Investigation of atypical cell parameter in the surveillance of patients with NMIBC; Initial outcomes of a single center prospective study

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Research Article

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Abstract

Objective: To investigate how well the Sysmex automated urine-analyzer's atypical-cell parameter can predict oncological outcomes when compared to cytology and pathology data in the follow-up of NMIBC patients.

Material and Methods: We prospectively collected clinical data from 273 patients who underwent cystoscopic examination due to benign and malign reasons in our center between June 2020 and March 2021. Patients were divided into 2 groups.(Group-1:Patients with no previous diagnosis of bladder cancer(BC),Group-2:Patients with previously diagnosed NMIBC).The atypical-cell parameter was determined by studying the urine sample given by the patient for urinalysis. The atypical-cell parameter's sensitivity, specificity, negative predictive value(NPV), and positive predictive value(PPV) were assessed.

Results: A total of 76(41.1%)patients underwent diagnostic procedures(Group-1) and remaining 109(58.9%)patients were NMIBC patients(Group-2) who subjected to control cystoscopy on follow-up. BC was detected in 70 patients, 28 of whom were newly diagnosed(Group-1). Remaining 42 patients had recurrence during their follow-up(Group-2).Atypical-cell values of 70 patients with BC were determined to be statistically significantly higher than those without malignancy.In Group-2, median atypical-cell values for those with no malignancy, those with low-grade BC reccurrence, and those with high-grade BC recurrence were 0.00(IQR:0.00-0.80), 0.25(IQR:0.10-1.10)and 1.20(IQR:0.70-2.15), respectively($p<0.001$). For a cut-off of 0.1 atypical cells/µL,sensitivity and specificity were measured as 83.33% and 53.73%,respectively(AUC:0.727; p-value<0.0001).

Conclusions: Atypical-cell parameter of the Sysmex-UF-5000 automated urine-analyzer is a newly introduced research parameter.The results of this study are promising.Based on our results, we presume that the atypical-cell parameter may be used in surveillance of the NMIBC patients.Multi-center studies with larger patient populations are required to prove its efficacy.

Introduction

Bladder cancer (BC) is one of the most commonly diagnosed cancer.[1] In 2018, over 550,000 new cases diagnosed worldwide.[2] Urothelial carcinoma (UC) is the most common histological type of BC (approximately 90%). Seventy five percent of patients with BC present with non-muscle invasive bladder cancer (NMIBC), while the rest presenting as muscle invasive BC (MIBC). The EAU Guidelines recommend the stratification of NMIBC patients into risk groups based on their probability of progression to MIBC to be able to facilitate the treatment recommendations.[1]

Patients with BC classically present with hematuria (macroscopic or microscopic), although lower urinary tract symptoms (dysuria, urgency, frequency) can be the initial symptoms. The diagnosis can be delayed due to the similarity of these symptoms to those of benign conditions (urinary tract infection, benign prostatic obstruction, prostatitis, ureteral or renal stone disease), and delays can lead to a worsened prognosis.[3] Hematuria is typically gross, intermittant and painless.

There is currently no screening program for bladder cancer. The diagnosis of BC is based on cystoscopic examination. However, patient history, physical examination, imaging and urinary cytology are helpful tools

for diagnosis.[1] Transurethral resection of the bladder tumor (TUR-BT) or cold-cup biopsy is mandatory to evaluate the tissue histologically. The objective of TUR-BT in NMIBC is to diagnose correctly and remove all lesions completely.[4]

Urinary cytology is useful, particularly is an adjunctive tool to the cystoscopy in patients with BC. Since, it has high sensitivity in high grade tumors (84%), but low sensitivity in low grade tumors (16%).[5] The sensitivity in detection of bladder carcinoma-in situ, which is also a high grade lesion, is 28–100%.[6]

Since urine cytology has low sensitivity, many urinary markers have been developed.[7] However, none of these markers have been approved for diagnosis or follow-up of patients with NMIBC. There is no guideline recommending the use of these markers in routine practice. Much research continues in this field which shows the necessity.

