

Association of the Preoperative Albumin-to-Globulin Ratio with Pathological Stage and Tumor Grade in Primary Bladder Tumor

Primer Mesane Tümörlerinde Ameliyat Öncesi Albumin-Globulin Oranının Patolojik Evre ve Tümör Derecesiyle İlişkisi

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ABSTRACT

Objective: This study aimed to investigate the association between the preoperative albumin-to-globulin ratio (AGR) and pathological stage and tumor grade in patients who underwent transurethral resection of bladder tumor (TURBT) for primary bladder tumors.

Materials and Methods: A total of 153 patients who underwent transurethral resection of bladder tumor (TURBT) for primary urothelial carcinoma between March 2017 and February 2020 were included in the study. AGR was calculated using preoperative serum albumin and total protein levels. Among the exclusion criteria were active infection, autoimmune disease, hepatic disease, and concomitant malignancy. The optimal AGR cut-off value was determined using receiver operating characteristic (ROC) curve analysis and the Youden index. Independent predictors of pathological stage (NMIBC/MIBC) and tumor grade (low/high Grade) were evaluated using logistic regression analysis.

Results: The median age of the patients was 68 years (IQR: 59–75), and 88.9% were male. The pathological stage distribution was Ta in 39.9%, T1 in 39.9%, and T2 in 20.3% of patients, while tumor grade distribution was low Grade in 54.2% and high Grade in 43.1%. The median AGR value of the cohort was 1.32 (IQR: 1.17–1.50). AGR was significantly lower in both the MIBC group ($p = 0.003$) and the high-grade group ($p < 0.001$) compared to their respective counterparts. ROC analysis identified an optimal cut-off value of 1.35 for both clinical outcomes (AUC=0.649, 95% CI: 0.542–0.755 for MIBC and AUC=0.656, 95% CI: 0.568–0.744 for high-grade disease). In multivariate logistic regression analysis, AGR was independently associated with both MIBC (OR=0.16, 95% CI: 0.03–0.97; $p = 0.046$) and high-grade disease (OR=0.18, 95% CI: 0.04–0.74; $p = 0.018$).

Conclusion: Preoperative AGR is independently associated with pathological stage and tumor grade in primary bladder tumors. The ability to calculate this parameter from routine biochemical tests without additional cost makes it clinically valuable for preoperative risk stratification. Further validation of these findings in larger patient cohorts is warranted.

Keywords: albumin-to-globulin ratio, neoplasm grading, neoplasm staging, transurethral resection of bladder tumor, urinary bladder neoplasms

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ÖZET

Amaç: Bu çalışma, primer mesane tümörü nedeniyle transüretral mesane tümörü rezeksiyonu (TURMT) geçiren hastalarda ameliyat öncesi albümin-globulin oranı (AGR) ile patolojik evre ve tümör derecesi arasındaki ilişkiyi araştırmayı amaçlamıştır.

Gereç ve Yöntemler: Mart 2017 ile Şubat 2020 tarihleri arasında primer ürotelyal karsinom nedeniyle transüretral mesane tümörü rezeksiyonu (TURMT) geçiren toplam 153 hasta çalışmaya dahil edilmiştir. AGR, ameliyat öncesi serum albümin ve toplam protein düzeyleri kullanılarak hesaplanmıştır. Dışlama kriterleri arasında aktif enfeksiyon, otoimmün hastalık, karaciğer hastalığı ve eşlik eden malignite yer almıştır. Optimal AGR eşik değeri, alıcı işletim karakteristik (ROC) eğrisi analizi ve Youden indeksi kullanılarak belirlenmiştir. Patolojik evre (NMIBC/MIBC) ve tümör derecesinin (düşük/yüksek derece) bağımsız prediktörleri lojistik regresyon analizi kullanılarak değerlendirilmiştir.

Bulgular: Hastaların medyan yaşı 68 yıl (IQR: 59–75) olup %88.9'u erkekti. Patolojik evre dağılımı hastaların %39.9'unda Ta, %39.9'unda T1 ve %20.3'ünde T2 iken, tümör derecesi dağılımı %54.2'sinde düşük dereceli ve %43.1'inde yüksek dereceliydi. Kohortun medyan AGR değeri 1.32 (IQR: 1.17–1.50) idi. AGR, hem MIBC grubunda ($p = 0.003$) hem de yüksek dereceli grupta ($p < 0.001$) ilgili karşıt gruplarına göre anlamlı derecede daha düşüktü. ROC analizi, her iki klinik sonuç için de 1.35'lik optimal bir kesme değeri belirledi (MIBC için AUC=0.649, %95 GA: 0.542–0.755 ve yüksek dereceli hastalık için AUC=0.656, %95 GA: 0.568–0.744). Çok değişkenli lojistik regresyon analizinde, AGR hem MIBC (OR=0.16, %95 GA: 0.03–0.97; $p = 0.046$) hem de yüksek dereceli hastalık (OR=0.18, %95 GA: 0.04–0.74; $p = 0.018$) ile bağımsız olarak ilişkili bulunmuştur.

Sonuçlar: Ameliyat öncesi AGR, primer mesane tümörlerinde patolojik evre ve tümör derecesi ile bağımsız olarak ilişkilidir. Bu parametrenin ek maliyet olmadan rutin biyokimyasal testlerden hesaplanabilmesi, ameliyat öncesi risk sınıflandırması için klinik olarak değerli kılmaktadır. Bu bulguların daha büyük hasta kohortlarında daha fazla doğrulanması gerekmektedir.

Anahtar Kelimeler: albümin-globulin oranı, mesane tümörü, transüretral mesane tümörü rezeksiyonu, tümör derecelendirmesi, tümör evrelendirmesi

INTRODUCTION

Bladder cancer is among the most common malignancies worldwide and represents one of the major challenges in urologic oncology (1). Approximately 70–80% of newly diagnosed bladder cancer cases are classified as non-muscle-invasive bladder cancer (NMIBC; Ta and T1), whereas muscle-invasive bladder cancer (MIBC; $\geq T2$) is detected in 20–25% of patients at diagnosis (2,3). Transurethral resection of bladder tumor (TURBT), the primary diagnostic procedure, is the fundamental surgical intervention that determines both diagnosis and treatment management by establishing the pathological stage and histological Grade of the tumor (4,5).

Pathological stage and tumor grade are the principal determinants of treatment selection and follow-up protocols in bladder cancer. The heterogeneous clinical course of NMIBC has necessitated the development of multivariable models, such as the European Association of Urology (EAU) risk classification; however, existing clinicopathological parameters may not always adequately reflect the individual patient risk profile (2,6). In this context, there is growing interest in novel biomarkers that can be obtained preoperatively, require no additional cost, and can be calculated from routine biochemical panels (7).

Systemic inflammation and nutritional status represent two fundamental physiological domains that directly influence tumor biology. The chronic inflammatory process accompanying tumor growth shapes the tumor microenvironment by promoting angiogenesis, tumor cell survival, invasion, and metastasis (8). In this process, proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) play key mediating roles. These cytokines suppress hepatic albumin synthesis while stimulating an increase in the globulin fraction, which includes acute-phase proteins (9). This mechanism establishes a direct relationship between inflammatory tumor burden and the serum protein profile, positioning the albumin-to-globulin ratio (AGR) as an integrated immunonutritional biomarker.

AGR is a simple biomarker calculated as the ratio of serum albumin to the difference between total protein and albumin [$AGR = \text{albumin} / (\text{total protein} - \text{albumin})$] and reflects both nutritional status and systemic inflammatory

activity within a single parameter (4). A low AGR simultaneously reflects hypoalbuminemia, indicating poor nutritional reserves, and elevated globulin levels, indicating chronic inflammatory stimulation. Since total protein comprises both components, individual changes may offset and thereby mask each other when assessed alone; this has been proposed as a rationale for why AGR may provide additional discriminatory value compared with either component alone (4,9).

