Original Article

Comparison Between Cytospin and Liquid-Based Cytology in Urine Specimens Classified According to the Paris System for Reporting Urinary Cytology

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BACKGROUND: The current study compared ThinPrep urinary cytology and conventional cytospin urinary cytology in the diagnosis of bladder cancer, applying the Paris System for Reporting Urinary Cytology. **METHODS:** Between January 2010 and December 2011, a total of 3659 urine samples were processed using conventional cytospin methods. Between January 2012 and December 2013, a total of 4186 urine cytological cases were analyzed using ThinPrep methods. In 131 cases (65 processed by conventional cytospin and 66 processed by ThinPrep), a subsequent biopsy was performed. The authors reclassified these cases according to the Paris System and an analysis between the 2 methods with regard to bladder biopsies was performed. **RESULTS:** No significant differences were observed in terms of sensitivity and specificity between the 2 methods in cases with positive cytology for high-grade carcinoma. According to the Paris System, cases of atypical urothelial cells (AUC) and atypical urothelial cells suspicious for high-grade carcinoma (AUC-H) that were processed using cytospin did not correlate with urothelial carcinoma or with negative biopsies; conversely, the AUC cases processed using ThinPrep appeared to correlate with negative histological biopsies or low-grade urothelial carcinoma. **CONCLUSIONS:** The results of the current study demonstrated that according to the Paris System, there were no significant differences in sensitivity or specificity for the diagnosis of high-grade urothelial carcinoma or AUC-H between the 2 methods. Cases of AUC should be easy to recognize using Thin Prep rather than cytospin and only AUCs diagnosed with ThinPrep were found to be statistically linked to negative cases for carcinoma or with low-grade urothelial carcinoma. ***Cancer Cytopathol* 2016;124:519–23**. *© 2016 American Cancer Society.*

KEY WORDS: cytospin; Paris System for Reporting Urinary Cytology; ThinPrep method; urinary cytology.

INTRODUCTION

Urothelial carcinoma of the bladder is the eighth most common cancer in the United States.[1] Bladder cancer recurrence is very high, ranging from 40% to 85% according to different studies.[2,3] Because of the risk of disease recurrence, regular follow-up is required after treatment and urinary cytology is one of the most important tests in the investigation of patients who are at risk of urothelial carcinoma.[4] Advantages of urinary cytology include high sensitivity and specificity for high-grade urothelial lesions that sometimes are cystoscopically occult,[5] whereas the low sensitivity and specificity noted in patients with low-grade urothelial tumors represent an important limitation of urinary cytology.[6,7] Another limitation is represented by the equivocal or atypical diagnosis, which can lead to management dilemmas for clinical collegues.[8,9]

The primary objective of urinary cytopathology is to detect and diagnose high-grade urothelial carcinoma (HGUC), and therefore urinary cytology is used to monitor patients with a history of urothelial neoplasms.[10] Liquid-based cytology has been developed as an alternative to conventional methods and most comparative

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studies have shown that ThinPrep is better than conventional preparations because it has a specificity of >90% in nongynecological specimens.

Over the years, the methods of urinary cytology have evolved as the histopathologic classification of bladder lesions has changed and the expectations of clinicians who send the urine specimen for study have changed. The modern challenge in urinary cytology is represented by the need for a method of reporting urinary cytology that is clinically useful, with a rigorous definition of validated cytological criteria for specific categories (in particular for the atypical category), and that is easy to use by the general pathology community.[5] In this article, we assessed the new classification system that was developed in Paris in 2014 with the participation of numerous experts. The Paris System for Reporting Urinary Cytology established a detailed description of 6 major diagnostic categories of urinary cytology and for what to our knowledge is the first time, included the rate of malignancy with follow-up suggestions for each group (Table 1).

