

# Risk Prediction of Bladder Carcinomas in Atypical Urine Specimens by Redefining Diagnostic Cytomorphological Criteria

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## Abstract

**Introduction:** Urine cytology in bladder tumors is challenging due to the lack of definite cytomorphological criteria in categorizing atypical cases. The study aims to re-evaluate atypia in the light of three definitive cytological parameters (high N/C ratio, hyperchromasia, and irregular nuclear contours) and correlate with the follow-up histopathology and cytopathology with an attempt to redefine the morphological features that would aid in distinguishing reactive atypia from malignancy. **Materials and Methods:** A retrospective analysis of 56 atypical urine specimens was performed considering three definitive cytomorphological parameters. All cases were classified into the following categories: atypical urothelial cells (AUCs)-favor reactive, AUCs-not otherwise specified (AUC-NOS), AUCs-favor neoplastic (AUC-FN), urothelial carcinoma (UC), and negative for malignancy (NEM). The cases reviewed were correlated with follow-up histopathology and cystoscopy. The sensitivity and specificity of urine cytology were then assessed. **Results:** Out of 56 cases, 20 cases (35.7%) were positive on urine cytology (AUC-FN/UC), and amongst them, 16 cases (80%) showed positive follow-up. Among the AUC-NOS category, 71% of the cases showed positive follow-up. The sensitivity and specificity of urine cytology in atypical cases were 89.8% and 87.5%, respectively. **Conclusions:** Urine cytology can be potentially useful in predicting the risk of bladder carcinomas when definite cytomorphological features are analyzed stringently. Moreover, urine cytology in conjunction with cystoscopy can increase the detection rate of malignancy with greater accuracy.

**Keywords:** Atypical, bladder tumor, cytomorphological, definitive criteria, urine

## INTRODUCTION

Urothelial carcinoma (UC) of the bladder is one of the most common malignancies encountered in urology clinics.<sup>[1]</sup> The gold standard for the primary diagnosis of bladder cancer is based on cystoscopy and histopathology, however, urine cytology acts as a specific complementary diagnostic modality for the surveillance of bladder cancers.<sup>[1]</sup> The sensitivity of urine cytology in the detection of high-grade UCs is comparatively high but low-grade urothelial tumors are often missed mainly due to subtle morphological differences mostly simulating normal urothelial cells.<sup>[2]</sup> Recognition of the distinct nature of “atypia” (reactive/malignant) in urine samples is challenging due to the lack of defined cytomorphological features relevant to subcategorizing atypia. Definitive interpretation of a urine sample is sometimes cumbersome due to varied conditions such as associated bladder inflammation, catheterized samples,

presence of calculi and surgical, and other treatment-related interventions.<sup>[3]</sup> Although a few studies have attempted to define and characterize atypia and its association with specific lesions, interobserver variability still persists. Therefore, a more acceptable method to evaluate certain objective cytological criteria is required for accurate diagnosis. The present study precisely focuses on three definitive parameters taken together to categorize atypia as favouring neoplasia and not as reactive/NOS. These are high nucleocytoplasmic ratio, hyperchromasia, and irregular nuclear contour.

All cases categorized either as equivocal (AUC-NOS) or suspicious of carcinoma (AUC-FN) need further investigations for the establishment of the diagnosis and a close follow-up

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for proper management. To the best of our knowledge, our study is the first Indian study to undertake this work with the aim to re-evaluate atypia in urine cytology and correlate with follow-up histopathology and cytopathology with an attempt to redefine the morphological features that would aid in distinguishing reactive atypia from malignancy and assist clinicians in therapeutic decision making.

## MATERIALS AND METHODS

### Study design and setting

A hospital-based cross-sectional study was conducted by selecting the data from the electronic medical record system of our institute, and a retrospective analysis of all voided urine cytology specimens diagnosed as “atypical” from January 2022 to June 2022 (6 months) was performed. Then, subsequent surgical and cytological follow-up of these specimens were retrieved for a minimum period of 1 year. All clinical information, including age, gender, chief complaints, and cystoscopic findings, were reviewed.