Sysmex UF-5000 (Sysmex Corporation, Kobe, Japan) fully automated urine analyzer introduced the "atypical cell" (Atyp.C,) parameter which uses the Fluorescence Flow Cytometry" (FFC) technology to differentiate between atypical and non-atypical cells, based on differences in the fluorescent staining of nucleic acids in urothelial cells. Based on the scattergram, Atyp.C parameter is able to report the number of atypical/potential malignant urothelial cells with abnormally high levels of nucleic acid contents.[8] This study aims to investigate to what extent the atypical cell parameter of the Sysmex automated urine analyzer can predict oncological results compared with cytopathology in the follow-up of NMIBC patients.

Material And Methods

Following the approval of institutional review board committee, we have prospectively collected clinical data from 273 patients who underwent cystoscopic examination due to benign and malign reasons in our center between June 2020 and March 2021. Patient characteristics, atypical cell parameters, pathological reports were recorded prospectively during the follow-up period. Seventy-one and 14 patients were excluded from the study due to cytopathological specimens were not obtained and they had concurrent urinary tract infections, respectively. Patients with suspicious cytology or pathology results for malignancy were excluded from the study. The upper urinary tract was not evaluated in the study. After excluding the patients with an upper urinary tract urothelial cancer history, 185 patients were enrolled the study. Patients were divided into 2 groups. (Group-1: Patients with no previous diagnosis of bladder cancer, Group-2: Patients with previously diagnosed NMIBC)

All patients underwent cystoscopic examination of the bladder and gave a urine sample to a sterile urine container a day before the cystoscopy procedure as recommended by the current EAU guidelines. Urine samples were transmitted to the laboratory and evaluated by the UF-5000 (Sysmex Corporation, Kobe, Japan) fully automated urine analyzer.

Biopsy or transurethral resection performed to patients with macroscopic mass lesion during cystoscopy, and hystopathological examination was performed. In the absence of macroscopic lesion, urine samples were taken and cytological examination was performed.

UF-5000 is a urine sediment analyzer developed by Sysmex Corporation (Kobe, Japan). It detects particles, cellular structures and bacteria in urine using “Fluorescence Flow Cytometry” (FFC) technology. In this technology, nucleic acids, RNA or DNA of the particles are marked with fluorescent markers and passed in front of a blue (480 nm) laser light. The "size", "internal granulity" and "nucleic acid content" information of the cells passing in front of the laser light are transferred to the "scattergram" distribution graphics and transmitted to the user as a numerical result. Atypical cells show side fluorescence and scattered light properties indicating their enlarged nuclei and increased nucleus/cytoplasm ratio. With the FFC technology it uses, the UF-5000 detects atypical cells in the urine with high sensitivity with low Limit of Blank, Limit of Detection, and Limit of Quantitation values.

The atypical cell parameter was determined by studying the urine sample given by the patient for urinalysis. In addition, this procedure did not have any additional costs to the patient or the hospital.

The primary endpoint was to investigate to what extent the atypical cell parameter can predict oncological results compared with cytopathology in the follow-up of NMIBC patients. For this purpose, we aimed to evaluate the sensitivity, specifity, negative predictive value (NPV) and positive predictive value (PPV) of the atypical cell parameter.

Statistical Analysis

Statistical analysis was performed with SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). Chi-Square test and Fisher’s exact tests were used to compare nominal variables between independent groups. Ordinal and non-normally distributed continuous variables are expressed as the median with interquartile ranges (IQR).

Different atypical cell values were determined as cut-off values in patients who were previously diagnosed with NMIBC and underwent cystoscopy for follow-up. Sensitivity, specificity, NPV and PPV for these different values were evaluated with the ROC curve and p value less than 0.05 considered significant.

The significance of the difference in the distribution of atypical cell values between the groups was evaluated with the Independent samples-Kruskal Wallis Test. P value less than 0.05 considered significant.