TABLE 1. The Paris System for Reporting Urinary Cytology: Implications for the Risk of Malignancy

Diagnostic Category	Risk of HGUC, %
Inadequate	0–5
Negative for HGUC	0–5
AUC of indeterminate categories	8–20
AUC-H	50–70
LGUC	10
HGUC	>90
Other malignancies	>90

Abbreviations: AUC, atypical urothelial cells; AUC-H, atypical urothelial cells suspicious for high-grade carcinoma; HGUC, high-grade urothelial carcinoma; LGUC, low-grade urothelial carcinoma.

MATERIALS AND METHODS

We examined urinary urine cytology cases (65 of which were processed by conventional cytospin and 66 of which were processed by ThinPrep) in patients at the time of first event of urinary symptoms such as hematuria or bladder irritation clinically suspected to be bladder cancer. The cytological diagnoses were classified according to the classification scheme proposed by Papanicolaou[11] and subsequently were reclassified according to The Paris System for Reporting Urinary Cytology (Table 1).

The results were correlated with bladder biopsies, which were obtained at the same time urinary cytology was performed or within 6 months after urinary cytology samples. In those cases with a positive cytology result but that were negative at the subsequent biopsy, no evidence of malignancy was observed during clinical investigations (ureteral washing, computed tomography scan of the urothelial tract) and during follow-up. All cytological samples and histological biopsies were evaluated by 2 different pathologists (G.F. and F.P.), and the cases were compared between the 2 methods.

Cytospin Method

Samples for conventional urinary cytology were centrifuged using the Thermo Scientific Shandon Cytospin (Shandon Scientific Ltd, Cheshire, UK). After this, 2 layers of cell smear samples were prepared. The cell smear samples were fixed in 95% alcohol for 30 minutes and were then stained by the standard Papanicolaou method following the manufacturer's instructions.

ThinPrep Method

For the ThinPrep method, slides were prepared using the ThinPrep 5000 automated slide processor (Hologic, Marlborough, Mass), fixed in 95% ethanol for 15 minutes, and stained by the standard Papanicolaou method following the manufacturer's instructions.

Statistical Analysis

Statistical analysis was performed using the chi-square test. A P value <0.05 was considered to be statistically significant.

RESULTS

According to the Paris System, each cytological diagnosis was reclassified as: 1) nondiagnostic or unsatisfactory; 2) negative for malignancy; 3) atypical urothelial cells of uncertain significance (AUC); 4) atypical urothelial cells suspicious for high-grade urothelial carcinoma (AUC-H); 5) low-grade urothelial carcinoma (LGUC); 6) HGUC; and 7) other malignancies (primary versus metastatic) and in the same cases a histocytological correlation was performed.

In particular, 39 and 41 cytological cases, respectively, processed by the conventional cytospin method and the ThinPrep method were diagnosed as being positive for carcinoma (HGUC), and >80% of urine cytological cases positive for carcinoma had a positive histological biopsy regardless of the cytological method of preparation. The sensitivity and specificity did not appear to differ significantly between the 2 techniques ($P<.05$; data not shown) (Table 2).

TABLE 2. Cytohistological Correlation Between the Cytospin and ThinPrep Methods

No. of Cases	Negative Biopsy	Positive Biopsy for Carcinoma
Cytospin (N = 65)		
Positive for HGUC carcinoma: 39	7 (18%)	32 (82%)
Suspicious: 26	14 (54%)	12 (46%)
ThinPrep (N = 66)		
Positive for HGUC carcinoma: 41	7 (17%)	34 (83%)
Suspicious: 25	17 (68%)	8 (32%)

Abbreviation: HGUC, high-grade urothelial carcinoma.

TABLE 3. Suspicious Cases Analyzed by the Cytospin and ThinPrep Methods According to the Paris System for Reporting Urinary Cytology

No. of Cases	Negative Biopsy	LGUC	HGUC
Cytospin Method (N = 26)			
AUC: 15	8 (53%)	2 (13%)	5 (33%)
AUC-H: 11	6 (55%)	0	5 (45%)
ThinPrep Method (N = 25)			
AUC: 15	13 (87%)	1 (7%)	1 (7%)
AUC-H: 10	4 (40%)	1 (10%)	5 (50%)

Abbreviations: AUC, atypical urothelial cells; AUC-H, atypical urothelial cells suspicious for high-grade carcinoma; HGUC, high-grade urothelial carcinoma; LGUC, low-grade urothelial carcinoma.