In recent years, the prognostic significance of AGR has been extensively investigated in various solid tumors. In the systematic review by Roberts et al. (7), which compiled data from 61 studies covering 18 different cancer types, AGR was shown to have a statistically significant association with at least one prognostic variable in multivariate analysis across all cancer types and can be calculated from routine preoperative biochemical panels without additional cost.

In the field of urologic oncology, the prognostic value of AGR has been investigated in both upper tract urothelial carcinoma (UTUC) and bladder cancer. Two meta-analyses by Xia et al. (10) one including 12 studies and 5,727 urothelial carcinoma (UC) patients, and another including 21 studies and 18,269 urologic cancer patients - consistently demonstrated that low pretreatment AGR was independently associated with worse overall survival, cancer-specific survival, and recurrence-free survival (11). In UTUC specifically, Xu et al. (n = 620), Otsuka et al. (n = 124), and Omura et al. (n = 179) each confirmed an independent association between low preoperative AGR and poor oncological outcomes after radical nephroureterectomy (9,12,13).

Regarding bladder cancer specifically, the majority of existing studies have focused on survival and recurrence outcomes. Niwa et al. (4) demonstrated that low pretreatment AGR (<1.60) was an independent risk factor for tumor recurrence in 364 patients with primary NMIBC, with a significant association with progression to muscle invasion also reported in univariate analysis. Quhal et al. (5) confirmed in a multicenter cohort of 1,096 patients that low AGR (<1.41) independently predicted progression from NMIBC to MIBC (HR=1.81, 95% CI: 1.22–2.68). Similarly, Schuettfort et al. (14) (n = 4,335) and Takada et al. (8) (n = 235) demonstrated independent associations between low preoperative AGR and adverse survival outcomes after radical cystectomy.

The common feature of all these studies is that they primarily focused on survival and recurrence outcomes as their main endpoints. To the best of our knowledge, no study in the current literature has specifically addressed the association between preoperative AGR and pathological stage and tumor grade obtained after TURBT as its primary hypothesis. However, pathological stage and Grade at diagnosis constitute the foundation of clinical decision-making. The distinction between NMIBC and MIBC fundamentally alters the treatment modality, while tumor grade directly shapes management across a broad spectrum, ranging from the selection of intravesical therapy to the intensity of follow-up (5,14).

In this study, we aimed to investigate the association between preoperative AGR and pathological stage (NMIBC/MIBC) and tumor grade (low grade/high Grade) in patients who underwent TURBT for primary bladder tumors. Determining the independent contribution of this parameter, which can be calculated from routine preoperative biochemical panels without additional cost, to conventional clinical variables may contribute to preoperative risk stratification and individualized patient management in bladder cancer.

MATERIALS AND METHODS

Study Design and Ethical Approval

This study was a single-center prospective cohort study conducted at the Department of Urology, University of Health Sciences İzmir Bozyaka Training and Research Hospital. Patients were prospectively enrolled at the time of TURBT between March 2017 and February 2020 and followed according to a pre-specified data collection protocol. Patient recruitment was terminated in February 2020 due to the COVID-19 pandemic, after which the prospectively

collected data were statistically analyzed. The study was approved by the Clinical Research Ethics Committee of the University of Health Sciences İzmir Bozyaka Training and Research Hospital (Decision No: 3; Date: March 15, 2017). Written informed consent was obtained from all patients before inclusion in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Selection

The inclusion criteria were defined as follows: patients who underwent transurethral resection of bladder tumor (TURBT) and were diagnosed with primary urothelial carcinoma on pathological examination; patients who underwent complete TURBT; patients with available laboratory data on preoperative serum total protein and albumin levels; and patients with sufficient clinical, operative, and pathological data.

The exclusion criteria were defined as follows: recurrent bladder tumor; history of preoperative chemotherapy or radiotherapy; known systemic inflammatory or autoimmune disease, such as rheumatoid arthritis or systemic lupus erythematosus; active infection or urinary tract infection in the preoperative period; history of concomitant malignancy; liver cirrhosis, nephrotic syndrome, poorly controlled diabetes mellitus, chronic kidney disease, or any disease directly affecting albumin metabolism; use of corticosteroids or immunosuppressive drugs; biochemical values obtained more than 30 days before surgery; incomplete resection or insufficient/pathologically unevaluable specimens.

According to these criteria, a total of 153 patients were included in the final analysis. A flow diagram summarizing the patient selection process is presented in Figure 1.

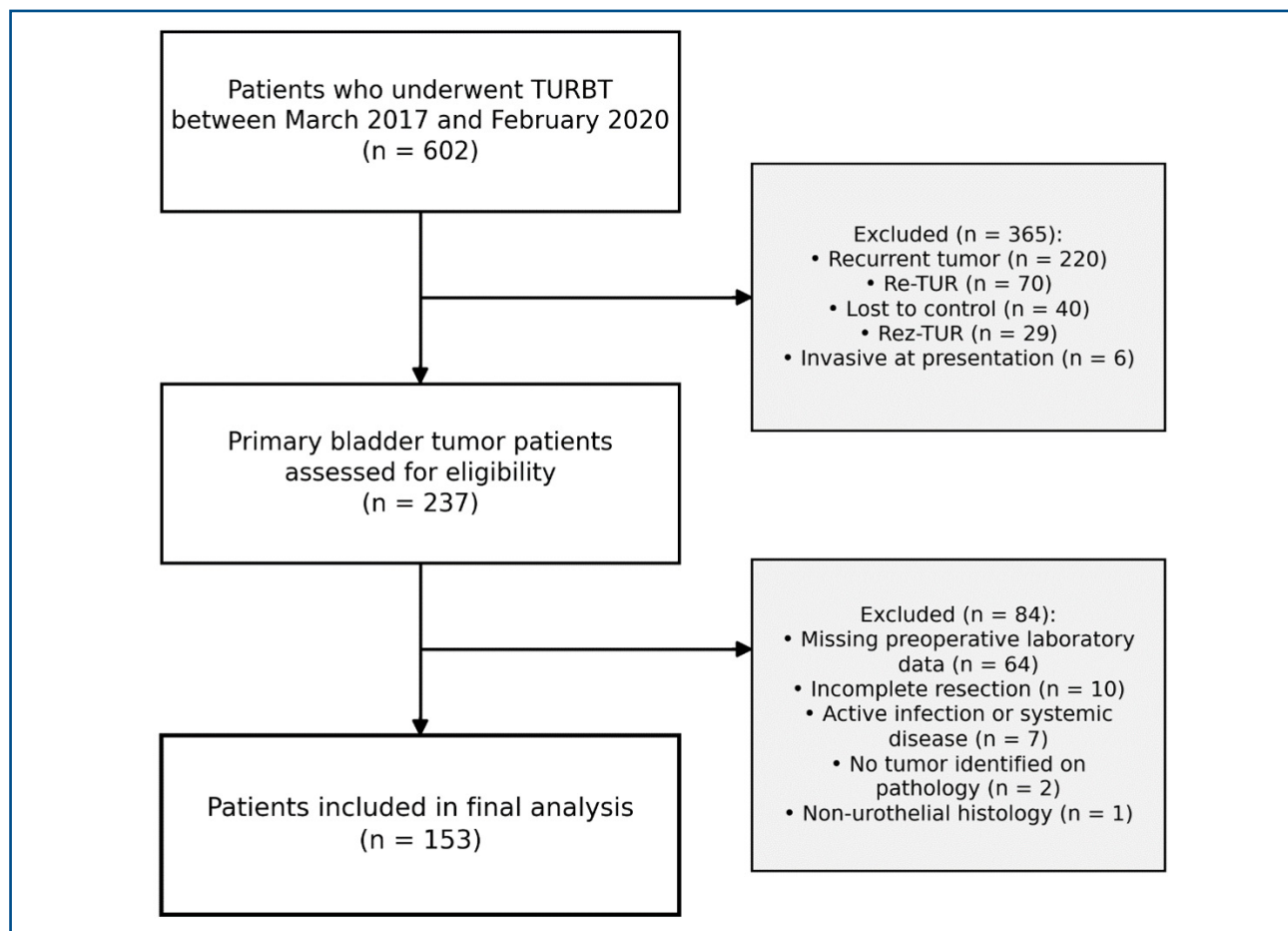


Figure 1. Flow diagram of patient selection

Surgical Procedure and Pathological Evaluation

All patients underwent macroscopically complete TURBT. Cases in which an incomplete resection was performed were not included in the study. Second look TURBT (re-TURBT) was routinely performed in patients with an indication in accordance with the recommendations of the EAU guidelines (15); the higher pathological result was considered for the determination of the final stage and Grade.