Results

A total of 76 (41.1%) patients underwent diagnostic cystoscopic examniation due to the various conditions (Group-1; Patients with no previous diagnosis of bladder cancer group) such as hematuria (bladder cancer suspected), lower urinary tract symptoms, recurrent urinary tract infections, chronic pelvic pain, and remaining 109 (58.9%) patients were the patients with NMIBC (Group-2; Patients with previously diagnosed NMIBC) who subjected to control cystoscopic examination on follow-up. Patient characteristics for gender, age, smoking status and BMI (body mass index) were well blanced and no significant differences in these variables were observed among groups (Table-1).

Bladder cancer was detected in 70 patients, 28 of whom were newly diagnosed (Table-2, Group-1). Remaining 42 patients had recurrency during their follow up (Table-2, Group-2). No malignancy was detected in the

cytopathological results of the remaining 115 patients. Atypical cell counts of 70 patients with bladder cancer were detected to be statistically significantly higher than those without malignancy. Median Atypical cells values among all patients were 0.7(IQR:0.00-1.45), 0.8(IQR:0.10–1.10) and 1.4(IQR:0.70–3.10) for no malignancy detected group, low-grade BC detected group and high-grade BC detected group, respectively (Independent samples-Kruskal Wallis Test; p:0.000).

Median atypical cell values in Group-1 which patients underwent cystoscopy for diagnostic purposes, were 1.15(IQR:0.70–1.90), 1.00(IQR:0.75–1.40) and 2.20(IQR:0.80–3.90) in those with no malignancy, those with low-grade BC, and those with high-grade BC, respectively. There was significant differences between those groups (Independent samples-Kruskal Wallis Test; p:0.033, Table-2).

In patients with previously diagnosed NMIBC (Group-2) who underwent cystoscopy for routine follow-up, median atypical cell values for those with no malignancy, those with low-grade BC reccurrence, and those with high-grade BC recurrence were 0.00 (IQR:0.00-0.80), 0.25 (IQR:0.10–1.10) and 1.20 (IQR:0.70–2.15), respectively. There was also significant differences between those groups (Independent samples-Kruskal Wallis Test; $p < 0.001$, Table-2). Patients who previously diagnosed high grade NMIBC were also evaluated within themselves. It was observed that statistical significance continued in that group (Independent samples-Kruskal Wallis Test; p:0.008, Table-2).

Figure-1 shows the Independent samples-Kruskal Wallis Test graphics and the distribution of atypical cells parameter for each group seperately.

Sensitivity, specificity, NPV and PPV were calculated for 0.1, 0.2, 0.5, 1.0 and 3.0 atypical cells/ µL. When the cut-off was determined as 0.1 atypical cells/ µL, sensitivity and specificity were measured as 83.33% and 53.73%, respectively (Table-3; Area under the ROC curve: 0.727; p value < 0.0001). This AUC and the p value indicates that the atypical cell parameter is successful in predicting recurrence. For this value, NPV and PPV were measured as 83.72% and 53.03%, respectively (Table-3). The measured sensitivity, specifity, NPV and PPV values and AUC (area under the ROC curve) value for the different cut-off values and for the subgroup diagnosed with high-grade NMIBC are shown in Table-3.

Discussion

Atypical cell parameter of the Sysmex UF-5000 (Sysmex Corporation, Kobe, Japan) is a research parameter and has not yet been validated.[9] For urine sediment analysis, the UF-5000 employs fluorescence flow cytometry technology and hydrodynamic focusing, in which particles are stained with specific fluorochromes for nucleic acids and surface structures before being passed through a semi-conductor laser beam. The characteristics of the particles are determined by counting and classifying signals of scattered light and fluorescence. Atypical cells have enlarged nuclei and a higher nucleus/cytoplasm ratio, as evidenced by side fluorescence and scattered light properties.[10] There is no subjective interpretation at any stage of this measurement. Therefore, it gives objective results. It creates quantitative results for each sample but is not transferred to the patient information system as it is not validated. The results are stored in the device's own memory.[9] The device already counts other cells such as leukocytes and erythrocytes in the urine with the same method for routine urinalysis.

