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Clinical-Bladder cancer

Objectifying grade in Ta-T1 urothelial carcinomas of the bladder using proliferative and quantitative markers: A multicentre study in 310 bladder tumors

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Abstract

Purpose: Histological grade is an important prognostic factor in patients with non-muscle−invasive bladder cancer (NMIBC). However, interobserver variability is high. Previous studies have suggested that quantification of histological features is useful to objectify grading. We evaluated whether quantification of the mean nuclear area of the 10 largest nuclei (MNA-10), degree of aneuploidy (DNA index or DI) and mitotic activity index (MAI) are of diagnostic value for NMIBC grade. Additionally, prognostic value of the 3 measures was assessed.

Material and methods: A consensus grade was determined by 3 uropathologists in 310 NMIBC tissues according to the World Health Organization (WHO) 1973 and the WHO2004. Logistic regression with forward selection was used to determine the optimal combination of measures (MNA-10, DI, and MAI) to diagnose grade 3 (G3) or high-grade (HG) NMIBC (WHO1973 and WHO2004, respectively).

Results: In 310 tumors of 215 patients at least 1 of the measures (MNA-10, DI, or MAI) had been determined. The combination of MNA-10 and MAI was selected as the most diagnostic combination and resulted in a sensitivity of 94% (95% confidence interval [CI]: 87−100) at a specificity of 72% (95% CI: 66−78) for G3 tumors. For the diagnosis of HG tumors sensitivity was 92% (95% CI: 86−97) at a specificity of 76% (95% CI: 70−93).

Conclusions: Determination of MNA-10 and MAI is promising for diagnosing G3 and HG bladder tumors. These findings warrant further studies on the diagnostic and prognostic value of proliferative and quantitative features in bladder cancer patients.

Keywords: DNA index; Grading; Interobserver variation; MAI; MNA-10; Urinary bladder neoplasm

1. Introduction

Management of non-muscle−invasive bladder cancer (NMIBC) is challenging as the disease recurs in 30% to 80% and progresses to muscle-invasive disease in 1% to 45% [1]. To predict progression and recurrence, several prognosticators are used. Based on these prognosticators, patients are stratified into different risk groups and each risk group is linked to specific treatment and follow-up recommendations [2]. Histological grade is 1 of these prognostic factors and can define risk group assignment [2,3].

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Therefore, adjuvant treatment partly depends on histological grade, ranging from a single immediate intravesical installation with chemotherapy to Bacillus Calmette-Guérin installations during 1 to 3 years or even radical cystectomy in the highest risk group [2].

The World Health Organization (WHO) 1973 classification distinguishes grade 1 (G1) to 3 tumors according to the level of cellular anaplasia with G1 being "the least" and grade 3 (G3) being "the most severe" degree of anaplasia, in the absence of clear criteria for assigning these different grades [4]. In the WHO2004, an attempt is made to define which histologic features suit the different tumor types (low- and high-grade [HG] carcinomas). For example, in low-grade (LG) tumors, mitoses are occasionally present and the nuclei are enlarged with some variation, whereas in HG tumors, mitoses may be atypical and are usually frequent at any level while the nuclei are enlarged with variation. Also, architectural changes in the papillae and the organization of cells are considered. [5] However, the descriptions on cytological and architectural features of the different grades leave room for interpretation, leading to interobserver variability in histological grading [6,7]. As a result, some patients might receive unnecessary aggressive treatments, while others are withheld from adequate therapy. Therefore, a more reproducible method is needed.

Several previous studies suggested that quantification of morphometric and proliferative features could fulfill this role in NMIBC [8−10]. In the current study, we investigate the diagnostic value of 3 potential quantifiable pathological features of NMIBC; nuclear size, DNA index, and mitotic rate. Giant nuclei, DNA aneuploidy, and a high mitotic rate are well-known characteristics of HG/G3 bladder cancer which were assessed by measurement of the mean nuclear area of the 10 largest nuclei (MNA-10), flow cytometry, and the mitotic activity index (MAI), respectively [9]. We analyzed the association of each of these features with grade as well as with clinical prognosis in NMIBC.