All resection specimens were evaluated in the institution's routine pathology laboratory. Pathological stage was determined according to the 2009 tumor-node-metastasis (TNM) classification system. Tumor grading was performed according to the World Health Organization/International Society of Urological Pathology (WHO/ISUP) 2004/2016 classification. The presence of concomitant carcinoma in situ (CIS) was also recorded separately (15).

Collection of Clinical and Pathological Data

The following data were prospectively recorded at the time of enrollment according to the study protocol, using a standardized data collection form, and subsequently verified against patient files and hospital information system records: age, sex, body mass index (BMI, kg/m²), smoking status (absent/present), intraoperative total tumor size (cm) and number of tumors, transurethral resection (TUR) specimen volume (mL), and the presence of concomitant CIS.

Non-muscle-invasive bladder cancer (NMIBC) was defined as Ta and T1 disease, whereas muscle-invasive bladder cancer (MIBC) was defined as T2 disease. In terms of tumor grade, patients were dichotomized into low-grade and high-grade urothelial carcinoma groups. Cases diagnosed as papillary urothelial neoplasm of low malignant potential (PUNLMP) were included in the low-grade group.

Calculation of the Albumin-to-Globulin Ratio

Preoperative serum albumin and total protein levels were obtained from routine biochemical panels performed within 30 days before surgery. Globulin level was calculated as the difference between total protein and albumin [globulin (g/dL) = total protein (g/dL) – albumin (g/dL)]. The albumin-to-globulin ratio (AGR) was calculated using the following formula: $AGR = \text{albumin (g/dL)} / [\text{total protein (g/dL)} - \text{albumin (g/dL)}]$.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean $\pm$ standard deviation (SD) or median (interquartile range, IQR), according to their distributional characteristics, whereas categorical variables were presented as frequencies and percentages (%). The normality of continuous variables was assessed for each group using the Shapiro-Wilk test. Variables with normal distribution were compared using the Student's t-test, while variables without normal distribution were compared using the Mann-Whitney U test. Categorical variables were analyzed using the Chi-square test; Fisher's exact test was applied when the expected cell frequency was less than 5.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the discriminatory ability of AGR for pathological stage (NMIBC/MIBC) and tumor grade (low grade/high Grade). The area under the curve (AUC) and 95% confidence intervals (CIs) were calculated. AUC values and their 95% confidence intervals were obtained in SPSS. The shaded confidence band displayed around the ROC curve was estimated via bootstrap resampling (2,000 resamples) in Python (SciPy, scikit-learn). The optimal AGR cut-off value was determined using the Youden index [$J = \text{sensitivity} + \text{specificity} - 1$], which maximizes the sum of sensitivity and specificity. According to this cut-off value, patients were divided into low-AGR and high-AGR groups, and the distribution of stage and Grade between the groups was compared using the Chi-square test.

Logistic regression analysis was performed to determine the independent predictive value of AGR for MIBC and high-grade tumors. Variables showing statistical significance at a threshold of $p < 0.10$ in univariate analysis were included in the multivariate model. Candidate variables for the multivariate models — age, sex, body mass index (BMI), tumor size, tumor number, and the presence of CIS — were defined a priori based on clinical relevance. Age, sex, and BMI were retained in both multivariate models as standard demographic covariates regardless of their univariate statistical significance, consistent with established practice in clinical prediction modeling, whereas tumor size, tumor number, and CIS were included based on their univariate significance and their established relevance to tumor burden. Total protein, albumin, and globulin were not entered into the regression models together with AGR because of their direct mathematical relationship with this ratio. TUR specimen volume was excluded from the candidate variable pool because of its strong collinearity with tumor size and its intraoperative, rather than strictly preoperative, measurement. The CIS variable was excluded from the high-grade multivariate model because of complete separation, as no CIS cases were observed in the low-grade group. Univariate logistic regression results for all candidate variables are presented in Tables 4 and 6. Given the relatively small sample size — particularly within the MIBC subgroup ($n = 31$) — and the known instability of stepwise selection methods in such settings, all pre-specified variables were entered into the multivariate model simultaneously (Enter method) rather than through a stepwise selection procedure. Results were reported as odds ratios (ORs) with 95% confidence intervals (CIs). A p -value of <0.05 was considered statistically significant in all analyses. The goodness-of-fit of each multivariable model was assessed using the Hosmer-Lemeshow test, and model discrimination was evaluated using the area under the receiver operating characteristic curve (AUC) with 95% confidence intervals, calculated from the predicted probabilities generated by each logistic regression model. To further explore an unexpected sign reversal observed for tumor number between univariable and multivariable analyses, a post hoc Spearman correlation analysis was performed to assess its association with tumor size and the presence of CIS. To evaluate whether AGR provided additional discriminatory value beyond its individual components, the AUC of AGR was compared with those of albumin, globulin, and total protein alone using DeLong's test for correlated ROC curves.

Given the early termination of patient recruitment due to the COVID-19 pandemic, a post hoc power analysis was performed (GPower 3.1, $\alpha=0.05$) using the observed effect sizes as the basis for calculation (Cohen's $d=0.594$ for stage comparison and Cohen's $d=0.582$ for grade comparison). The existing sample of 153 patients was calculated to provide 84% statistical power for stage comparison and 94.6% statistical power for grade comparison, confirming that the 80% power threshold was exceeded for both analyses.

RESULTS

A total of 153 patients who underwent TURBT were included in the study. AGR was calculated from preoperative serum albumin and total protein values using the formula $AGR = \text{albumin} / (\text{total protein} - \text{albumin})$. The overall demographic, clinical, and biochemical characteristics of the cohort are summarized in Table 1.

The median age was 68 years (IQR: 59–75; mean $\pm$ SD: 67.3 ± 10.9). The majority of patients were male ($n = 136$, 88.9%). The median BMI was 26.6 kg/m^2 (IQR: 23.7–29.4), and active smoking was observed in 58.8% of patients. The median tumor size was 4.5 cm (IQR: 3.0–6.0), the median number of tumors was 2 (IQR: 1–4), and the median TUR specimen volume was 1.8 mL (IQR: 0.8–5.6). Concomitant carcinoma in situ (CIS) was present in 8 patients (5.2%).

Regarding pathological stage distribution, Ta, T1, and T2 disease were identified in 61 (39.9%), 61 (39.9%), and 31 (20.3%) patients, respectively. Tumor grade distribution was as follows: PUNLMP in 4 patients (2.6%), low-grade disease in 83 patients (54.2%), and high-grade disease in 66 patients (43.1%).