In summary, this parameter provides objective results which indicates the atypia by evaluating the urine sample which is normally eveluated and reported by pathologists.

Considering all these, we can interpret the results of our study. Malignancy was detected in 28 patients from Group-1 and 42 patients from Group-2. The median atypical cell count of 70 patients with malignancy was statistically significantly higher when compared to those without malignancy. High grade urothelial cancer was detected in 43 of these 70 patients. According to The Paris System (TPS) urinary cytology classification, in order to be reported as suspicious for high-grade urothelial carcinoma (SHGUC) or high-grade urothelial carcinoma (HGUC), the nucleus/cytoplasm ratio must be greater than 0.7.[11] Furthermore, in order to be able to identify atypicalal urothelial cells (AUC), this ratio must be greater than 0.5, according to TPS.[11] These 43 patients had an increased nucleus/cytoplasm ratio, which was detected by the device, and their malignancy was confirmed by cytopathology results.

As indicated in Table-2, statistical significance continued when groups are evaluated within themselves. The median atypical cell counts of those with high grade carcinoma in group-1 were found to be statistically significantly higher (p:0.033, Table-2). The interesting thing here was that the median atypical cell values of the patients without any carcinoma were found to be higher than those with low grade carcinoma (1.15 Atyp.C/µL vs 1.00 Atyp.C/µL). This could be due to a variety of factors. Low cellular atypia in low-grade urothelial carcinomas could also be a factor.[11] In patients who have a positive test result, there may be a pathology that has gone unnoticed. It may be a urothelial cancer originating from the upper urinary tract. This may have been overlooked, since our study did not evaluate the upper urinary system. Alternatively, a flat lesion in the bladder could have gone undetected. We don't know, but it's possible that these patients will require another cystoscopy. This is one of our study's limitations. These are some of the possible causes. On the other hand, there is no cut-off value determined for the Atypical cell parameter until now. Any atypical cell value above zero was considered positive. No malignancy was detected in every patient with a positive value. So that, this group may represent false positives. One cause of false positives, particularly in female patients, may be vaginal clue cells composed of squamous cells covered with bacteria. In a study by Aydın et al, vaginal clue cells were observed when the urine sample of a patient with atypical cell value of 13.7/µL was examined by manual microscopy.[10] Another possibility is that malignancy may be detected in these patients in the future as mentioned above. For this reason, we continue to follow up these patients to perform cystoscopy again if necessary. We also plan to share the long-term results after 3 years of follow-up.

The median atypical cell count of the Group-1 who underwent cystoscopy for diagnostic purposes and was diagnosed with high grade BC was found to be higher than the median atypical cells of the patients in Group-2 and high grade recurrence detected in the follow-up cystoscopy (2.20 vs. 1.2 Atypical cells/µL, Table-2). This may be related to the high tumor burden in some patients in the newly diagnosed group and therefore the high number of atypical cells in the urine. On the other hand, patients in group-2 were routine follow-up patients. Since, these patients were under close follow-up, the tumor burden was low even in those who relapsed. Therefore, the median atypical cell count of positives in this group may be relatively lower. In Group-1, 28 patients underwent TUR-B or punch biopsy with a preliminary diagnosis of bladder cancer. Low-grade NMIBC and High-grade NMIBC was detected in 7 and 21 patients, respectively. In 8 of 21 patients with high-grade

NMIBC, the tumor size was greater than 3 cm. The median number of atypical cells per microliter of these 8 patients was 3,1. Whereas in Group-2, all those with recurrence had a tumor burden less than 3 cm.

In this study, we compared the results of the atypical cell parameter with the results of cytology and pathology. The sensitivity of cytological examination of voided urine or bladder-washing specimens for high-grade and low-grade tumours are 84% and 16%, respectively.[5] The sensitivity in detection of CIS (which is also a high grade flat tumour) is 28–100%. Another critical point about urine cytology is that cytological interpretation is dependent on the cytopathologist.[12,13] However, the atypical cell parameter is not dependent on the person making it because the device gives the result directly. On the other hand, in our study, cytology and pathology results were interpreted by a single uropathologist. In addition, urinary stone diseases, urinary tract infections, intravesical instillations may reduce the quality of cytological examination by making examination difficult.[13] Although there is no study on this condition, it should be kept in mind that these conditions may also affect the atypical cell parameter. Patients with urinary tract infections were not included in this study. None of the patients included in the study were found to have kidney stones in the examinations.