### Sample size

During this period, 112 cases of urine samples were diagnosed as atypical, out of which 52 recently diagnosed cases with inadequate follow-up were excluded from the study. From the remaining 60 cases, an additional four cases without follow-up details were also excluded from the study. Thus, a total of 56 cases formed the basis of this study group.

### Ethical declaration

The study was approved by the institutional ethical committee (IEC/IRB NO: HIMS/IRB/2021-22/3360). Informed consent was not applicable as the research deals with pathology slides only, demographic information is anonymized, and the submission does not include images that may identify the person.

### Sample preparation

All urine samples were processed by conventional methods either by direct smear preparation from sediment after centrifugation or by cytospin method using Shandon Cytospin-4. Before smear preparation, the slides were albuminized to improve the cell adhesion so as to minimize the cell loss in cases with scanty sediment on centrifugation. The smears were fixed in 100% methanol and stained by Papanicolaou stain.

### Cytological analysis

The cytological diagnoses of “Atypia” were reclassified as atypical urothelial cells-favor reactive (AUC-FR), AUC-NOS, AUCs-favour neoplastic (AUC-FN), UC, and negative for malignancy (NEM). An extensive literature search revealed that three cytomorphological features, namely, high N/C ratio, nuclear membrane irregularity, and hyperchromasia, were consistently studied by many authors as the most relevant objective criteria for the diagnosis of atypia/malignancy. Therefore, these features were considered for evaluation in this study. In addition, cellularity and

presence of single atypical cells and clusters/papillae were also studied in each case.

The category of AUC-FR was assigned to those cases displaying only a high N/C ratio ( $>0.5$ ) but no hyperchromasia and/or nuclear membrane irregularity. AUC-NOS showed two of the three features, with one of the features as high N/C ratio. The diagnosis of AUC-FN was assigned in cases which had all the three features, however, the number of atypical cells was  $<5$ . When the number of atypical cells was more than 5, it was diagnosed as UC. When all the three features were absent, the smears were diagnosed as NEM.

Based on the above described features, a redefined criterion for the grade of malignancy was attempted by an algorithmic approach. The cases were reviewed by two trained cytopathologists who were blinded regarding the primary diagnosis and clinical history of the cases.

### Follow-up

The cases reviewed were correlated with follow-up histopathology, cystoscopy with or without histopathology, and subsequent urine cytology. The histopathology was the gold standard and considered positive in cases of papillary urothelial neoplasm of low-malignant potential, low-grade urothelial carcinoma (LGUC), and high-grade urothelial carcinoma (HGUC). The diagnosis of cystitis, hyperplasia, metaplasia, and atypia of undetermined significance were considered negative/benign. The presence of a growth or any mucosal abnormalities on cystoscopy except treatment induced changes bacillus calmette-guerin induced - cystitis (BCG-Cystitis) was contemplated as positive. Follow-up urine samples were considered positive in cases which were diagnosed as AUC-FN and UC. After an initial diagnosis on urine cytology was made, three to 15 consecutive urine samples were received in all cases within a minimum duration of 1 year.

### Statistical analysis

The overall sensitivity and specificity of urine cytology were calculated in cases with bladder carcinoma.

## RESULTS

### Clinical profile

A total of 56 cases were included in the study. Out of these, 38 cases (68%) were diagnosed as high-grade and 18 cases (32%) as low-grade bladder carcinomas. The age of these patients ranged between 35 and 79 years, with a mean age of 57 years. Most patients were males (89%) with a male-to-female ratio of 8.3:1. Majority of these patients presented with hematuria ( $n = 28$ ; 51%) followed by urgency and increased frequency of micturition ( $n = 15$ ; 27%), dysuria and abdominal pain ( $n = 07$ ; 12%), urinary retention ( $n = 03$ ; 05%), and incontinence ( $n = 03$ ; 05%).

Overall, 1280 urine samples were received during the study period, of which the atypical category accounted for an incidence of 8.75%. Of these cases, follow-up histopathology

was available in 30 cases, whereas subsequent urine cytology records and cystoscopic findings were obtainable in all the cases.

**Cytological evaluation**

The initial diagnosis of atypia in 56 cases of urine samples could be revised based on the algorithmic approach that was designed for the reclassification of the cases [Figure 1], as shown in Table 1.