In the biochemical evaluation, the mean total protein level was $6.91 \pm 0.66 \text{ g/dL}$, the mean albumin level was $3.91 \pm 0.49 \text{ g/dL}$, and the mean globulin level was $3.00 \pm 0.47 \text{ g/dL}$. The median AGR value of the cohort was 1.32 (IQR:

1.17–1.50; mean $\pm$ SD: 1.34 ± 0.28 ; minimum: 0.700, maximum: 2,294).

The NMIBC (Ta+T1, $n = 122$) and MIBC (T2, $n = 31$) groups were compared in terms of demographic and clinical characteristics. No statistically significant differences were observed between the two groups with respect to age, BMI, or smoking status ($p = 0.784$, $p = 0.538$, and $p = 0.781$, respectively). Sex distribution differed significantly between the groups, with a higher proportion of female patients in the MIBC group ($p = 0.048$). Tumor size and TUR specimen volume were significantly higher in the MIBC group ($p < 0.001$ for both). No statistically significant differences were observed in terms of tumor number or the presence of CIS ($p = 0.329$ and $p = 0.054$, respectively) (Table 2).

When biochemical parameters were evaluated, total protein and albumin levels alone did not reach statistical significance between the groups ($p = 0.806$ and $p = 0.076$, respectively). In contrast, globulin levels were significantly higher in the MIBC group (3.20 vs. 3.00 g/dL, $p = 0.041$). AGR was found to be markedly lower in the MIBC group compared with the NMIBC group (1.21 ± 0.24 vs. 1.37 ± 0.28 ; $p = 0.003$) (Table 2).

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of AGR in predicting MIBC (Figure 2). According to the Youden index, the optimal AGR cut-off value was determined as 1.35 [AUC=0.649, 95% CI: 0.542–0.755; sensitivity 74.2%, specificity 50.8%]. When patients were divided into two groups according to this cut-off value, the rate of MIBC was 27.7% (23/83) in the low-AGR group (<1.35), whereas it was 11.4% (8/70) in the high-AGR group (≥ 1.35) ($p = 0.022$; Table 3).

Table 1. Overall Cohort Characteristics

	N = 153
Age (years), median (IQR); mean $\pm$ SD	68 (59–75); 67.3 ± 10.9
Sex, n (%)	
Male	136 (88.9%)
Female	17 (11.1%)
BMI (kg/m ²), median (IQR)	26.6 (23.7–29.4)
Smoking, Active, n (%)	90 (58.8%)
Tumor size (cm), median (IQR)	4.5 (3.0–6.0)
Number of tumors, median (IQR)	2 (1–4)
TUR specimen volume (mL), median (IQR)	1.8 (0.8–5.6)
CIS (+), n (%)	8 (5.2%)
Pathological stage, n (%)	
Ta	61 (39.9%)
T1	61 (39.9%)
T2	31 (20.3%)
Pathological Grade, n (%)	
PUNLMP	4 (2.6%)
Low Grade	83 (54.2%)
High Grade	66 (43.1%)
Total protein (g/dL), mean $\pm$ SD	6.91 ± 0.66
Albumin (g/dL), mean $\pm$ SD	3.91 ± 0.49
Globulin (g/dL), median (IQR)	3.00 (2.60–3.30)
AGR, median (IQR); mean $\pm$ SD	1.32 (1.17–1.50); 1.34 ± 0.28

AGR: albumin-to-globulin ratio; BMI: body mass index; CIS: carcinoma in situ; IQR: interquartile range; PUNLMP: papillary urothelial neoplasm of low malignant potential; SD: standard deviation; TUR: transurethral resection.

Table 2. Comparison of the NMIBC (Ta+T1) and MIBC (T2) Groups

	NMIBC (Ta+T1) (n=122)	MIBC (T2) (n=31)	p
Age (years), mean $\pm$ SD	67.2 $\pm$ 10.6	67.8 $\pm$ 12.0	0.784*
Gender, n (%)			
Male	112 (%91.8)	24 (%77.4)	0.048**
Female	10 (%8.2)	7 (%22.6)	
BMI (kg/m ²), med (IQR)	26.4 (24.2–29.4)	27.0 (21.5–29.4)	0.538***
Smoking (Active), n (%)	73 (%59.8)	17 (%54.8)	0.781****
Tumor size (cm), med (IQR)	4.0 (2.5–6.8)	6.0 (5.0–8.0)	<0.001***
Number of tumors, med (IQR)	2 (1–3)	2 (1–3)	0.329***
TUR volume (mL), med (IQR)	1.6 (0.6–5.0)	6.8 (5.4–10.5)	<0.001***
CIS (+), n (%)	4 (%3.3)	4 (%12.9)	0.054**
Total protein (g/dL), mean $\pm$ SD	6.92 $\pm$ 0.64	6.89 $\pm$ 0.74	0.806*
Albumin (g/dL), mean $\pm$ SD	3.95 $\pm$ 0.45	3.75 $\pm$ 0.59	0.076*
Globulin (g/dL), med (IQR)	3.00 (2.60–3.30)	3.20 (2.85–3.50)	0.041***
AGR, mean $\pm$ SD	1.37 $\pm$ 0.28	1.21 $\pm$ 0.24	0.003*

AGR: albumin-to-globulin ratio; BMI: body mass index; CIS: carcinoma in situ; IQR: interquartile range; MIBC: muscle-invasive bladder cancer; NMIBC: non-muscle-invasive bladder cancer; SD: standard deviation; TUR: transurethral resection.

*Student's t-test, **Fisher's exact test, ***Mann-Whitney U test, ****Chi-square Test

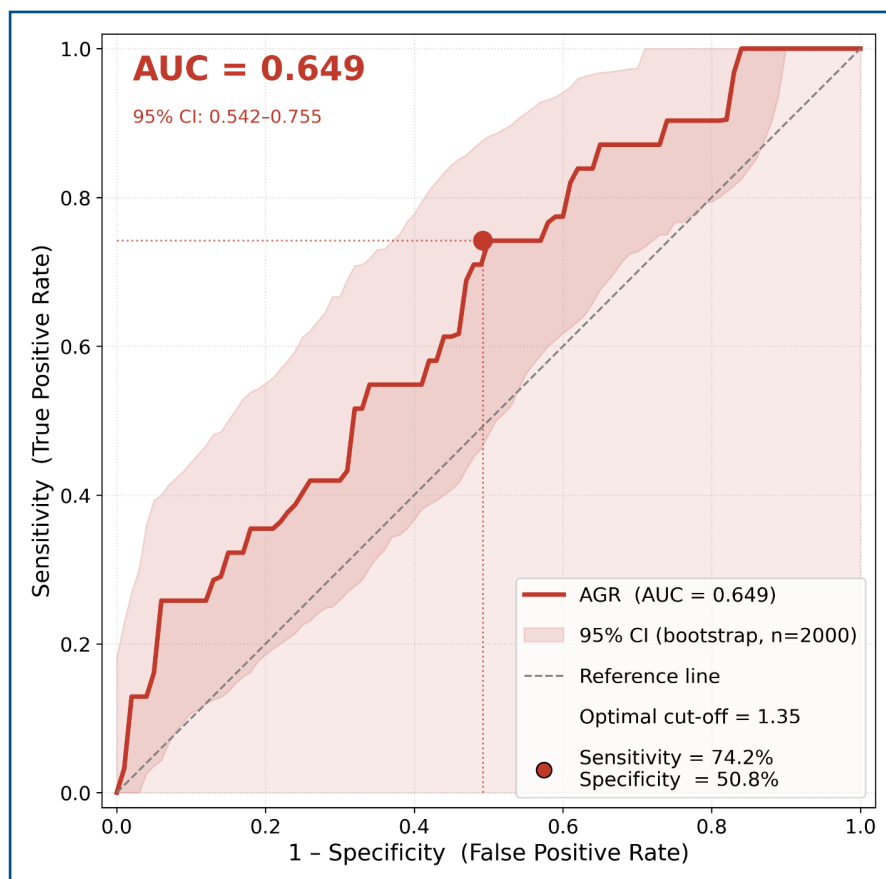


Figure 2. ROC curve of the albumin-to-globulin ratio (AGR) for predicting muscle-invasive bladder cancer. Optimal cut-off: 1.35 (AUC = 0.649, 95% CI: 0.542–0.755; sensitivity 74.2%, specificity 50.8%). Shaded area represents the 95% confidence band estimated by bootstrap resampling (2,000 resamples).

