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ORIGINAL PAPER

# Atypical urothelial cells investigation with automated urinalysis and expert review of microscopic sediment as early parameter for suspected bladder cancer patients

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**Summary** *Introduction and objectives: Automated urinalysis offers rapid and efficient evaluation of urine sediment; however, most systems do not routinely report epithelial cell atypia. This study aimed to evaluate the clinical relevance of the atypical cell (Atyp.C) parameter generated by the Sysmex UF-4000 analyzer as an early indicator of suspected urothelial carcinoma.*

*Materials and methods: A prospective study was conducted using the UF-4000 analyzer to detect Atyp.C in urine specimens from high-risk patients. Atyp.C results were collected simultaneously for all samples. Specimens with positive Atyp.C findings underwent urinary tract cytology, which was independently reviewed by board-certified cytopathologists and categorized into four diagnostic groups. Statistical analyses were performed using IBM SPSS version 21.*

*Results: Among 332 specimens, 20 samples (6.02%) showed Atyp.C values > 0.0/μL. The mean Atyp.C value was 0.6/μL (95% CI 0.064-1.135) in males and 1.20/μL (95% CI 0.608-1.791) in females, with no significant difference between sexes ($p = 0.2549$). Of the 20 Atyp.C-positive samples, 5 (25%) demonstrated abnormal cytology, including 2 cases of atypical urothelial cells, 1 case suspicious for high-grade malignancy, and 2 cases of confirmed high-grade urothelial carcinoma. Atyp.C values were significantly higher in abnormal compared with benign cytology ($p < 0.01$).*

*Conclusions: The Atyp.C parameter may have potential as an adjunctive screening marker for urothelial abnormalities; however, further validation studies are required.*

KEY WORDS: *Atypical cell; Urinalysis; Urine cytology; Urothelial carcinoma; UF-4000.*

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## INTRODUCTION

The evolution of urinalysis technology is a fascinating development (1). While manual microscopy in urine sediment analysis remains highly regarded as the standard, the debate between automation and manual examination appears to have shifted decisively in favor of machines. Automated systems offer speed, cost-effectiveness, and efficiency. Interestingly, some aspects of machine work, particularly those carried out by automated urinalysis instruments, can be described as artificial intelligence. These instruments' software systems are programmed to identify specific cell types in urine, akin to the cognitive process of the human brain. Some utilize optic lenses for visual detection, while others employ laser beams and voltage sensors to simulate vision. The visual data is then analyzed by software systems, which label the cells and generate reports. Notably, the latest generation of devices even provide clinical diagnosis estimations in their reports.

Bladder cancer ranks as the eighth most common cancer worldwide, with incidence of 9.6/100.000 among men and 2.4/100.000 among women (2). The bladder, acting as a reservoir for waste products, accumulates harmful chemicals filtered from the bloodstream by the kidneys. External factors such as smoking and prolonged exposure to aromatic amines are known to contribute to the neoplastic progression of urinary epithelium. Microscopic examination of urine samples is a common method for diagnosing various conditions affecting the urinary tract. By observing fresh urinary sediments, diverse cellular types associated with various pathologies, including carcinomas, can be identified (3).

Automated instruments are designed to report several parameters such as red blood cells, white blood cells, and epithelial cells as part of routine laboratory analysis. Although some instruments differentiate epithelial cells based on their origin, the reporting of "*atypia*" in these cells is not a common parameter.

Sysmex UN-Series automated urinalysis instruments present a challenge in diagnosing urinary tract neoplasms. "*Atypical cells*" serve as a research parameter, reported by the instrument but not yet validated or included in patient reports. This study aims to contribute to the ongoing efforts to evolve this research parameter, with the hope of enhancing patient care.

## METHODS

### *Study design and patients*

This prospective observational study was conducted at

*Saiful Anwar General Hospital, Malang, Indonesia, between August 9 and October 7, 2023*. Urine specimens were collected from patients considered at high risk for bladder cancer, defined as age over 50 years, history of active or passive smoking, occupational exposure, or positive family history of malignancy. Passive smoking exposure was included as part of the high-risk criteria, which contributed to the high prevalence of smoking-related history in the study population. Patients with a prior diagnosis of bladder cancer were excluded.