An important advantage of the atypical cell parameter is that it has no additional cost. Atypical cell values were obtained from the device throughout the study. However, this did not cost the patient, the hospital, or the team performing the study. The Sysmex UF-5000 and similar automated urine analyzers are already available in many hospital biochemistry labs. Routine urine analysis is performed with these devices. The advantage of the device is to give the atypical cell count while performing routine urine analysis without any extra cost. The cost is very important in the follow-up of bladder cancer. NMIBC is treated with TUR-B (transurethral resection of bladder tumor) and adjuvant intravesical therapy, depending on the risk classification. Surveillance relies on long-term follow-up and repeated cystoscopies. Bladder cancer is a disease that requires close follow-up. The most important component of this close follow-up is regular cystoscopies. However, these procedures and treatments have a very high economic burden. Bladder cancer is the cancer with the highest cost per patient from diagnosis to death. [14] Cumulative costs of treatment over a 5-year period for a base case were $52,125 for low-risk, $146,250 for intermediate-risk, and $366,143 for high-risk NMIBC. [15] In order to reduce both the cost and the frequency of cystoscopy, cost analyzes were also performed with many biomarkers. [16] There are studies showing that the frequency of cystoscopies and cost of surveillance can be reduced by using various biomarkers, however these studies are based on overconfident estimates of sensitivity and specificity and do not include data specific to recurrent bladder cancer. [16] Another study by Kamat et al revealed that cystoscopy is still the most cost-effective method for surveillance of NMIBC.

We found that sensitivity, specificity, NPV and PPV of Atypical cell parameter for the surveillance of the NMIBC patients are 83.33%, 53.73%, 83.72% and 53.03% for the 0,1 Atypical cell per microliter, respectively. The performance of urine biomarkers depends on their sensitivity, specificity, NPV and PPV. The high negative predictive value can be counted as an advantage. Thus, a negative atypical cell value in the surveillance of NMIBC patients will indicate that the probability of recurrence is very low and may perhaps reduce the number and frequency of cystoscopies in follow-up.

Five urinary biomarker tests (NMP22 BladderChek Test, NMP22 test kit, BTA stat, BTA TRAK, and UroVysion) have been approved by the United States Food and Drug Administration (FDA) in the diagnosis and

surveillance of BC. One of the other biormarker tests; The uCyt + test is only approved in the follow-up of NMIBC patients. In the summary of the phase II–IV and phase II–III marker performances in the study by Soria et al, it has been seen that the sensitivity, specificity, NPV and PPV values of these six markers are not much superior to those of the atypical cell parameter.[7] However, none of these tests have been recommended in both EAU and AUA guidelines.[1,17,18] Because it has been shown that existing commercially available urinary biomarker tests are not adequately validated to be properly used in clinical practice.[7] For this reason, we have to perform cystoscopies at regular intervals.

This study had some limitations. The sample size was not large enough, however, it is the first study with the largest sample size on this subject. Bladder biopsy was not taken from all patients and atypical cell values of some patients were compared with cytology alone. Nevertheless, no macroscopic pathology was observed at the cystoscopy in these patients. Another limitation of this study is that it was not investigated whether there was a difference between those who received intravesical BCG therapy and those who did not. It is unknown whether BCG treatment will affect the atypical cell count. However, it should also be noted that urine samples of patients receiving intravesical BCG therapy were obtained at least 4 weeks after the completion of BCG therapy in this study.

Conclusion

Atypical cell parameter of the Sysmex UF-5000 automated urine analyzer is a newly introduced research parameter. The results of this study are promising. Based on our results, we presume that the atypical cell parameter may be used in surveillance of the NMIBC patients. Multi-center studies with larger patient populations are required to prove its efficacy.