2. Material and methods

2.1. Patients

Histological grade was based on data from a previous study on interobserver variability [11]. In that study, 3 uropathologists assessed histological grade according to both the WHO1973 and the WHO2004 classification. Discrepancies between the WHO1973 and WHO2004 grade assigned by the 3 pathologists were resolved in consensus meetings. The pathologists were blinded for the initially assigned grade and clinical information. The eventual T-category and grades assigned in consensus meetings were defined as "true" histological diagnosis. All patients in whom MNA-10, DNA index (DI), or MAI were determined were included in the current study. Histologic tissues were obtained from 3 hospitals in the Netherlands (Amsterdam UMC, location VUmc; Amsterdam UMC, location AMC, and Amstelland Hospital).

2.2. Determination of morphometric and proliferative features

As part of standard clinical practice, the resected tumor tissues were fixed in formalin, embedded in paraffin, and 4 μm slides were cut and stained with hematoxylin and eosin. For this study, tissue slides were collected from the archive. The MNA-10 was determined using a protocol previously described by another study [12]. Briefly, MNA-10 was calculated by determining the mean of the 10 largest nuclei in the worst differentiated area of the tumor, as marked by the pathologist, at a magnification of 400×. DNA flow cytometry of the formalin-fixed paraffin-embedded tissues was used to assess the DI as an extent of cell ploidy. Both MNA-10 and DI were determined as part of standard daily practice by a single technician. The MAI was determined according to a protocol previously described by others [13]. Briefly, mitotic figures were counted in 10 consecutive fields of vision. All fields of vision consisted of urothelial tumor cells for at least 75%.

2.3. Follow-up and treatment

Cystoscopy was performed every 3 months in the first year, and every 6 to 12 months thereafter in case of no recurrence. A computed tomography scan of the upper urinary tract was conducted with a 2-year interval in high-risk patients, or in case of clinical suspicion for an upper urinary tract tumor.

2.4. Statistical analysis

Continuous data were summarized with mean, first, and third quartiles. The χ^2 test was used in the analysis of cross-tables. The independent samples t test was used to compare means of continuous data between groups. Associations between the consensus grade and the measures were analyzed using the Kruskal−Wallis test (WHO1973) and the Mann-Whitney U test (WHO2004). Receiver operating characteristic (ROC) curves were plotted with the values of each measurement and the area under the curve (AUC) was calculated. Cut-offs were obtained by Youden's J index [14].

To determine the best combination of measures for the diagnosis of a G3 or HG tumor, multivariable binary logistic regression was used with forward selection. The WHO1973 was converted to a 2-tiered system (G1/2 vs. G3) and the MAI and DI were analyzed as categorical variables. The MAI was categorized as 0, 1 to 5, 6 to 10, 11 to 20, and >20 mitoses. The DI was categorized into diploid (DI of 1), near diploid (DI >1−1.3), low aneuploid (DI

>1.3–1.9), tetraploid (DI >1.9–2.1), and high aneuploid (DI >2.1) [9]. The *P* value for entry was <0.05. From the final combination of measures and the associated predicted probabilities, ROC curves were plotted and the AUC was determined. The final combination of measures was considered positive if at least 1 of the measures was positive ("believe-the-positive") [15]. Additionally, the discriminative value of the measures for the diagnosis of a G2-HG vs. a G2-LG tumor was determined in the same manner.