Among 24 cases of the AUC-FN category, 12 cases retained the diagnosis of AUC-FN owing to less number of atypical cells (<5) with the presence of all the three definite diagnostic features including high N/C ratio, hyperchromasia, and irregular nuclear contours [Figure 2]. However, in 5 cases, the number of atypical cells was >5 and showed highly pleomorphic clusters, papillae as well as scattered single cells. Thus, on review these cases were upgraded to UC.

Out of 56 cases, a total of 20 cases were observed in the category of AUC-FN/UC with highly cellular smears, cell clusters and

papillae. High N/C ratio (>0.5) and hyperchromasia with coarse nuclear chromatin were observed in all 20 cases. India-ink-type nuclei and comet cells were observed in 6 cases. Background necrosis was seen in 8 cases. In three cases, the additional finding of the thickened nuclear membrane was also observed along with a high N/C ratio, hyperchromasia, and irregular nuclear contour.

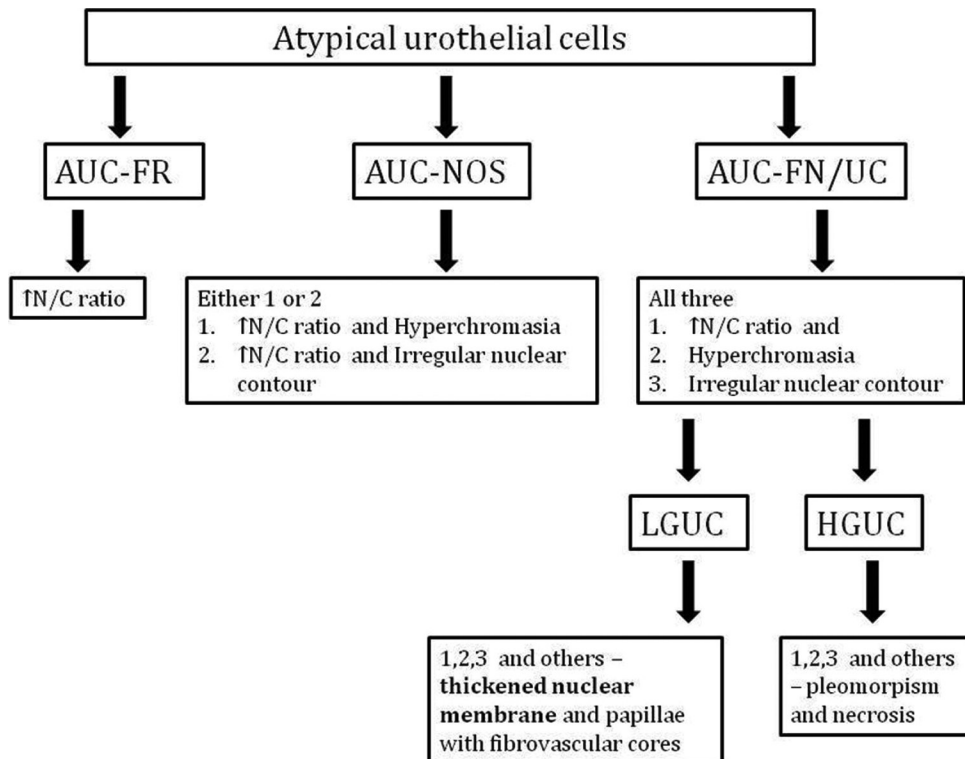
**Cystoscopy–histology–cytology follow-up**

Out of 30 available follow-up histopathological records, 19 cases (63%) were positive. Out of these 19 cases, 14 cases (85%) were of HGUC and 5 cases (15%) showed LGUC. The remaining 11 cases (37%) were NEM and showed metaplasia and inflammation. Follow-up cystoscopy revealed positive findings in 16 cases (29%) out of total 56 cases. Out of these 16 cases, 15 cases (94%) were positive on histology also. Follow-up urine cytology was positive in 7 cases (1.3%). Out of these 7 cases, 4 cases (57%) showed both positive histology and cystoscopy, whereas one case showed positive histology only [Table 2].

