RESEARCH ARTICLE

Clinical Value of Dividing False Positive Urine Cytology Findings into Three Categories: Atypical, Indeterminate, and Suspicious of Malignancy

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Abstract

Background: The aim of this study was to evaluate 10 years of false positive urine cytology records, along with follow-up histologic and cytologic data, to determine the significance of suspicious urine cytology findings. Materials and Methods: We retrospectively reviewed records of urine samples harvested between January 2002 and December 2012 from voided and catheterized urine from the bladder. Among the 21,283 urine samples obtained during this period, we located 1,090 eligible false positive findings for patients being evaluated for the purpose of confirming urothelial carcinoma (UC). These findings were divided into three categories: atypical, indeterminate, and suspicious of malignancy. Results: Of the 1,090 samples classified as false positive, 444 (40.7%) were categorized as atypical, 367 (33.7%) as indeterminate, and 279 (25.6%) as suspicious of malignancy. Patients with concomitant UC accounted for 105 (23.6%) of the atypical samples, 147 (40.1%) of the indeterminate samples, and 139 (49.8%) of the suspicious of malignancy samples (p<0.0001). The rate of subsequent diagnosis of UC during a 1-year follow-up period after harvesting of a sample with false positive urine cytology initially diagnosed as benign was significantly higher in the suspicious of malignancy category than in the other categories (p<0.001). The total numbers of UCs were 150 (33.8%) for atypical samples, 213 (58.0%) for indeterminate samples, and 199 (71.3%) for samples categorized as suspicious of malignancy. Conclusions: Urine cytology remains the most specific adjunctive method for the surveillance of UC. We demonstrated the clinical value of dividing false positive urine cytology findings into three categories, and our results may help clinicians better manage patients with suspicious findings.

Keywords: Urine cytology - false positive - urothelial carcinoma - diagnosis

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Introduction

Although cystoscopy is the current standard for the diagnosis of urothelial carcinoma (UC), urine cytology remains the most specific adjunctive method for the surveillance of bladder UC. (Kanagawa Urological Research, 2012) The specificity of urine cytology for UC is high, but its sensitivity varies and is generally low. (Sternberg et al., 2011) Sample type can affect the interpretation of urine cytology: voided samples are more specific but slightly less sensitive than catheterized samples. (Raab et al., 2007) This implies that positive urine cytology is generally compatible with diagnosis of UC, but a benign interpretation of a urine sample does not exclude the presence of cancerous lesions. Because of these sensitivity and specificity issues, definitive interpretation of a urine sample as either malignant or benign may not always be possible.

Various conditions and procedures can affect the cellularity and nuclear morphology of urine samples, including catheterization, inflammation, infection, surgical manipulation, and calculi, making the discrimination of malignant cells difficult even for experts. (Deshpande et al., 2005; Kapur et al., 2008) Samples collected under such conditions are often categorized as false positive, and many urologists view a suspicious urine cytology finding as requiring examination of the entire urinary tract. If no lesions are detected in the urinary tract, the suspicious sample might be re-categorized as essentially benign; however, most cytopathologists consider such samples to indicate at least moderate risk. (Powsner et al., 2000; Deshpande et al., 2005; Sternberg et al., 2011) Whereas clinical discrepancies between specialties exist, the prognostic value of a false positive urine cytology

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finding remains unclear.

The aim of this study was to review 10 years of records of false positive urine cytology findings in samples harvested at our institution and determine the significance of the suspicious findings by evaluating follow-up histologic and cytologic data. The ultimate goal is to provide better guidance for clinicians on how to more effectively manage patients with false positive urine cytology findings.

Materials and Methods

We retrospectively reviewed records for urine cytology samples classified as false positive between January 2002 and December 2012. Urine cytology diagnoses were based on commonly accepted morphologic criteria. (Cibas et al., 2009) The urine samples were collected from voided and catheterized urine from the bladder and were evaluated by board-certified cytopathologists using a three-class classification (i.e., negative, false positive, or positive urine cytology). During this period, 21,283 urine samples were harvested; of these, 1197 (5.6%) were categorized as false positive. Of these, we excluded 60 samples collected specifically for examination of the upper urinary tract and 47 samples collected from patients with other types of cancer (including cervical, ovarian, colon cancer, and tumors that invaded the bladder from advanced prostate cancer, leukemia, and lymphoma). The remaining 1090 urine samples, which had been collected for the purpose of confirming UC, were classified as false positive. In this study, we categorized the false positive specimens as (1) atypical (strongly suspected to be benign, but malignancy could not be ruled out), (2) indeterminate (discrimination between benignancy and malignancy was difficult), and (3) suspicious of malignancy (strongly suspected to be malignant but benignancy could not be ruled out).