Prognostic value of both classifications (WHO1973 and WHO2004) and the 3 measures (MNA-10, DI, and MAI) for progression and recurrence were analyzed in a Cox proportional-hazard model. Recurrence was defined as tumor growth in the bladder, treated by resection (and histological evaluation), cauterization, or laser. Progression was defined as the development of muscle-invasive/metastatic disease. To account for confounding, multivariable Cox models were used. For progression, taken into account the small number of events ($n = 30$), forward-stepwise-selection was used to avoid overfitting. Confounders tested were: T-category, number of tumors (single/multiple), primary/recurrence, and tumor size (<3 cm/≥3 cm). For recurrence there was no problem with overfitting ($n = 97$ events). Therefore, all aforementioned variables were corrected in the models.

3. Results

3.1. Patient and tumor characteristics

The MNA-10, DI, or MAI was known in 218 patients. In 3 patients, the consensus diagnosis changed the T-category from T1 to T2. These patients were excluded. Baseline characteristics of the remaining 215 patients are shown in Table 1. In these 215 patients, at least 1 out of 3 measures (MNA-10, DI, or MAI) was known in 310 tumors. The MNA-10 was determined in 84% (259/310), DI in 55% (171/310), and MAI in 97% (302/310) of these tumors, respectively.

3.2. Association between the measures and consensus grade

There was a significant association between MNA-10, MAI, DI, and both grades (WHO1973 and WHO2004) (Fig. 1). The AUC's, sensitivities, and specificities of each measure are presented in Table 2. The highest AUC was observed for MNA-10. The ROC-curves per measure are shown in Fig. 2.

3.3. Discrimination of multiple measures

The combination of MNA-10 and MAI was selected by forward selection for both the WHO1973 and the WHO2004 grade. For all patients in whom both measures were known, the ROC-curves of the predicted probabilities of MNA-10 and MAI are shown in Fig. 3. The AUC for MNA-10 and MAI was 0.92 (95% confidence interval (CI): 0.88–0.96) for G3 tumors as well as for HG tumors. For G3 tumors, the sensitivity of the combination of both the

Table 1
Baseline characteristics of all included patients.

Characteristic	Result
Sex, *n* (%)	
Male	153 (71)
Female	62 (29)
Age, y, median (IQR)	71 (62–78)
Prior recurrence rate, *n* (%)	
Primary tumor	191 (89)
≤1 recurrence/y	20 (9.3)
>1 recurrence/y	4 (1.9)
Number of tumors, *n* (%)	
Solitary	145 (67)
Multiple	70 (33)
Tumor size, *n* (%)	
<3 cm	117 (54)
≥3 cm	74 (34)
Missing	24 (11)
T category, *n* (%)	
Ta	194 (90)
T1	21 (9.8)
CIS, *n* (%)	
No	215 (100)
Yes	0
Grade WHO1973, *n* (%)	
G1	34 (16)
G2	129 (60)
G3	52 (24)

CIS = carcinoma in situ; G = grade; IQR = interquartile range; WHO = World Health Organization.

Table 2
Morphometric measures evaluated as binary markers for grade 3 (WHO1973) and high-grade (WHO2004) tumors. Cut-offs were determined with Youden's J index.

WHO1973 (G1-2 vs. G3)						
Measure	AUC	95% CI	Sens	95% CI	Spec	95% CI
MNA-10	0.88	0.84–0.93	86	76–95	82	77–88
DI	0.83	0.74–0.93	77	62–92	89	83–94
MAI	0.85	0.79–0.91	74	62–86	84	79–88

WHO2004						
Measure	AUC	95% CI	Sens	95% CI	Spec	95% CI
MNA-10	0.87	0.82–0.92	78	70–86	88	83–93
DI	0.76	0.67–0.85	66	53–79	81	74–88
MAI	0.83	0.78–0.88	70	61–79	84	79–89

DI = DNA Index; G = grade; MAI = mitotic activity index; MNA-10 = mean nuclear area of the 10 largest nuclei; WHO = World Health Organization.