Of 26 suspicious cases processed by conventional methods, we found at the time of histological biopsies 10 cases that were positive for HGUC, 2 cases that were positive for LGUC, and 14 cases that were negative for neoplasia.

Moreover, in 25 suspicious cases that were processed using ThinPrep, the histological diagnoses included 6 cases that were positive for HGUC, 2 cases that were positive for LGUC, and 17 cases diagnosed as negative for neoplasia.

In accordance with the Paris System for Reporting Urinary Cytology, 26 cases processed using the cytospin method were classified as 15 cases of AUC and 11 cases of AUC-H, whereas in 25 urine samples processed using the ThinPrep method, 15 cases were classified as AUC and 10 cases were classified as AUC-H. No diagnosis of LGUC was made among those cases processed by cytospin nor those that were processed using ThinPrep.

Finally, when correlating the results with histological biopsies, we found that the cases of AUC and AUC-H did not correlate with HGUC ($P = .689$), whereas only in cytology processed by the ThinPrep method did the diagnosis of AUC appear to correlate with negative histological biopsies or LGUC ($P = .022$) (Table 3).

DISCUSSION

Urothelial carcinoma is the fourth most common tumor diagnosed in the United States and Europe, representing a heterogeneous group of cancers. Urothelial carcinoma can be located in the urothelial epithelium of the entire urinary tract. Urinary cytology as a method for diagnosing bladder carcinoma was introduced in 1945 by Papanicolaou and Marshall and, to the best of our knowledge, represents the first method with which to investigate urothelial tumors, and often is used as an adjunct in combination with cystoscopy and biopsy.[10–17]

In particular, urinary cytology is useful for performing a mass screening of a group of patients considered to be at high risk, allowing for the diagnosis of intraepithelial cancer and the detection of cancer in the diverticulum.[18]

However, urinary cytology has its limitations, with a mean sensitivity of nearly 50% for detecting urothelial carcinoma,[19] and new methods of collection and preparation have been studied to improve sensitivity and specificity, especially for the detection of low-grade cancerous lesions.

In the 1990s, liquid-based cytology was developed as a replacement for the conventional method because of reportedly higher cell recovery rates and better cell preservation.[19]

Numerous studies have compared the ThinPrep method with conventional methods of specimen processing in urinary cytology,[19–25] and in these same studies conflicting conclusions were present. Some authors preferred ThinPrep over conventional methods in the majority of nongynecologic cytology, including urinary cytological cases, with results demonstrating that the ThinPrep method resulted in great cellularity, cell preservation, and a cleaner background compared with conventional methods.[20,21] Papillo and Lapen demonstrated increased cell recovery using the ThinPrep technique, particularly for cytological specimens with low cellularity.[25] Luthra et al demonstrated an improved cellular quantity and better cellular morphology for the ThinPrep method, with less time taken for screening the smears and a reduction in the number of unsatisfactory samples.[21] Moreover, it has been demonstrated that nuclear details are more evident in the ThinPrep slides than in cytospin preparations, and Wright and Halford concluded that a 1-slide thin-layer urine preparation is comparable to 4 conventional slides in the detection of urothelial abnormalities.[24]

However, other studies have demonstrated that conventional methods in urinary cytologic samples are superior in terms of interpreting the cytomorphological details in cases of malignancy.[19,22,23] Nassar et al concluded that in cytospin preparations, malignant cells were frequently clumped together, thereby conserving papillary architecture, whereas in ThinPrep preparations, the diagnostic cells were small, shrunken, and dispersed and the distinction between normal cells, reactive cells, and atypical cells was easier to make with cytospin preparations.[23]

Classification systems in urinary cytology have evolved in parallel with the progress in our understanding of bladder cancer and the Paris classification represents what to our knowledge is the most recent effort to address the problem of urinary cytological classification. In this classification, to our knowledge for the first time, diagnostic categories based on pathologic evidence and risk of disease recurrence and progression have been identified to distinguish those patients who need immediate cystoscopy versus those who can be followed at an interval based on risk stratification for their diagnostic category.