### *Reference standard and study design*

This study was designed as an exploratory pilot study to assess the potential clinical relevance of the Atyp.C parameter generated by the UF-4000 analyzer. Due to the study design, only specimens with positive Atyp.C findings underwent further evaluation with urinary tract cytology and, when indicated, cystoscopy with biopsy. Therefore, a full diagnostic accuracy assessment (including sensitivity, specificity, and predictive values) could not be performed. Instead, the study focused on evaluating the association between Atyp.C positivity and cytological abnormalities.

### *Urine sample collection and processing*

A total of 332 urine specimens were collected in sterile containers, including voided urine, catheterized urine, and bladder washing samples. Specimens were processed within 2 hours after collection. Each sample was divided into two aliquots: one for automated urinalysis and one for urinary tract cytology.

### *Automated urinalysis and atypical cell assessment*

Automated urinalysis was performed using the Sysmex UF-4000 analyzer (*Sysmex Corporation, Kobe, Japan*). The Atyp.C parameter detects cells with increased nucleic acid content based on fluorescence intensity. Cells with fluorescence intensity ≥ 0.1/μL were classified as atypical, as previously described. All specimens were analyzed under standardized conditions without staining or centrifugation.

### *Definition of Atyp.C positivity*

Atyp.C positivity was defined as a fluorescence intensity of ≥ 0.1/μL, based on the manufacturer's research parameter and previous studies. This threshold was used as an exploratory cut-off due to the absence of standardized clinical criteria. The diagnostic criteria and cytological classification used in this study are summarized in Table 1.

***Table 1.***
*Diagnostic criteria used in this study.*

| Parameter | Definition |
|---|---|
| Parameter | Definition |
| Atyp.C positivity | Fluorescence intensity ≥ 0.1/μL |
| Benign cytology | No atypical or malignant urothelial features |
| Atypical urothelial cells | Increased N:C ratio with mild atypia |
| SHGUC | Suspicious for high-grade urothelial carcinoma |
| HGUC | High-grade urothelial carcinoma |

### *Urinary tract cytology and histopathology*

Urinary tract cytology slides from specimens with positive Atyp.C findings were prepared using the ThinPrep T3000 processor (*Hologic, Marlborough, MA, USA*) and stained with Papanicolaou stain. Cytological evaluation was performed by board-certified cytopathologists with more than 15 years of experience and classified into four categories: benign, atypical urothelial cells, suspicious for high-grade urothelial carcinoma, and high-grade urothelial carcinoma, in accordance with established cytological criteria. Patients with abnormal cytology findings underwent cystoscopic examination and biopsy. Morphological features considered suggestive of atypical or malignant urothelial cells included increased nucleus-to-cytoplasm ratio, irregular nuclear borders, hyperchromasia, irregular chromatin pattern, and cellular pleomorphism. Histopathological diagnosis served as the reference standard for confirmation of urothelial carcinoma. In cases of diagnostic uncertainty, consensus evaluation between cytopathologists was performed.

### *Statistical analysis*

Statistical analyses were performed using IBM SPSS Statistics version 21. Continuous variables were expressed as mean values with 95% confidence intervals.

Comparisons between groups were performed using the Wilcoxon test. A p-value < 0.05 was considered statistically significant. A formal sample size calculation was not performed because this exploratory study included all eligible patients during the study period.

## RESULTS

The UF-4000 analyzer generated valid results for all 332 urine specimens included in this study. The demographic and clinical characteristics of the study population are summarized in Table 2. Among the 332 patients, 20 specimens (6.02%) demonstrated detectable Atyp.C values (> 0.0/μL) on automated urinalysis. There was no statistically signifi-