The institutional medical record for each patient with any UC in his or her urinary tract was reviewed for the following pertinent information: prior history of UC; cystoscopic findings (e.g., tumor size, multiplicity, location); and subsequent histologic and cytologic follow-up data. From follow-up data, subsequent UC was defined as either histologically or cytologically proven UC (i.e., positive urine cytology) within 1 year after harvest of a false positive urine sample. Multiple false positive urine samples that led to multiple end points, such as a false positive urine sample with a consequent diagnosis of UC and a second false positive urine sample from the same patient after diagnosis and treatment of the UC, were recorded as multiple records.

Statistical analysis of the data was performed using chi-square tests and Fisher's exact test. All analyses were performed with StatView (ver. 5.0, SAS Institute, Cary, NC, USA), and $p<0.05$ was considered to be statistically significant.

Results

The 1090 false positive urine samples that were initially identified were obtained from 592 patients (434 males and 158 females). The mean patient age was 69 years (range, 26-96 years). Eligible samples were categorized as follows: 444 (40.7%) as atypical, 367 (33.7%) as indeterminate, and 279 (25.6%) as suspicious of malignancy (Table 1).

Of the urine samples categorized as atypical, indeterminate, and suspicious of malignancy, 105 (23.6%), 147 (40.1%), and 139 (49.8%), respectively, were taken from patients with concomitant UC (Table 1); that is, the detection rate of UC was higher in the suspicious of malignancy category than in the other categories ($p<0.0001$). The UC detection rate was significantly higher ($p=0.009$) for catheterized urine samples than for voided urine sample only in the atypical category; there were no significant differences in UC detection rate between the sample types in the other two categories.

UCs of the bladder were detected in 308 (28.3%) samples, and UCs of the upper urinary tract were detected in 83 (7.6%). Among the bladder UCs, 237 (76.9%) samples were assigned pathologic grades as follows: Grade 1, 45 (19.0%); Grade 2, 132 (55.7%); and Grade 3, 60 (25.3%). In terms of pathologic stage, 84 (35.4%) were Ta, 26 (11.0%) were carcinoma in situ, 86 (36.3%) were T1, and 41 (17.3%) were muscle invasive or extravesical cancers. Among the upper urinary tract UCs, 62 (74.7%) samples were assigned pathologic grades as follows: Grade 1, 8 (12.9%); Grade 2, 37 (59.7%); and Grade 3, 17 (27.4%). In terms of pathologic stage, 3 (4.8%) were Ta, 5 (8.1%) were carcinoma in situ, 20 (32.3%) were T1, and 34 (54.8%) were muscle-invasive or non-organ-confined cancers.

Data on prior history of UC and subsequent diagnosis of UC (Table 2) indicated that the rate of prior history of

Table 1. Initial Diagnoses for Patients with Suspicious Urine Cytology

	Benign (%)	Concomitant UC (%)	Total samples (%)
Atypical	339 (76.4)	105 (23.6)	444
Voided	213 (81.0)	50 (19.0)	263 (59.2)
Catheterized	126 (69.6)	55 (30.4)	181 (40.8)
Indeterminate	220 (59.9)	147 (40.1)	367
Voided	122 (63.2)	71 (36.8)	193 (52.6)
Catheterized	98 (56.3)	76 (43.7)	174 (47.4)
Suspicious of malignancy	140 (50.2)	139 (49.8)	279
Voided	85 (51.8)	79 (48.2)	164 (58.8)
Catheterized	55 (47.8)	60 (52.2)	115 (41.2)

*UC: urothelial carcinoma

Table 2. Prior History of UC and Subsequent UC Diagnoses in Samples Initially Diagnosed as Benign

	Benign samples (%)	Prior history of UC (%)	Subsequent UC (%)
Atypical	339	131 (38.6)	45 (13.3)
Voided	213 (62.8)	52 (24.4)	21 (9.6)
Catheterized	126 (37.2)	79 (62.7)	24 (19.0)
Indeterminate	220	109 (49.5)	66 (30.0)
Voided	122 (55.5)	51 (41.8)	32 (26.2)
Catheterized	98 (44.5)	58 (59.2)	34 (34.7)
Suspicious of malignancy	140	85 (60.7)	60 (42.9)
Voided	85 (60.7)	39 (45.9)	34 (40.0)
Catheterized	55 (39.3)	46 (83.6)	26 (47.3)

*UC: urothelial carcinoma

UC was higher in samples in the suspicious of malignancy category than in the other categories (p<0.0001). The rate of prior history of UC was higher in catheterized samples than in voided urine samples in all three sample categories. UC was subsequently diagnosed in 45 (13.3%) of samples categorized as atypical, 66 (30.0%) categorized as indeterminate, and 60 (42.9%) categorized as suspicious of malignancy. Subsequent UC diagnosis was significantly higher in samples categorized as suspicious of malignancy than in samples in the other categories (p<0.0001). Catheterized urine samples showed a significantly higher rate of subsequent UC diagnosis than voided urine samples only for samples categorized as atypical (p=0.02); there were no significant differences between sample types in the other sample categories.