**Table 1: Revised diagnostic categories based on redefined morphological features**

Diagnostic category	Initial number of cases	Revised cytodiagnosis			
		AUC-FR	AUC-NOS	AUC-FN/UC	Negative
AUC-FR	9	4	1	-	4
AUC-NOS	23	7	3	3	10
AUC-FN/UC	24	-	3	(12+5) 17	4
Total (%)	56	11 (20)	7 (13)	20 (35)	18 (32)

AUC-FR: Atypical urothelial cells - favor reactive, AUC-NOS: Atypical urothelial cells - not otherwise specified, AUC-FN/UC: Atypical urothelial cells - favor neoplastic urothelial carcinoma



**Figure 1:** An algorithmic approach to classify atypical urothelial cells based on definite cytomorphological features

All cases revised as either AUC-FN or UC were correlated with follow-up cystoscopy, histopathology and/or consecutive urine cytology. A total of 20 cases (36%) were noted in this category with follow-up histopathology positive in 14 cases (70%) only. In 4 cases (22%), all three diagnostic methods (cystoscopy, histopathology, and urine cytology) were positive. In 7 cases (35%), both histopathology and cystoscopy showed positive findings, while in one case, histopathology and urine cytology were positive. In two cases, only follow-up urine cytology was positive. Thus, out of 20 cases, a total of 16 cases (80%) showed positive follow-up. Four cases (20%) that did not show any positive findings on follow-up were further investigated. In one of the cases, radiology showed a soft-tissue mass at the region of sacral promontory, which was diagnosed as sarcoma on biopsy.

Among 11 cases of the AUC-FR category [Figure 3], none of the cases showed positive findings on follow-up. In 18 cases revised as NEM, follow-up urine cytology was negative in all cases. However, one case revealed suspicious areas on cystoscopy and on histopathology, HGUC was detected.

**Table 2: Follow-up histopathology - cystoscopy - cytology correlation with the diagnostic categories**

Diagnostic category	Positive follow-up		
	Histopathology	Cystoscopy	Urine cytology
UC	3/5	2/5	3/5
AUC-FN	11/15	9/15	4/15
AUC-NOS	4/7	4/7	0/7
AUC-FR	0/11	0/11	0/11
Negative	1/18	1/18	0/18
Total	19/56	16/56	07/56

UC: Urothelial carcinoma, AUC-FN: Atypical urothelial cells - favor neoplastic, AUC-FR: Atypical urothelial cells - favor reactive, AUC-NOS: Atypical urothelial cells - not otherwise specified

In 7 cases of the AUC-NOS category [Figure 4], a total of 5 cases (71%) showed positive follow-up. Out of these 5 cases, three cases (38%) showed positive follow-up histopathology and cystoscopy. One case revealed positive histopathology only and another case showed positive cystoscopy only.

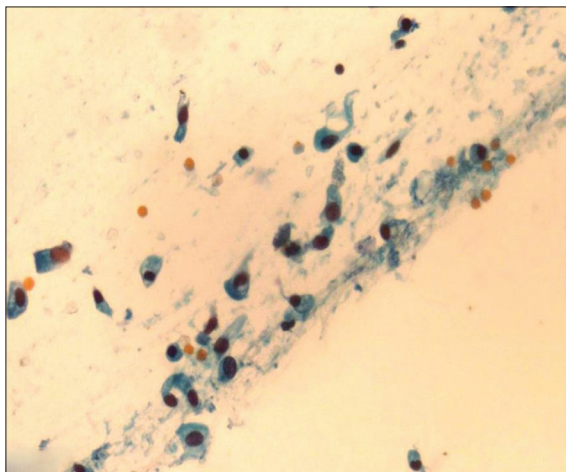
Thus, in this study, out of 56 cases diagnosed as atypical, 16 cases (29%) were true positive, and 28 cases (50%) were true negative. The sensitivity of urine cytology was 89.8%, whereas 87.5% was the specificity.