Table 3. Distribution of Stage and Grade According to AGR Groups (Cut-off: 1.35)

	Low AGR (<1.35) (n=83)	High AGR (≥1.35) (n=70)	p
NMIBC (Ta+T1), n (%)	60 (72.3%)	62 (88.6%)	0.022*
MIBC (T2), n (%)	23 (27.7%)	8 (11.4%)	
Low Grade, n (%)	36 (43.4%)	51 (72.9%)	<0.001*
High Grade, n (%)	47 (56.6%)	19 (27.1%)	

AGR: albumin-to-globulin ratio; MIBC: muscle-invasive bladder cancer; NMIBC: non-muscle-invasive bladder cancer.

* Chi-square Test

In the univariate logistic regression analysis, AGR was identified as a significant predictor of MIBC (OR=0.10, 95% CI: 0.02–0.52, $p = 0.007$). In the multivariate model including age, sex, BMI, tumor size, tumor number, and CIS, AGR remained independently associated with MIBC (OR=0.16, 95% CI: 0.03–0.97, $p = 0.046$). In the same model, tumor size (OR=1.34, 95% CI: 1.12–1.60, $p = 0.002$), tumor number (OR=0.79, 95% CI: 0.63–0.98, $p = 0.035$), the presence of CIS (OR=7.19, 95% CI: 1.34–38.56, $p = 0.021$), and female sex (OR=3.50, 95% CI: 1.02–12.06, $p = 0.047$) were also independently associated with MIBC (Table 4). The MIBC prediction model demonstrated adequate calibration (Hosmer-Lemeshow test: $\chi^2=6.085$, $df=8$, $p = 0.638$) and acceptable discriminative ability (AUC=0.773, 95% CI: 0.682–0.865). In a post hoc exploratory analysis performed to investigate the sign reversal observed for tumor number, Spearman correlation analysis revealed a moderate positive correlation between tumor number and tumor size ($r = 0.522$, $p < 0.001$), and a weak positive correlation between tumor number and the presence of CIS ($r = 0.167$, $p = 0.039$).

For the purpose of grade-based comparisons, PUNLMP cases ($n = 4$) were included in the low-grade group, resulting in 87 low-grade and 66 high-grade patients. Low-grade ($n = 87$) and high-grade ($n = 66$) groups were compared. Patients in the high-grade group were significantly older ($p = 0.007$). No significant differences were observed between the groups in terms of BMI or smoking status ($p = 0.801$ and $p = 0.634$, respectively). Sex distribution did not differ significantly between the two groups ($p = 0.100$). Tumor size, tumor number, and TUR specimen volume were significantly higher in the high-grade group ($p < 0.001$, $p = 0.035$, and $p < 0.001$, respectively). CIS was observed only in the high-grade group ($p = 0.001$) (Table 5).

When biochemical parameters were evaluated, total protein levels did not differ significantly between the groups ($p = 0.559$). In contrast, albumin levels were significantly lower ($p = 0.022$), whereas globulin levels were significantly higher in the high-grade group ($p = 0.031$). AGR was markedly lower in the high-grade group ($p < 0.001$) (Table 5).

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of AGR in predicting high-grade tumors (Figure 3). According to the Youden index, the optimal AGR cut-off value was determined as 1.35 [AUC=0.656, 95% CI: 0.568–0.744; sensitivity 69.7%, specificity 60.9%]. The rate of high-grade tumors was 56.6% (47/83) in the low-AGR group (<1.35), whereas it was 27.1% (19/70) in the high-AGR group (≥1.35) ($p < 0.001$) (Table 3).

In the univariate logistic regression analysis, AGR was identified as a strong predictor of high-grade tumors (OR=0.10, 95% CI: 0.03–0.40, $p = 0.001$). Since the CIS variable resulted in complete separation in the high-grade group, as no CIS cases were present in the low-grade group, it was excluded from the multivariate model. In the multivariate model including age, sex, BMI, tumor size, and tumor number, AGR remained independently associated with high-grade disease (OR=0.18, 95% CI: 0.04–0.74, $p = 0.018$). Tumor size was also independently associated with high-grade disease in this model (OR=1.19, 95% CI: 1.02–1.37, $p = 0.023$; Table 6). The high-grade prediction model similarly demonstrated adequate calibration (Hosmer-Lemeshow test: $\chi^2=10.940$, $df=8$, $p = 0.205$) and acceptable discriminative ability (AUC=0.736, 95% CI: 0.657–0.815).

Table 4. Logistic regression, MIBC (T2) predictors.

	Univariable OR (95%CI)	p	Multivariable OR (95%CI)	p
AGR	0.10 (0.02–0.52)	0.007	0.16 (0.03–0.97)	0.046
Tumor size (cm)	1.24 (1.08–1.42)	0.002	1.34 (1.12–1.60)	0.002
Number of tumors	1.01 (0.87–1.18)	0.879	0.79 (0.63–0.98)	0.035
Presence of CIS	4.37 (1.03–18.59)	0.046	7.19 (1.34–38.56)	0.021
Sex (female vs. male)	3.27 (1.13–9.45)	0.029	3.50 (1.02–12.06)	0.047
Age (years)	1.01 (0.97–1.04)	0.766	0.99 (0.95–1.03)	0.506
BMI (kg/m ²)	0.98 (0.90–1.07)	0.707	0.97 (0.89–1.07)	0.583

AGR: albumin-to-globulin ratio; BMI: body mass index; CI: confidence interval; CIS: carcinoma in situ; OR: odds ratio.

Table 5. Comparison of low-grade and high-grade groups.

	Low Grade (n=87)	High Grade (n=66)	p
Age (years), median (IQR)	64 (59–71)	71 (61–80)	0.007*
Gender, n (%)			
Male	81 (%93.1)	55 (%83.3)	0.100**
Female	6 (%6.9)	11 (%16.7)	
BMI (kg/m ²), med (IQR)	26.3 (24.1–29.4)	27.2 (23.2–29.7)	0.801*
Smoking (Active), n (%)	53 (%60.9)	37 (%56.1)	0.634**
Tumor size (cm), med (IQR)	3.0 (2.0–5.5)	5.0 (4.0–8.0)	<0.001*
Number of tumors, med (IQR)	2 (1–3)	2 (1–4)	0.035*
TUR volume (mL), med (IQR)	1.3 (0.5–4.2)	5.1 (2.5–8.9)	<0.001*
CIS (+), n (%)	0 (%0.0)	8 (%12.1)	0.001***
Total protein (g/dL), mean ± SD	6.94 ± 0.65	6.88 ± 0.68	0.559****
Albumin (g/dL), med (IQR)	4.00 (3.80–4.30)	3.90 (3.43–4.28)	0.022*
Globulin (g/dL), med (IQR)	3.00 (2.60–3.20)	3.10 (2.80–3.48)	0.031*
AGR, mean ± SD	1.40 ± 0.28	1.25 ± 0.25	<0.001****

AGR: albumin-to-globulin ratio; BMI: body mass index; CIS: carcinoma in situ; IQR: interquartile range; PUNLMP: papillary urothelial neoplasm of low malignant potential; SD: standard deviation; TUR: transurethral resection.