The results of the current study indicated that despite differences in quality, the techniques studied appear to have no impact on the diagnosis of malignancy as evaluated by the rate of abnormalities (nuclear features, cytomorphologic details, and architectural pattern). The criteria proposed by the Paris System to identify HGUC appear to be easily recognizable both in cytospin preparations and in specimens processed using the ThinPrep method. Greater than 80% of urinary cytological cases with a diagnosis of HGUC were found to demonstrate histological diagnoses of HGUC regardless of the cytological method of preparation, and the sensitivity and specificity did not appear to differ significantly between the 2 techniques.[26]

Conversely, a difference can be observed in the "suspect" category if the specimen has been processed using a conventional or ThinPrep method. To the best of our knowledge, the Paris System is the first time the "atypical cells" are distinct in AUC and AUC-H to identify those patients at a higher risk of a subsequent aggressive disease, particularly in the follow-up period after conservative treatment. AUC were defined as nonsuperficial cells, and are few in number with cytologic criteria that include an increased nuclear/cytoplasmic ratio ($\leq$0.7), a centrally located nucleus, slight to moderate nuclear hyperchromasia, homogenous chromatin, and a regular nuclear shape, whereas for the diagnosis of AUC-H, a nuclear/cytoplasmic ratio $>$0.7, severe nuclear hyperchromasia, dense chromatin, and an irregularly outlined nuclear shape are required.

The data from the current study demonstrated that the AUCs diagnosed by the Thin Prep method correlated in 93% of cases with negative histological biopsies or LGUC ($P = .022$), whereas approximately 67% of the AUCs in samples processed with the cytospin method correlated with negative biopsies or LGUC. For the AUC-H category, the probability of noting a HGUC in histological biopsies was approximately 50% and the 2 different methods of preparation appear to have no impact on the accuracy of the diagnosis. The ThinPrep, when applied to urinary cytology, was found to improve cell yield and cell preservation and reduce background artifacts, and specimens appear cleaner than those prepared with conventional preparations; in particular, nuclear detail appeared clearer and nucleoli more obvious in ThinPrep compared with conventional cytospin slides.

In the Paris System, the difference between AUC and AUC-H is based mainly on nuclear details and on characteristics of chromatin, but the degree of nuclear hyperchromasia (moderate vs severe) or the nuclear shape (regular, round nuclear shape vs irregularly outlined nuclear shape) could be very difficult to observe if the urothelial cells are overlapped, clustered, and/or deformed as observed in the majority of conventional cytospin preparations. Moreover, any constituents that may interfere with the diagnostic procedure such as blood, mucus, and inflammatory cells present in cytospin preparations are removed with the liquid-based monolayer cell preparation system.

When comparing ThinPrep urinary cytology and conventional cytospin urinary cytology for the diagnosis of bladder cancer applying the Paris System, no difference between the 2 techniques in terms of their ability to identify HGUC was noted. The Paris System category of AUC was easily recognized using the Thin Prep method compared with conventional cytospin preparations and only those AUC cases diagnosed with the ThinPrep method appeared to correlate with negative cases for carcinoma or with LGUC.

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CONFLICT OF INTEREST DISCLOSURES

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AUTHOR CONTRIBUTIONS

Patrizia Straccia: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing-original draft, writing–review and editing, and visualization. **Tommaso Bizzarro**: Formal analysis and investigation. **Guido Fadda**: Conceptualization, methodology, and writing-review and editing. **Francesco Pierconti**: Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing-original draft, writing–review and editing, visualization, supervision, project administration, and funding acquisition.

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