Declarations

Ethical Approval and Consent to participate

All data is gathered and analysed after geting ethical approve from Ankara University Ethics Comitee and consents from the patients for participation.

Human and Animal Ethics

No animal data has been used in our study. All the data gethered from human participants are applicable in accordance with the 1964 Helsinki Declaration

Consent for publication

All the authors of this paper, Murat Can Karaburun, Mehmet Fatih Özkaya, Berrin İmge Ergüder, Evren Süer have consents fort his paper to be published in Journal of Medical Systems.

Availabilty of the Data

Patients' cystoscopy and cytology data are accessible through the hospital databse Avicenna v2.0® Sysmex UF 5000 Urine analyses can be demanded from Ankara University Biochemistry Department.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that

could have appeared to influence the work reported in this paper.

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Authors Contributions

Murat Can Karaburun, wrote the main parts of manuscirpt, performed most of the cystoscoy procedures

Mehmet Fatih Özkaya, wrote other parts of the manuscript, evaluate the data from the surgical notes and cytology records

Berrin İmge Ergüder, did the Sysmax UF-5000 urine cytology evaluation

Evren Süer also did the big part on writing and correcting the manuscript and did the procedures

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Tables

Table-1: Patient characteristics

	Total of patients (n:185)	**Group-1:** Patients with no previous diagnosis of bladder cancer group (n:76)	**Group-2:** Patients with previously diagnosed NMIBC (n:109)	P value
Gender, n (%)				
Male:	126	50 (65.8%)	76 (69.7%)	0.78
Female:	59	26 (34.2%)	33 (30.3%)	
Age, yr, median (range):	62(48-77)	58 (48-74)	64 (53-77)	0.624
Smoking, n (%)				
Never:	52	23 (30.3%)	29 (26.6%)	
Former:	42	18 (23.7%)	24 (22.0%)	0.572
Current:	91	35 (46.0%)	56 (51.4%)	
BMI, (kg/m^2)	30.9	32.1	29.9	0.356

BMI=Body mass index; NMIBC=non-muscle invasive bladder cancer

Table-2: Comparison of atypical cell and pathology results within groups

	Cytology or pathology report at cystoscopy: n (%)		Median Atypical cells (/µL) (IQr)	Mean Atypical cells (/µL ± StD)	P value (Independent samples -Kruskal Wallis Test)
Group-1: Patients with no previous diagnosis of bladder cancer: n (%)	No malignancy detected	48 (63.2%)	1.15 (0.70 – 1.90)	1.30 ± 0.09	0.033
	Low grade BC detected	7 (9.2%)	1.00 (0.75 – 1.40)	1.40 ± 0.43	
	High grade BC detected	21 (27.6%)	2.20 (0.80 – 3.90)	3.58 ± 0.99	
Group-2: Patients with previously diagnosed NMIBC: n (%)	No recurrence detected	67 (61.4%)	0.00 (0.00 – 0.80)	0.53 ± 0.10	<0.001
	Low grade BC recurrence +	10 (9.1%)	0.25 (0.10 – 1.10)	0.81 ± 0.40	
	High grade BC recurrence +	32 (29.5%)	1.20 (0.70 – 2.15)	1.88 ± 0.39	
Group-2.A: Patients with previously diagnosed **High-Grade** NMIBC: n (%)	No recurrence detected	49 (55.6%)	0.10 (0.00 – 1.10)	0.72 ± 0.13	0.008
	Low grade BC recurrence +	7 (7.9%)	1.00 (0.25 – 1.10)	1.12 ± 0.54	
	High grade BC recurrence +	32 (36.5%)	1.20 (0.70 – 2.15)	1.88 ± 0.39	

IQR=interquartile ranges; NMIBC=non-muscle invasive bladder cancer; UC=urothelial cancer

Table-3: Sensitivity, specificity, Negative predictive value and Positive predictive values according to different cut-off values in patients with previously diagnosed NMIBC