PUNLMP cases (n = 4) were included in the low-grade group (total low-grade n = 87).

*Mann-Whitney U test, **Chi-square Test, ***Fisher's exact test, ****Student's t-test

ROC analyses performed for both clinical endpoints, namely MIBC and high-grade disease, yielded an AUC of 0.649 (95% CI: 0.542–0.755) for the prediction of MIBC and 0.656 (95% CI: 0.568–0.744) for the prediction of high-grade tumors. Notably, the optimal cut-off value determined by the Youden index overlapped at 1.35 in both analyses. Binary group comparisons according to the optimal cut-off value (AGR=1.35) are presented in Table 3.

DISCUSSION

In this study, preoperative AGR was shown to be independently associated with pathological stage (NMIBC/MIBC) and tumor grade (low grade/high Grade) in 153 patients who underwent TURBT for primary bladder tumor. AGR was significantly lower in the MIBC group (1.21 ± 0.24 vs. 1.37 ± 0.28 ; $p = 0.003$), and multivariate logistic regression analysis demonstrated that AGR was independently associated with MIBC (OR=0.16, 95% CI: 0.03–0.97; $p = 0.046$). Similarly, AGR was markedly lower in patients with high-grade tumors (1.25 ± 0.25 vs. 1.40 ± 0.28 ; $p < 0.001$), and AGR remained independently associated with high-grade disease (OR=0.18, 95% CI: 0.04–0.74; $p = 0.018$). The overlap of

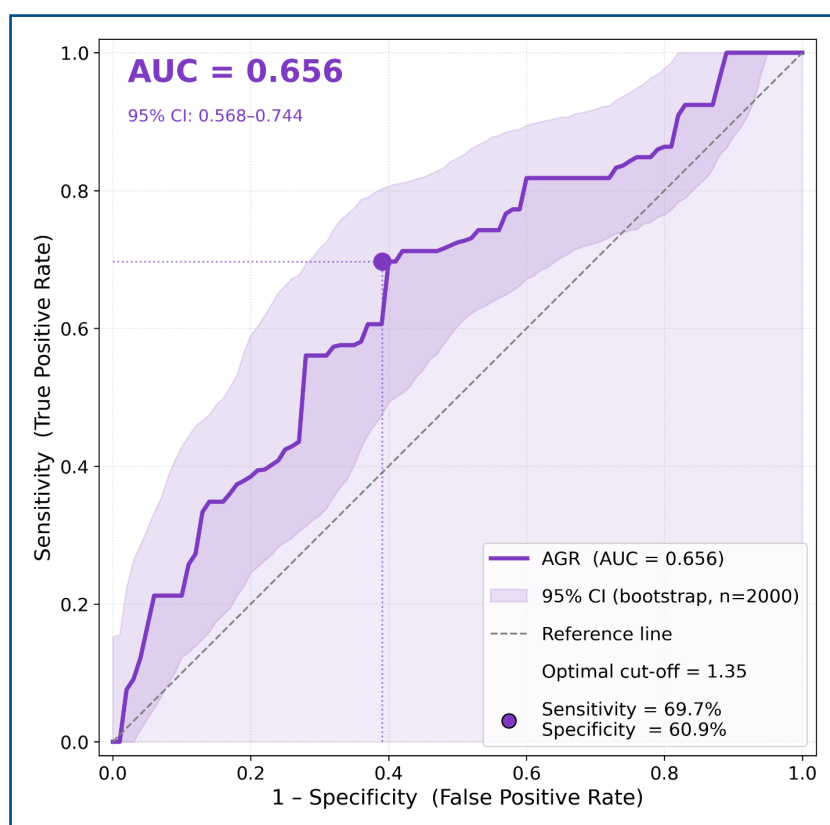


Figure 3. ROC curve of the albumin-to-globulin ratio (AGR) for predicting high-grade bladder tumor. Optimal cut-off: 1.35 (AUC = 0.656, 95% CI: 0.568–0.744; sensitivity 69.7%, specificity 60.9%). Shaded area represents the 95% confidence band estimated by bootstrap resampling (2,000 resamples).

Table 6. Logistic regression, high-grade predictors.

	Univariable OR (95%CI)	p	Multivariable OR (95%CI)	p
AGR	0.10 (0.03–0.40)	0.001	0.18 (0.04–0.74)	0.018
Tumor size (cm)	1.23 (1.09–1.38)	0.001	1.19 (1.02–1.37)	0.023
Number of tumors	1.14 (1.00–1.30)	0.050	1.00 (0.84–1.18)	0.959
Presence of CIS	Not estimable*	—	Excluded*	—
Sex (female vs. male)	2.70 (0.94–7.73)	0.064	2.87 (0.85–9.70)	0.091
Age (years)	1.04 (1.01–1.08)	0.009	1.03 (0.99–1.07)	0.098
BMI (kg/m ²)	1.01 (0.94–1.09)	0.714	1.03 (0.95–1.12)	0.456

AGR: albumin-to-globulin ratio; BMI: body mass index; CI: confidence interval; OR: odds ratio.

*CIS could not be estimated in the univariable analysis and was excluded from the multivariable model because no CIS cases were observed in the low-grade group (complete separation).

the optimal cut-off value at 1.35, as determined by the Youden index in both ROC analyses, provides clinicians with a single and practical threshold value.

Tumor size remained independently associated with both outcomes in the multivariate models, suggesting that AGR may provide a more meaningful predictive profile when used in combination with established clinical parameters rather than as a stand-alone marker. This finding also indicates that while AGR reflects systemic alterations associated with the biological aggressiveness of the tumor, it maintains an independent association with clinicopathological factors.

An additional statistical observation warrants comment: the number of tumors showed no significant association with MIBC in univariable analysis (OR=1.01, 95% CI: 0.87–1.18, $p = 0.879$), yet became significantly and independently associated with MIBC, with a reversed direction of effect. In this post hoc, exploratory analysis, the number of tumors was moderately correlated with tumor size (Spearman $r = 0.52$, $p < 0.001$) and, to a lesser extent, with the presence of CIS ($r = 0.17$, $p = 0.039$), both of which were themselves strong positive predictors of MIBC. In the unadjusted analysis, this positive confounding by tumor size and CIS masked the true negative partial association between tumor number and MIBC; once tumor size and CIS were accounted for in the multivariable model, this masked association became apparent. Given the relatively low events-per-variable ratio in the MIBC model (31 events for 7 covariates), this specific finding should nonetheless be interpreted with caution, as coefficient estimates for covariates with modest individual effect sizes may be sensitive to sample size constraints in addition to genuine suppression effects.

AGR is a biomarker whose prognostic significance has been extensively investigated in various malignancies. Roberts et al. (7) reviewed 61 studies including 18 different cancer types and reported that AGR showed a statistically significant association with at least one prognostic variable in multivariate analysis. In a meta-analysis conducted by Xia et al. (10) in patients with urothelial carcinoma, including 12 studies and 5,727 patients, low preoperative AGR was shown to have independent prognostic significance for overall survival (HR=1.99, 95% CI: 1.45–2.75), cancer-specific survival (HR=2.01, 95% CI: 1.50–2.69), and recurrence-free survival (HR=1.39, 95% CI: 1.12–1.72). These findings were further confirmed in a meta-analysis of urologic cancers, including 21 studies and 18,269 patients, in which low AGR was significantly associated with overall survival (HR=1.93, 95% CI: 1.56–2.39), cancer-specific survival (HR=2.22, 95% CI: 1.67–2.96), and recurrence-free survival (HR=1.69, 95% CI: 1.29–2.22) (11). However, survival and recurrence outcomes were the primary endpoints in all of these studies. To our knowledge, the present study is the first to evaluate pathological stage and tumor grade after TURBT as primary outcomes in relation to preoperative AGR.