***Table 2.***
*Patient characteristics.*

| | No. |
|---|---|
| Total patients | 332 |
| Gender | |
| Male | 57 (17.2%) |
| Female | 275 (82.8%) |
| Mean Age (Range), years | |
| Male | 55 (50-71) |
| Female | 58 (50-73) |
| Patients with Hypertension | 157 (47.3%) |
| Patients with DM | 81 (24.4%) |
| Overweight | 88 (26.5%) |
| Obese | 57 (17.2%) |
| History of smoking (active & passive) | 298 (89.8%) |
| Positive microscopic hematuria | 52 (15.7%) |
| Positive leukocyturia | 98 (29.5%) |

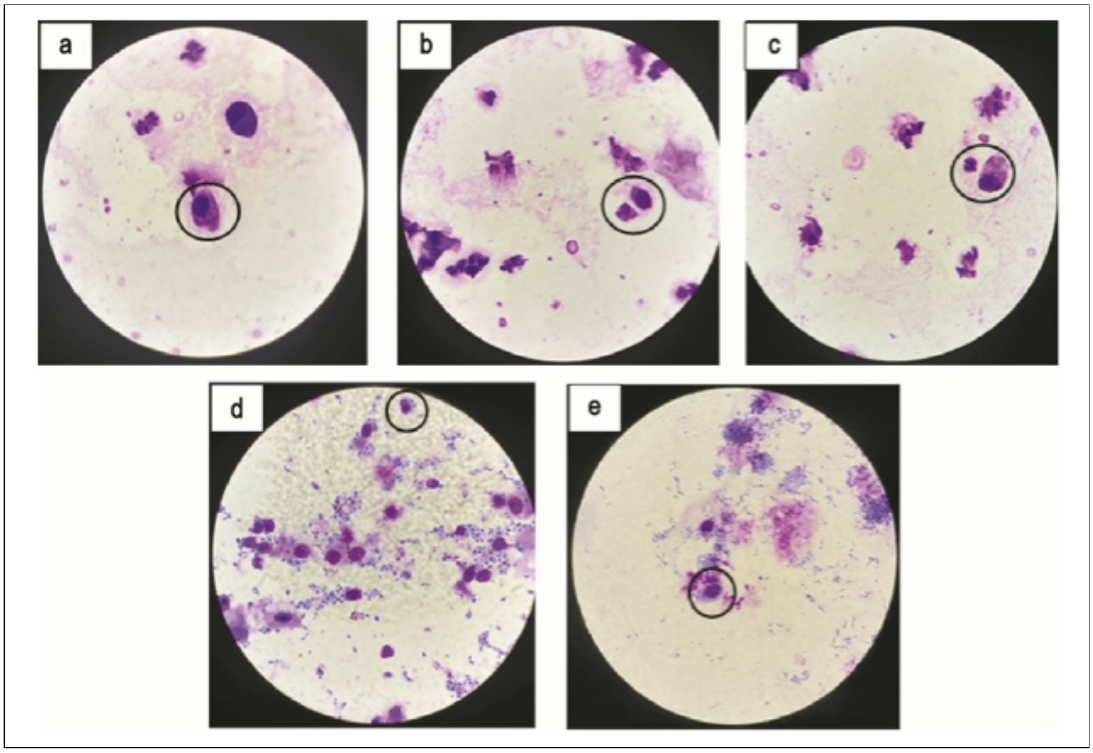



***Figure 1.***
*(a-e) Manual microscopic evaluation showed 5 positive AtypC from 20 positive AtypC samples by UF-4000 analyzer.*

cant difference in Atyp.C values between male and female patients (p = 0.2549). Manual microscopic evaluation of these 20 Atyp.C-positive specimens confirmed the presence of atypical cells in five samples, while the remaining specimens were cytologically benign. Representative microscopic findings are illustrated in Figure 1.

### *Follow-up cytopathology diagnosis associated with the UF- 4000*

Follow-up urinary cytology revealed abnormal cytological findings in 5 of the 20 Atyp.C-positive specimens (25%), including atypical urothelial cells, *suspicious high-grade urothelial carcinoma* (SHGUC), and *high-grade urothelial carcinoma* (HGUC). Detailed cytological classification and associated Atyp.C intensities are presented in Table 3, and representative cytological images are shown in Figure 2.