	Cut-off value for Atypical cell (/ µL)	Sensitivity (%)	%95 CI	Specificity (%)	%95 CI	Area under the ROC curve (AUC) (%95 CI)	P value	NPV (%)	PPV (%)
Group-2: Patients with previously diagnosed NMIBC	≥ 0,1	83.33	68.6-93.0	53.73	41.1-66.0	0.727 (0.634-0.808)	<0.0001	83.72	53.03
	≥ 0,2	76.19	60.5-87.9	64.18	51.5-75.5			81.13	57.14
	≥ 0,5	71.43	55.4-84.3	67.16	54.6-78.2			78.95	57.69
	≥ 1,0	52.38	36.4-68.0	76.12	64.1-85.7			71.83	57.89
	≥ 3,0	19.05	8.6-34.1	94.03	85.4-98.3			64.95	66.67
Group-2.A: Patients with previously diagnosed **High-Grade** NMIBC	≥ 0,1	84.62	69.5-94.1	40.82	27.0-55.8	0.685 (0.577-0.780)	0.0011	76.92	53.23
	≥ 0,2	82.05	66.5-92.5	51.02	36.3-65.6			78.13	57.14
	≥ 0,5	76.92	60.7-88.9	55.10	40.2-69.3			75.0	57.69
	≥ 1,0	56.41	39.6-72.2	67.35	52.5-80.1			66.0	57.89
	≥ 3,0	20.51	9.3-36.5	91.84	80.4-97.7			59.21	66.67

NMIBC=non-muscle invasive bladder cancer; CI=confidence interval; AUC=area under the curve; NPV=negative predictive value; PPV=positive predictive value;

Figures

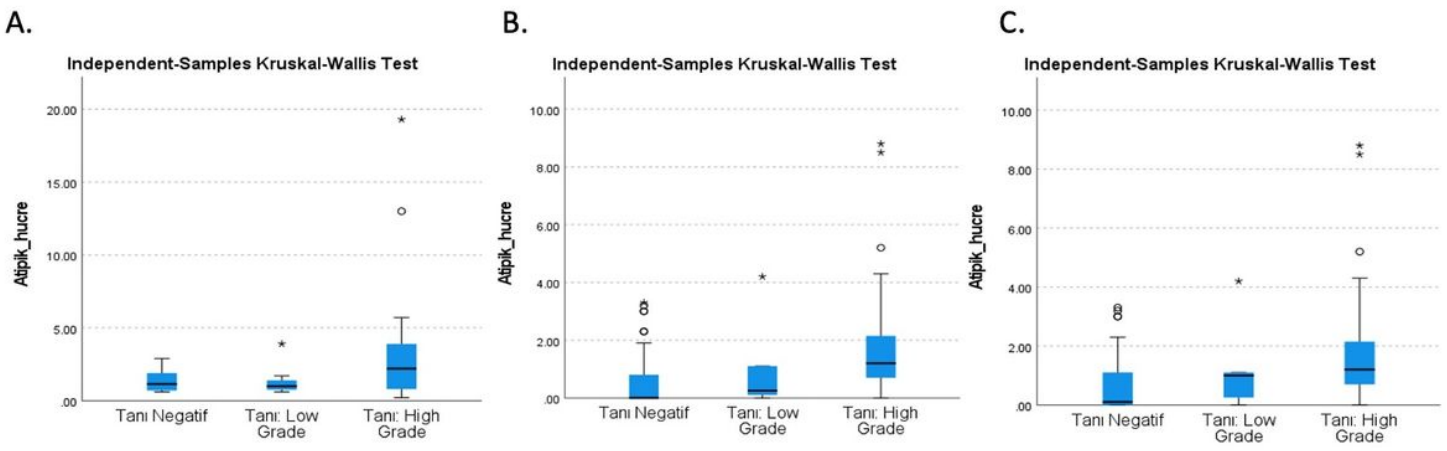

Figure 1

Independent samples-Kruskal Wallis Test Graphics for each groups

1-A: Group-1

1-B: Group-2

1-C: Group-2.A