The globulin fraction includes acute-phase proteins such as immunoglobulins, complement components, C-reactive protein, and fibrinogen. The chronic inflammatory process accompanying tumor growth increases the synthesis of these proteins, thereby elevating globulin levels. Moreover, tumor-related immunosuppression and inflammatory stimulation may also enhance immunoglobulin secretion from B lymphocytes, further contributing to increased globulin levels (9).

AGR is an integrated biomarker that reflects both nutritional reserve and the immunoinflammatory balance within a single parameter. Since total protein includes both components, individual changes may offset and thereby mask each other when total protein is evaluated alone. In the present study, total protein did not differ significantly in either the MIBC or high-grade groups ($p = 0.806$ and $p = 0.559$, respectively); however, globulin levels were significantly elevated in both groups (MIBC: $p = 0.041$; high Grade: $p = 0.031$), while albumin levels were significantly decreased in the high-grade group ($p = 0.022$). To formally test this, the ROC-AUC of AGR was compared against albumin, globulin, and total protein alone using DeLong's test. AGR showed a numerically higher AUC than each individual component for both MIBC (0.649 vs. 0.591, 0.619, and 0.509, respectively) and high-grade disease (0.656 vs. 0.608, 0.602, and 0.489, respectively); however, this difference reached statistical significance only when AGR was compared with total protein in the high-grade model ($p = 0.009$), while differences from albumin and globulin individually did not reach significance (all $p \geq 0.11$), likely reflecting the limited statistical power of this single-center cohort. These findings offer only partial support for the superiority of AGR as a ratio over its individual components and should be interpreted with appropriate caution.

Niwa et al. (4) suggested that combining albumin and globulin into a single ratio may reveal alterations that could be missed when each component is assessed separately. Takada et al. (8) emphasized that blood-based biomarkers such as AGR, neutrophil-to-lymphocyte ratio (NLR), and C-reactive protein-to-albumin ratio (CAR) may provide complementary information regarding physiological reserve and biological tumor burden that cannot be captured

by imaging or conventional pathological staging, as they reflect both nutritional and inflammatory status. They also highlighted that chronic inflammation directly shapes the tumor microenvironment by promoting angiogenesis, tumor cell survival, invasion, and metastasis.

No study in the literature has investigated the association between AGR and pathological stage after TURBT in primary bladder tumor as a primary endpoint. Therefore, our findings were evaluated within the framework of studies that examined similar mechanisms but used different methodologies and outcome measures.

In their multicenter study including 1,096 patients with NMIBC, Quhal et al. (5) demonstrated that low preoperative AGR (<1.41) was an independent predictor of progression to MIBC (HR=1.81, 95% CI: 1.22–2.68; $p = 0.003$). Unlike the present study, pathological T-stage distribution and tumor grade did not differ between AGR groups at baseline in that cohort ($p = 0.77$ and $p = 0.21$, respectively), as its design evaluated progression risk during longitudinal follow-up rather than pathological stage at diagnosis.

Niwa et al. (4) evaluated 364 patients with primary NMIBC and found that low AGR (<1.6) was an independent predictor of tumor recurrence (multivariate HR=0.53, 95% CI: 0.36–0.78; $p < 0.01$). In the same study, low AGR was also reported to be significantly associated with progression to muscle invasion in univariate analysis (HR=0.24, 95% CI: 0.10–0.56; $p < 0.01$); however, multivariate analysis for progression was not performed. In that study, pathological T category and tumor grade did not differ significantly between AGR groups at baseline; however, the effect of AGR on the biological course of the disease was reflected in follow-up outcomes. In the present study, by contrast, this strong association was directly reflected in pathological staging at diagnosis, further emphasizing the predictive value of pre-TURBT AGR measurement for estimating pathological outcomes.

Schuettfort et al., (14) in a large multicenter series of 4,335 patients, reported a significant association between low AGR and pathological $\geq pT3$ disease after radical cystectomy (OR=1.15, 95% CI: 1.01–1.31; $p = 0.04$). However, pathological T-stage distribution did not differ significantly between AGR groups in that study ($p = 0.23$). This finding pertains to a radical cystectomy cohort and therefore has limited direct comparability with our TURBT-based results. In addition, the incorporation of AGR into existing clinical models was reported not to provide a meaningful improvement in AUC ($\Delta AUC < 1\%$). In contrast, in the present study, AGR was independently associated with MIBC in multivariate analysis, together with tumor size, tumor number, the presence of CIS, and sex. This discrepancy may partly be attributable to differences in patient population heterogeneity, as patients undergoing radical cystectomy generally have more advanced tumors at baseline, and to the fact that the distinction between NMIBC and MIBC can be more clearly captured in the TURBT setting.

Omura et al. (13) evaluated 179 patients with UTUC and demonstrated, using the Chi-square test, that low preoperative AGR (<1.25) was significantly associated with pathological T stage $\geq T3$ ($p < 0.01$) and lymph node involvement N1–3 ($p < 0.01$). Despite differences in tumor location and treatment modality, namely radical nephroureterectomy, these findings are conceptually consistent with those of the present study. They suggest that the association between low AGR and more advanced pathological stage may not be limited to bladder cancer but may reflect a common biological pattern across urothelial carcinoma.

No study in the current literature has evaluated the association between AGR and pathological tumor grade in primary bladder tumor as a primary endpoint. In this context, the closest basis for comparison is provided by studies reporting the relationship between AGR and Grade as a secondary pathological finding in patients with UTUC.

Xu et al., (12) in their study including 620 patients with UTUC, investigated the association between low preoperative AGR (<1.45) and adverse pathological outcomes using multivariate logistic regression. They demonstrated that low

AGR was an independent predictor of high-grade tumor (OR=2.35, 95% CI: 1.62–3.42; $p < 0.001$), $\geq$ pT2 stage (OR=1.34; $p < 0.001$), and lymphovascular invasion (OR=1.91; $p = 0.005$). This study provides one of the strongest pieces of evidence in the literature demonstrating the relationship between AGR and pathological Grade at the multivariate level. In the present study, a similar association was observed for high-grade disease, with an OR of 0.18. Despite the difference in tumor location, the consistency of the underlying biological mechanism is noteworthy.

In the study by Omura et al. (13), tumor grade alone was not significantly associated with AGR < 1.25 , whereas the dominant association was observed through pathological T stage. This finding suggests that the AGR-grade relationship may not have the same strength under all conditions and may vary according to patient population, cut-off value, and tumor characteristics.

The observation that CIS was present only in the high-grade group ($p = 0.001$), thereby causing complete separation in the multivariate grade model, should be considered an important methodological point. Since CIS is, by WHO/ISUP definition, classified as high-grade disease, this finding is an expected consequence of this classification rather than an independent biological validation, and should not be interpreted as additional evidence beyond what is already implied by the grading system itself.

In this study, the optimal AGR cut-off value determined by the Youden index was 1.35 for the prediction of both MIBC and high-grade disease. When the AGR cut-off values used in the literature are reviewed, they appear to range widely between 1.25 and 1.60: Xu et al. (12) reported 1.45, Otsuka et al. (9) 1.40, Schuettfort et al. (14) 1.42, Quhal et al. (5) 1.41, Omura et al. (13) 1.25, and Niwa et al. (4) 1.60. The cut-off value of 1.35 identified in the present study lies within the middle of this range. Notably, the cut-off of 1.35 identified in the present study closely approximates the mean AGR cut-off of 1,356 reported for overall survival across 61 studies in the systematic review by Roberts et al. (7), suggesting cross-methodological consistency.

