, such as the presence or absence of nuclear contour irregularities, that can affect diagnostic accuracy in upper urinary tract cytology specimens compared to lower urinary tract specimens. Modification of diagnostic criteria based on the source of urinary cytology specimens may enhance the utility of urine cytology and eventually help develop clinically meaningful management guidelines for patient care.

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