***Table 3.***
*Comparison of Atyp.C intensity between benign and abnormal cytology groups.*

| Cytology grade | N | Atyp.C mean (95% CI) | p |
|---|---|---|---|
| Benign | 15 | 0.65 (0.46-0.85) | < 0.01 |
| Abnormal* | 5 | 2.36 (0.67-4.04) | |

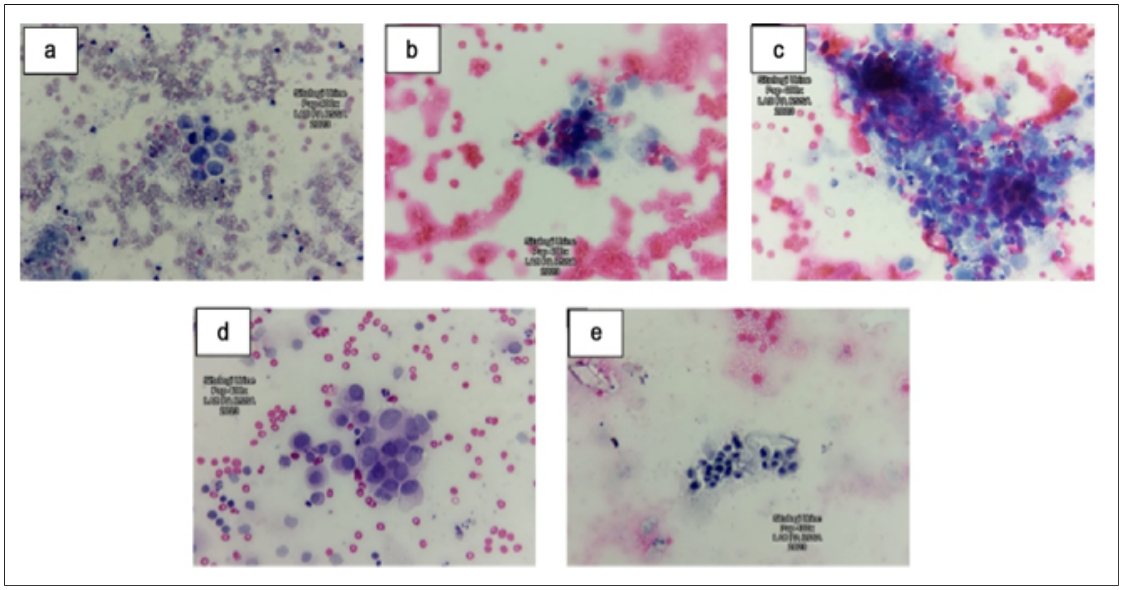



***Figure 2.***
*(a, b) Atypical cells showed increase of nucleus-to-cytoplasmic ratio and hyperchromatic nuclei. (c) Suspicious for high-grade urothelial carcinoma, the cells have hyperchromatic nuclei, irregular nuclear membranes, and high nucleus-to-cytoplasmic ratio. (d, e) High-grade urothelial carcinoma, cluster of cells with high nucleus-to-cytoplasmic ratio, hyperchromatic nuclei, irregular nuclear membranes, and coarse chromatin.*

Comparison between abnormal and benign cytology groups demonstrated a significant difference in mean Atyp.C intensity ($p < 0.01$), as summarized in Table 4. The distribution of Atyp.C intensity across cytological groups is further visualized in Figure 4, showing higher Atyp.C levels predominantly in specimens with abnormal cytology, particularly in the HGUC subgroup.

A schematic overview of specimen processing and diagnostic outcomes is presented in Figure 3. Cystoscopic biopsy was performed in patients with abnormal cytology. Histopathological examination confirmed high-grade urothelial carcinoma in one specimen.