**DISCUSSION**

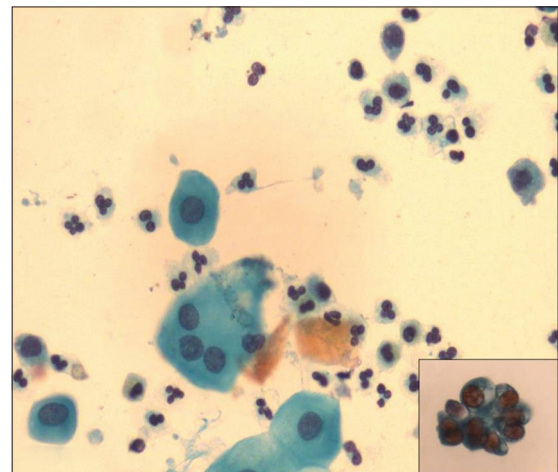
In 1864, Sanders first emphasized the significance of microscopic evaluation of urine specimens in detecting bladder carcinomas.<sup>[2]</sup> Later in 1945, the work of Papanicolaou and Marshall on urine sediments for diagnosing urinary tract malignancies was recognized greatly.<sup>[3]</sup> Since then, urine cytology has been widely accepted as a reliable, noninvasive technique for screening, diagnosing, and follow-up of bladder cancers. However, the accuracy depends largely on the grade of tumor, sampling technique, nature of urine specimens, and interpretation skills of the pathologist.

In 2004, the Papanicolaou Society of Cytopathology attempted to standardize the diagnostic categories and recommended to include the term “AUC” for AUCs followed by a descriptive comment. In the present study, the incidence of AUC was 8.75% which was similar to the findings of Muus Ubago *et al.*, who reported an incidence of 8.1%.<sup>[4]</sup> However, a study from Saudi Arabia described a higher rate (16%) of atypical urine diagnosis in voided specimens.<sup>[1]</sup>

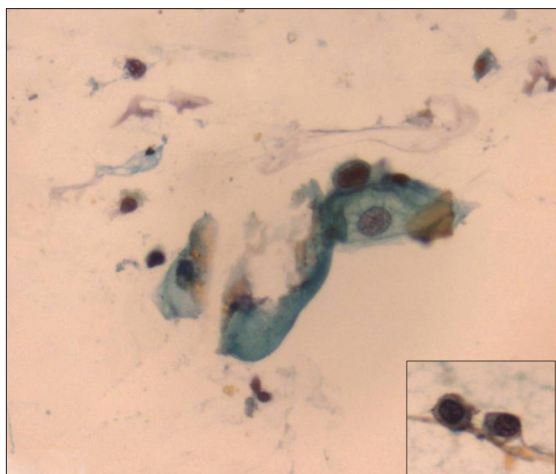
In our study, the mean age of presentation was 57 years, with a higher predilection in males. However, in a study by Nabi *et al.* and Ton Nu *et al.*, the mean age of presentation was 61.7 years and 74 years, respectively.<sup>[5,6]</sup> Most patients presented with



**Figure 2:** A case of atypical urothelial cells-favour neoplastic showing urothelial cells with high N/C ratio, hyperchromasia, and irregular nuclear membranes in a necrotic background along with few comet cells and cells with india-ink nuclei (x400, Papanicolaou stain)



**Figure 3:** A case of atypical urothelial cells-favor reactive showing urothelial cells with high N/C ratio and clusters of atypical cells with cytoplasmic vacuolations (inset) in an inflammatory background (x400, PAP)



**Figure 4:** A case of atypical urothelial cells-not otherwise specified showing few benign urothelial cells and few cells with high N/C ratio and hyperchromasia (inset) ( $\times 400$ , PAP)

hematuria and the vast majority of them were previously diagnosed as HGUC on histopathology (70%).