We also evaluated the numbers of concomitant UC current diagnoses plus the number of subsequent UC diagnoses within a follow-up period of 1 year for each sample type in all three sample categories. The total numbers of UCs were 150 (33.8%) for atypical samples, 213 (58.0%) for indeterminate samples, and 199 (71.3%) for samples categorized as suspicious of malignancy.

Discussion

The significance of false positive urine cytology remains unknown, both for samples from patients with a clinically meaningful lesion and for samples from patients without lesions. When presented with false positive urine cytology findings, many urologists are confused about treatment and follow-up strategies.

The reported rate of suspicious urine cytology ranges from 1.9% to 28.7%, (Bhatia et al., 2006; Raab et al., 2007; Kapur et al., 2008; Brimo et al., 2009; Mokhtar et al., 2010) and the rate in our study (5.6%) fell within this range. This wide range reflects the fact that the category of false positive urine cytology is poorly defined, unlike, for example, the atypical category for squamous cells in the Bethesda system, for which the diagnostic criteria are well defined and for which guidelines exist for the proportion of cases that should fall into that category. The lack of a rigorous definition and a conventional guideline leads to great interobserver variability among cytopathologists and makes comparing data from different studies difficult.

Several research groups have investigated the role of false positive or suspicious urine cytology. For example, Sternberg et al. (Sternberg et al., 2011) reviewed data on class III urine cytology obtained from a consecutive cohort and showed that approximately one-half of cases categorized as class III were diagnosed with UC upon initial evaluation. In a study of 1320 suspicious urine samples, Muus Ubago et al. (Muus Ubago et al., 2013) reported that 21% of patients with suspicious urine cytology progressed to positive urine cytology or surgically pathologic results. Deshpande et al. (Deshpande et al., 2005) observed that 47 of 201 suspicious samples (23.4%) progressed to a positive diagnosis. Bhatia et al. (Bhatia et al., 2006) reported that 18 of 58 samples (31%) progressed to cancer during follow-up. In our study, we found that among the samples categorized as atypical, indeterminate, and suspicious of malignancy, the numbers of concommitant cancerous lesions were 105 (23.6%), 147 (40.1%), and 139 (49.8%), respectively. Raitanen et al. (Raitanen et al., 2002) addressed the question of whether, in a population of patients diagnosed with bladder cancer, class III urine cytology should be considered as a negative or positive indication for the presence of cancerous lesions; these investigators recommended that class III cytology should be considered as positive. Several reports have shown that assuming class III reports to be positive instead of negative improves sensitivity while maintaining acceptable specificity. (Whisnant et al., 2003; Deshpande et al., 2005; Turco et al., 2011) Although the design of the current study did not allow us to draw a similar conclusion from our data, our findings support this notion, at least for the category of suspicious of malignancy.

In a study of 282 urine cytology samples with follow-up data, Brimo et al. (Brimo et al., 2009) analyzed the association of cytology with histology in atypical urine cytology and analyzed subgroups designated as "atypical, unclear if reactive or neoplastic" and "atypical, favor a reactive process." Their results showed that compared to a benign diagnosis, a diagnosis of "atypical urothelial cells" was not associated with a significantly increased risk of UC. In addition, although a diagnosis of "atypical, unclear if reactive or neoplastic" had a higher rate of detection of high-grade cancer than did a diagnosis of "atypical, favor a reactive process" or benign, this difference was not significant. In our study, subclassification was associated with the detection of concomitant UC. The detection rate of UC was higher in samples categorized as suspicious of malignancy than in the other sample categories. These results call into question the value of the category of false positive urine cytology. We highly recommended that patients with samples categorized as suspicious of malignancy be investigated further and followed closely.

We observed that the rate of false positive urine cytology in patients with upper tract UC (7.6%) was lower than that in patients with bladder UC (28.3%). Over half of the patients showed histologic grade 2 for both UCs. Approximately 70% of patients with false positive urine cytology had non-muscle-invasive bladder UC, and over half of the patients who had suspicious malignancy and pathologically revealed upper tract UC had a muscle-invasive lesion or non-organ-confined disease. These results indicate that urologists and cytopathologists need to pay close attention to malignancy when presented with false positive urine cytology from bladder urine samples and clinical signs of an upper tract tumor, and that they should consider more aggressive clinical management for these patients.