***Table 4.***
*Cytological diagnosis in patients associated with UF-4000 Atyp.C result.*

| Cytology grade | N | Atyp.C mean (intensity range) |
|---|---|---|
| HGUC | 2 | 3.8 (0-4.1) |
| SHGUC | 1 | 1.7 (0-1.70) |
| Atypical | 2 | 1.25 (0-1.5) |
| Benign | 15 | 0.65 (0-1.2) |

***Figure 3.***
*Study flow diagram showing specimen selection, cytological classification, and histopathological confirmation.*

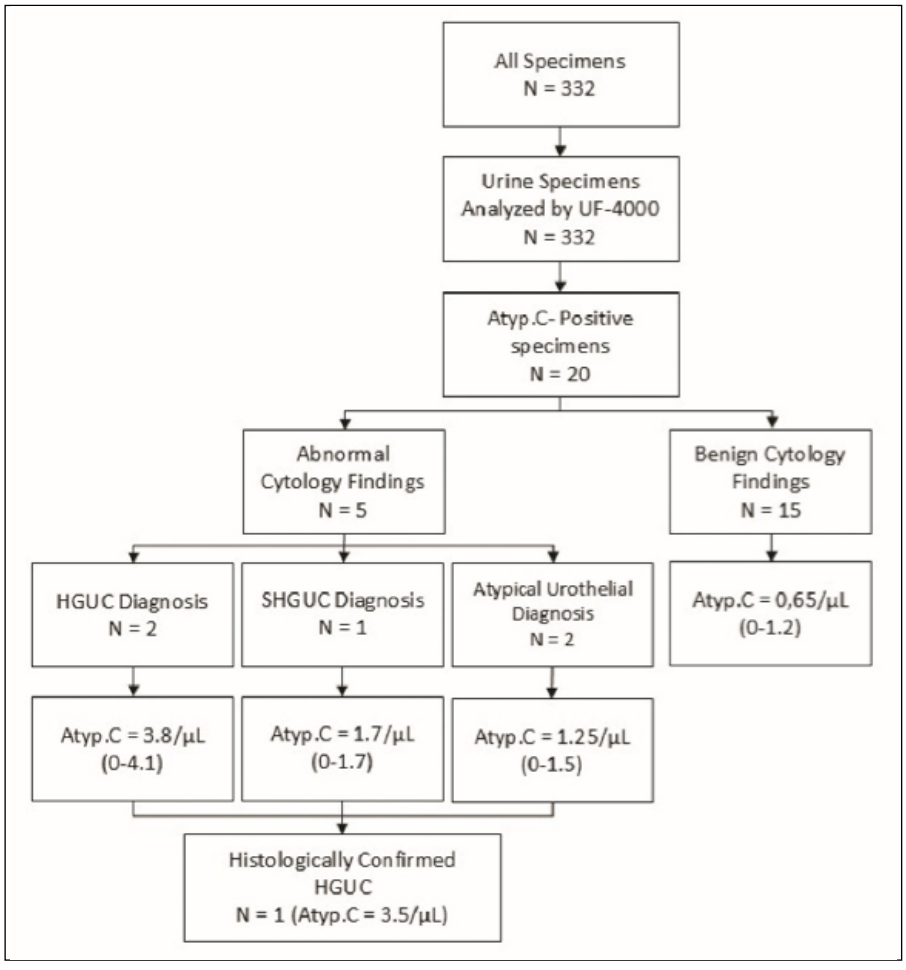
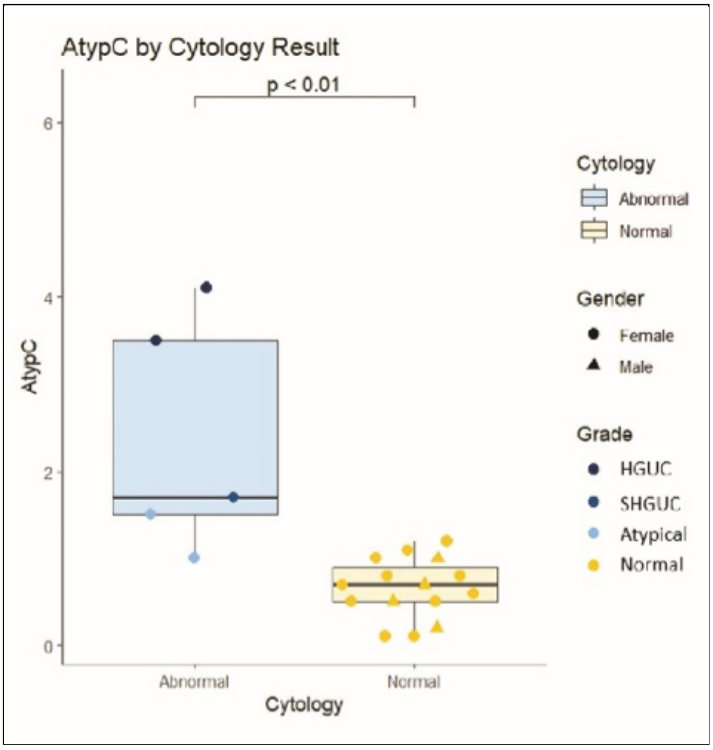