Cytology smears that are diffusely cellular and show cell clusters and necrosis are primarily indicative of malignancy.<sup>[7]</sup> High-grade malignancies usually display the following features: More than 5 AUCs with a high N/C ratio ( $>0.7$ ), moderate-to-severe hyperchromasia, irregular nuclear membrane, coarse chromatin, cell clusters, India-ink type nuclei, and comet cells. On the contrary, low-grade tumors show subtle changes with variable cellularity from few cells to large papillae, minimal nuclear enlargement, dense cytoplasm, elongated nuclei, and thickened nuclear membrane.<sup>[8]</sup> In this study, three frequently studied parameters, including high N/C ratio, hyperchromasia, and irregular nuclear margins, were picked up to categorize atypical urine specimens. When all the three diagnostic features were present but the number of atypical cells was less than 5, cases were classified as AUC-FN and when the number of atypical cells exceeded 5, these were labeled as UC. Based on these criteria, maximum cellularity was observed in the category of AUC-FN/UC, whereas variable cellularity was noted in reactive/negative cases. Cell clusters, papillae, hyperchromasia, coarse chromatin, India-ink nuclei, and comet cells were noted exclusively in malignant cases. In this present study, apart from a high N/C ratio, hyperchromasia and irregular nuclear contour, thickened nuclear membrane was an additional finding observed in low-grade malignancies. These findings were in concordance with the findings of other authors.<sup>[5-10]</sup>

It was observed that 28 cases were over-diagnosed due to the presence of decoy cells in the majority of the cases, leading to misinterpretation. Similarly, underdiagnosis was noted in 9 cases because of various reasons such as low cellularity, lack of definite criteria, and interobserver variability.

In the past, many large series have shown that urine cytology has a low sensitivity rate ranging from 42% to 66% and a high

specificity rate not exceeding 97% in routine practice.<sup>[5,11-16]</sup> In the present study, the rate of sensitivity and specificity of the atypical category at our institute was 89.8% and 87.5%, respectively. Of all 56 cases, 22 cases (39%) showed a positive follow-up, of which 19 cases (34%) were positive on follow-up biopsy. However, in a study by Deshpande *et al.*, follow-up biopsy was positive in only 23.4% of the specimens.<sup>[7]</sup> In the present study, most cases showed high-grade malignancy on follow-up biopsy (55%). This finding was similar to the findings of Mokhtar *et al.* and Kapur *et al.*<sup>[1,17]</sup>

Out of 20 positive cases from the AUC-FN/UC category, follow-up biopsy showed high-grade malignancy in 13 cases (65%) and low-grade malignancy in 3 cases (15%). In 4 cases (20%), no positive follow-up was recorded. This discrepancy can be attributed to the presence of flat lesions in the bladder, which were not detected on cystoscopy and so biopsy was not attempted, or it could be due to the presence of lesions higher up in the urinary tract system or any soft-tissue pelvic mass directly invading the urinary tract. In one of the cases, further investigations revealed a soft-tissue mass lesion at the level of sacral promontory diagnosed as sarcoma on histopathological examination. In three other cases, no lesion could be detected, and the presumption of flat lesions was favored.

None of the cases falling in the category of AUC-FR turned out to be positive in our study. However, one case (11%) diagnosed as NEM on cytology came out positive on follow-up biopsy. Thus, the malignancy rate was 11% in the AUC-FR/NEM category. This finding was comparatively low compared to a study by Brimo *et al.*, who described malignancy rate of 29% among reactive cases.<sup>[18]</sup> The reason for this discrepancy could be due to the presence of flat lesions in the bladder wherein neoplastic cells did not shed in urine and were detected on biopsy only.

The category of AUC-NOS showed a positive follow-up in 71% of the cases. Moreover, cell clusters were frequent in this category with moderate cellularity but lacking all three definite diagnostic features. Therefore, the category of AUC-NOS lies in the gray-zone area, and such cases need a close follow-up.

## CONCLUSIONS

Although urine cytology has its limitations, it can be useful if interpretation errors are minimized by evaluating definitive cytological features. The terminology 'atypia' should be used with caution and all possible causes leading to reactive cellular changes must be ruled out before labeling any urine specimen as atypical. Furthermore, all cases including equivocal (AUC-NOS) and positive (AUC-FN/UC) category must be thoroughly investigated. Moreover, urine cytology in conjunction with cystoscopy can increase the detection rate of malignancy with greater accuracy. Therefore, it becomes crucial for the clinician to understand the diagnostic terminology and its implications to make appropriate decisions in the management of bladder carcinomas.

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## Conflicts of interest

There are no conflicts of interest.

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