The variation in cut-off values reported in the literature may be attributable to heterogeneity in patient populations, tumor location and biology, differences in statistical methods such as ROC-based approaches versus reference range-based thresholds, and interinstitutional variation in laboratory measurement conditions. Takada et al. (8) emphasized that ROC-based cut-off determination involves data-specific optimization and may therefore lead to optimism bias when applied to different cohorts; they further suggested that thresholds based on institutional reference ranges may be more reliable in terms of interinstitutional consistency and reproducibility. Establishing a universal AGR cut-off value specific to urothelial carcinoma requires large-scale prospective studies.

The considerable variability in the AGR cut-off values reported across the literature is noteworthy; values in existing studies range from 1.25 to 1.60. This variability can largely be attributed to differences in the patient populations studied: higher thresholds have generally been reported in NMIBC cohorts (4,5), whereas lower values have been observed in upper urinary tract urothelial carcinoma (9,16) and radical cystectomy series (17,18), with an intermediate value reported in a grade-specific cohort (19). Given this variability, it appears more realistic to regard AGR not as a fixed, binary cut-off but as a continuous risk indicator to be interpreted alongside established clinicopathological parameters. Indeed, in the present cohort, a low AGR value (particularly when accompanied by large tumor size, multiple tumors, or the presence of CIS) may raise preoperative suspicion for MIBC or high-grade disease, prompting closer intraoperative assessment during TURBT and, when warranted, earlier multidisciplinary discussion. On the other hand, given the modest sensitivity and specificity observed in our study, we wish to emphasize that a high AGR value alone should not be used to exclude the possibility of advanced-stage or high-grade disease. Until the cut-off value is validated in external cohorts and, ideally, institution-specific reference ranges are established, we suggest that AGR be used not as an independent decision-making tool, but as a supportive indicator contributing to existing preoperative risk assessment.

To date, no study has formally incorporated AGR into existing risk stratification tools (such as the EAU risk groups (15) or the EORTC (20) and CUETO (21) scoring systems) by adding it as a variable and recalibrating these nomograms accordingly. The closest evidence available comes from two sources. In a large NMIBC cohort, low AGR was not significantly associated with either recurrence-free survival ($p = 0.59$) or progression-free survival ($p = 0.22$) within any EAU risk group once patients were stratified accordingly (5). Along similar lines, a panel of systemic inflammatory biomarkers that included AGR was tested, using machine-learning methods, for predicting upstaging to MIBC, lymph node involvement, and advanced pathological stage in a radical cystectomy cohort, but was found to add only limited discriminative value beyond established clinicopathological variables (22). Together, these findings suggest that AGR may not offer an independent contribution beyond what existing risk stratification systems already provide; it should be kept in mind, however, that this evidence is drawn from a small number of studies and does not directly test integration into the EAU, EORTC, or CUETO frameworks. Whether incorporating AGR into such models would add value specifically for predicting pathological stage and grade (the focus of the present study) has not yet been tested, and this remains an open question for future prospective, externally validated research.

This study has several important methodological strengths. First, its prospective design allowed the implementation of a standardized protocol for patient selection and data collection, thereby minimizing retrospective selection bias. The early termination of patient recruitment due to the COVID-19 pandemic does not substantially compromise the methodological strength of the prospective design, as the post hoc power analysis demonstrated that the 80% threshold was exceeded for both comparisons.

The inclusion of only primary tumors eliminated the confounding effect arising from the distinct biological behavior of recurrent tumors and the potential influence of prior intravesical therapies on AGR. Similarly, the systematic exclusion of patients with active infection, autoimmune disease, hepatic pathology, nephrotic syndrome, or immunosuppressive drug use enabled control of the major confounders that could directly affect AGR levels independently of tumor biology. In this respect, the design of our study provided a setting in which the relationship between AGR and tumor biology could be evaluated in as purified a context as possible. Quhal et al. (5) also applied similar exclusion criteria in their multicenter design.

For stage and grade determination, re-TURBT was performed in accordance with EAU guideline recommendations, and the final pathological result was used as the basis for analysis. This approach helps overcome the common problems of insufficient muscle-containing specimens and understaging encountered in initial TURBT specimens, particularly with respect to stage and grade determination, thereby increasing the reliability of pathological assessment (4). Finally, the fact that the Youden index indicated the same cut-off value (1.35) in independent analyses for both clinical endpoints provide clinicians with a single threshold that is easy to remember and apply.

Several important limitations of this study should be considered. First, its single-center design limits the external validity and generalizability of the findings. This limitation becomes more evident when compared with multicenter designs such as the multicenter study by Quhal et al. ($n = 1,096$) and the large multicenter cohort by Schuetthfort et al. ($n = 4,335$) (5,14). Multicenter prospective studies and external validation cohorts are required to confirm the validity of our findings in different populations. Second, the exclusion criteria applied in this study were comprehensive: as shown in the flow diagram (Figure 1), 84 of 237 eligible primary tumor patients (35.4%) were excluded, most commonly due to missing preoperative laboratory data ($n = 64$), followed by incomplete resection ($n = 10$) and active infection or systemic disease ($n = 7$). While these criteria were necessary to minimize confounding of the AGR measurement, this comprehensive exclusion process may have resulted in a selected cohort that does not fully represent the broader, real-world population of patients undergoing TURBT for primary bladder tumor, which should be considered when interpreting the generalizability of our findings.

Early termination of recruitment due to the COVID-19 pandemic resulted in a smaller-than-intended sample, which may have reduced statistical power for subgroup analyses and limits the generalizability of findings — particularly for the MIBC subgroup ($n = 31$). Post hoc power analysis confirmed that the 80% threshold was exceeded for both primary comparisons; nevertheless, certain associations may remain undetected in this sample. Furthermore, given the relatively low events-per-variable ratio in the MIBC multivariate model (31 events for 7 covariates), coefficient estimates — including that of AGR — should be interpreted with appropriate caution, as they showed some sensitivity to covariate specification in sensitivity analyses.

The AUC values of AGR in both analyses (MIBC: 0.649, 95% CI: 0.542–0.755; high Grade: 0.656, 95% CI: 0.568–0.744) indicate moderate discriminatory ability. This finding suggests that AGR would be insufficient as a stand-alone decision-making biomarker and should instead be evaluated in combination with existing clinicopathological variables. This result is also consistent with the findings of Schuettfort et al. (14), in which the addition of AGR to existing models did not provide a meaningful improvement in AUC.

The higher proportion of female patients in the MIBC group ($p = 0.048$) indicates a potential demographic imbalance. Although this variable was controlled for in the multivariate model, the interpretation of the association between sex and MIBC requires caution in a predominantly male cohort ($n = 136$, 88.9%). Furthermore, the absence of follow-up data precludes evaluation of the association between preoperative AGR and long-term clinical outcomes such as recurrence, disease progression, and survival. This naturally limits the scope of interpretation of the present findings.

CONCLUSION

Preoperative AGR was independently associated with MIBC and high-grade disease in this single-center cohort; however, its incremental clinical value and generalizability require external validation. The absence of previous studies addressing this question as a primary endpoint in the current literature provides an original contribution to our findings.

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Conflict of Interest: The authors declare no conflict of interest.

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Ethical Approval: This study was approved by the Clinical Research Ethics Committee of University of Health Sciences İzmir Bozyaka Training and Research Hospital (Decision No: 3; Date: March 15, 2017). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Informed Consent: Written informed consent was obtained from all patients prior to inclusion in the study.

Author Contributions: Concept and Design: MEA, İB, DB, AA — Data Collection: MEA, İB, DB — Data Analysis and Interpretation: GC, İHB, SÇ, DB, TD — Writing the Manuscript: MEA, İB, GC, AA — Review of Manuscript Content: GC, İHB, EŞ, SÇ, TD — Statistical Analysis: MEA, İHB, EŞ, SÇ, AA, TD — Supervision: MEA, İB, İHB, EŞ, DB, AA

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