***Figure 4.***
*Dot plot showing Atyp.C intensity in abnormal and normal groups identified by cytology.*




## Discussion

Automation in laboratory evolves at an enormous scale. The laboratories host automated urine analyzers with an exuberant welcome. Apparently, human medical care service in laboratory is time-consuming, requires well trained personnel and is subject to subjectivity and intra-observer variability. Automation in urinalysis has been relatively late in development and manufacturers face extra problems compared to other systems. Urine sediment analysis requires identification and decision steps which can very well be classified as artificial intelligence (4).

Sysmex UN-Series (*Sysmex Corporation, Kobe, Japan*) automated urine analyzer has recently introduced the "*atypical cells*" parameter. Sysmex UN is the single instrument studying this parameter. Atypical cells show side fluorescence and scattered light properties indicating their enlarged nuclei and increased nucleus/cytoplasm ratio (5). Atypical cells are supposed to be epithelial cells with features of suspicion for a neoplastic proliferation, most likely urothelial carcinoma of the urinary tract (6). A detailed microscopic examination of urine sediment is conducted to detect atypical bladder can-

cer cells (7). Several previous studies from Japan, Italy and Turkey have compared the usefulness of the Atypical Cell parameter with that of cytological and histological findings (4, 7, 8). In 2015 *Anderlini et al.* presented an inspiring study in which expert pathologists reviewed digital images of specimens from an automated urine analyzer (8). The analyzer used a very similar flow cytometry technology to Sysmex UN but lacked the atypical cells parameter so that the experts had to review a bulk of "*unclassified*" images. In a 5-year period, they reviewed 1,635, 287 samples and reported atypical cells in 162 patients: representing an incidence of 0.01/100 samples.

In our series with UF-4000, 332 patients at high risk (for family history, tobacco smoking, occupational hazards, chronic inflammation, infection, and previous bladder carcinoma diagnosis) underwent baseline ultrasonography before urine sampling, and no documented bladder mass was previously identified at the initial evaluation.

These cases gave valuable information about features that were possibly deceptive for the instrument in this parameter.

We found 20 samples with positive atypical cell parameter with 5 confirmed positive urine cytology during a 3-consecutive day study period. Two specimens were diagnosed as atypical urothelial cells, one specimen was classified as suspicious for high grade malignancy, and two specimens were confirmed as high-grade malignancy, while the rest 15 were diagnosed as benign.

The difference of the Atyp.C intensity between normal and abnormal group was statistically significant ($p < 0.01$).

From bladder biopsy one specimen was further confirmed as a high-grade urothelial carcinoma, representing an incidence of atypical cell positivity of 20/332 (6%) and an incidence of bladder cancer in high-risk patients of 3/332. The cut off value to define patients to be included in the study was empirical with a fluorescence intensity value of ≥ 0.1/nL used to classify the specimen as atypical. Otherwise, *Shukuya et al.* mentioned a cut off 0.4/nL which obtained a sensitivity of 79.5% and a specificity of 85.3% with a AUC of 0.87 (7). However, until now there has not been a standardized positive cut-off value used to classify atypical cells by Sysmex UN.