Our data also provide further details about the outcome of false positive urine cytology with a negative initial work-up. Approximately 30% of the total number of patients developed UC during further follow-up within 1 year. Furthermore, samples categorized as suspicious of malignancy demonstrated a higher detection rate of subsequent UC than did samples in the other categories. In a study of the natural history of suspicious urine cytology in 102 patients with negative initial evaluation, Nabi et al. (Nabi et al., 2004) reported a significantly higher rate (36.3%) of subsequent UC during follow-

up. These investigators emphasized that patients with persistent suspicious cytology or hematuria require further evaluation and follow-up. However, it is difficult to recommend how long such patients should be followed. Because most of the malignant findings in our series presented in the first 12 months (70.7%, data not shown), regular follow-up for a period at least 12 months after the harvest of a false positive urine sample initially diagnosed as benign seems reasonable.

Unlike other forms of cytological assessment (e.g., breast cytology), urine cytology does not target an abnormal area directly and instead relies on neoplastic cells being shed in the urine. High-grade UCs are recognized as being more likely to shed abnormal cells into a urine sample than are low-grade UCs. The cells that are shed vary on slightly from normal urothelial cells, apart from a tendency to be shed in clusters rather than as single cells. Interpretation of these subtle changes is difficult, particularly when comparing normal cells and cells from low-grade UCs. Additional problems in urine cytology are that stones, infections, an instrumented urinary tract, chemotherapy and so on can mimic malignancy, leading to an erroneous diagnosis. (Deshpande et al., 2005; Kapur et al., 2008) In addition, a prior history of UC also affected the results of false positive urine cytology in this study. (Sternberg et al., 2011) The rate of a prior history of UC was higher in samples categorized as suspicious of malignancy than in the other sample categories. This result may be due to morphologic changes in the normal urothelium once UC has occurred in the urinary tract. Another possibility is that patients with bladder UC were partly treated with intravesical instillation of BCG for prophylaxis and prevention of UC recurrence. Although the possibility that inflammatory conditions can result in misinterpretation of urine cytology has been widely discussed, there is little evidence in the literature about the influence of urinary tract infection on suspicious urine cytology results. (Sullivan et al., 2010)

The sample type may have affected the association between cytologic findings and pathologic diagnosis. Exclusively in the atypical category, catheterized urine samples were associated with the detection of concomitant UC; the rate of concomitant UC was not affected by sample type in the other two categories. Although artifacts introduced by instrumentation are difficult to distinguish from malignant cytology, (Bastacky et al., 1999) many more urothelial cells (including normal, infected, or malignant cells) are harvested by catheterization than by voiding, leading to precious cytologic examination, particularly in the atypical category.

Our study was limited in several ways. First, it was retrospective, and the data were collected from only one academic institution; therefore, extrapolation of the data to different academic centers need to be done with caution. Although this variability is a limitation, the diversity of the findings may increase their generalizability to clinical practice. Second, UC was the only type of cancer that was studied. Although several patients who had been diagnosed with other types of cancers were excluded from this study, some of the suspicious urine samples may have indicated some other type of cancer, such as that resulting from invasion from gastrointestinal or gynecological lesions into the urinary tract. Third, urine cytology was the only urinary marker we considered. Other urinary markers may collectively increase the accuracy of UC diagnosis; for example, a panel of markers (including bladder tumor antigen and nuclear matrix protein-22) along with suspicious urine cytology might improve the accuracy of UC diagnosis. Despite the limitations, we believe that this study of a large cohort of patients with pathologically or cytologically confirmed UC and available clinical data provides evidence for the clinical significance of false positive urine cytology.

In conclusion, urine cytology is still one of the standard diagnostic tools for UC. Urine cytology results classified as false positive should not be categorized as negative results. False positive urine cytology findings may require close follow-up, and urologists and cytopathologists need to discuss the meaning of this finding to avoid missing significant lesions. Samples categorized as suspicious of malignancy showed a significantly high rate of detection of UC, both concomitant and subsequent UC, whereas samples categorized as atypical showed a relatively low incidence of UC. However, the UC detection rate of approximately 30% in the atypical samples indicates that each clinician should determine the significance of findings that fall into this category. Our results demonstrate the clinical value of categorizing false positive urine cytology findings into three categories, and this categorization scheme may help clinicians better manage patients with suspicious urine cytology findings.

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