Using follow-up urinary cytology and histopathological findings as confirmatory evaluations, we observed that the UF-4000 was capable of detecting abnormal cells in unstained and uncentrifuged urine specimens, with an agreement of 25.0% (5/20) compared to urine cytology. The practical advantage of the UF-4000 system is its ability to analyze fresh, unstained, and uncentrifuged urine specimens in a rapid and automated manner. The Atyp.C result was higher in *high-grade urothelial carcinoma* (HGUC) compared to *suspicious high-grade urothelial carcinoma* (SHGUC) and benign groups, with a range of 0-4.1 *vs* 0-1.7 *vs* 0-1.5. This suggests that higher Atyp.C intensity may serve as a valuable indicator for cytopathological diagnosis of HGUC. Histopathological confirmation of HGUC in one specimen by cystoscopic biopsy further supported the ability of Atyp.C in detecting abnormal urinary cytopathology findings.

The nuclear to cytoplasmic (N:C) ratio above 0.5 together with one of other three minor criteria (irregular nuclear membranes, hyperchromasia, and irregular clumpy chromatin) define an atypical cell. *Barkan et al.* described that atypical cells have a lower risk of urothelial carcinoma than SHGUC (8%-35% vs 50%-90% risk of malignancy) (9). The definition of Suspicious High-Grade Urothelial Carcinoma requires an N:C ratio of 0.5 with moderate to severe hyperchromasia, and one of the minor criteria like marked irregular nuclear membranes and irregular clumpy chromatin (10). A cytologic diagnosis of HGUC requires a minimum of 5 to 10 cells meeting all major criteria: N:C ratio greater than 0.7, moderate to severe hyperchromasia, marked irregular nuclear membranes, and coarse chromatin. Additional features, such as nuclear pleomorphism, eccentric nuclei, mitotic figures, apoptotic bodies, prominent nucleoli, and enlarged nuclei, are commonly observed but are not required for diagnosis (11).

In this study the average Atyp.C intensity value for males was 0.6/µL (95% CI 0.064-1.135/µL) and 1.20 (95% CI 0.608-1.791) for females. The difference in percentages between genders was statistically not significant ($p < 0.2549$). Although no statistically significant difference was observed between males and females, subgroup analysis was limited by the small sample size. This shows further research with larger populations are required to explore potential differences across sex and age groups.

A major limitation of this study is that only Atyp.C-positive samples underwent further cytological and histopathological evaluation. As a result, the study does not allow calcu-

## Declarations

***Ethical approval and consent for participate:*** *This prospective study was conducted in accordance with institutional policies and the ethical principles of the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Dr. Saiful Anwar General Hospital, Malang, Indonesia (Ref No: 400/026/CR/102.7/2023).*

***Availability of data and material:*** *The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.*

***Competing interests:*** *The authors declare that they have no competing interests.*

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***Authors' contributions:*** *DEP contributed to study conception and design, data analysis, statistical analysis, and manuscript drafting; KPS contributed to study conception and design, study supervision, and critical revision of the manuscript; BD contributed to data collection, study supervision, and critical revision of the manuscript; FKD contributed to data collection and manuscript drafting; HS contributed to cytopathological analysis and critical revision of the manuscript; KWA contributed to cytopathological analysis and critical revision of the manuscript. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.*

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lation of diagnostic accuracy parameters such as sensitivity, specificity, or predictive values. This limits the ability to establish Atyp.C as a standalone diagnostic tool. Therefore, the present study should be interpreted as a pilot study aimed at exploring the potential clinical utility of Atyp.C rather than providing definitive diagnostic validation. Furthermore, the absence of clear guidelines for urine cytology sample collection or how specimens should be preselected or pre-processed for the Atyp.C assay to ensure its clinical value may introduce variability and uncertainty in the data interpretation, thereby restricting the study's ability to draw definitive conclusions.

## Conclusions

Regular urine tests are conducted in both symptomatic and asymptomatic individuals, so it's crucial to pay more attention to the presence of Atyp.C, regardless of any symptoms related to urothelial carcinoma. Our research indicates that the Atyp.C parameter may serve as a promising adjunctive screening marker; however, further large-scale studies with complete diagnostic verification are required before clinical implementation. This is particularly advantageous for identifying high-risk patients who may require more frequent monitoring or additional medical intervention.

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