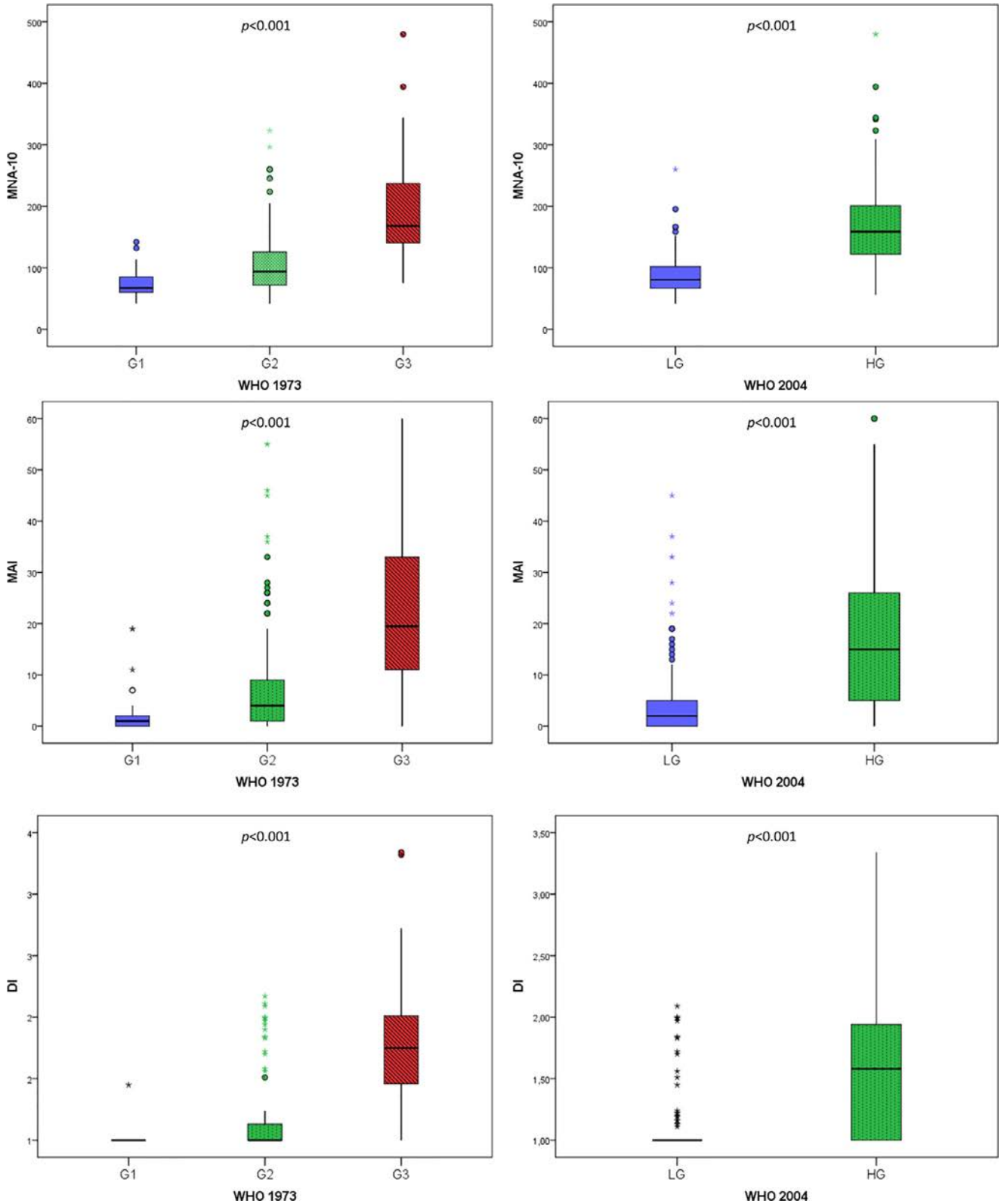

Fig. 1. Association between mean nuclear area of the 10 largest nuclei (MNA-10) (a), mitotic activity index (MAI) (b), DNA index (DI) (c), and the consensus grade according to the World Health Organization (WHO) 1973 and the WHO2004.

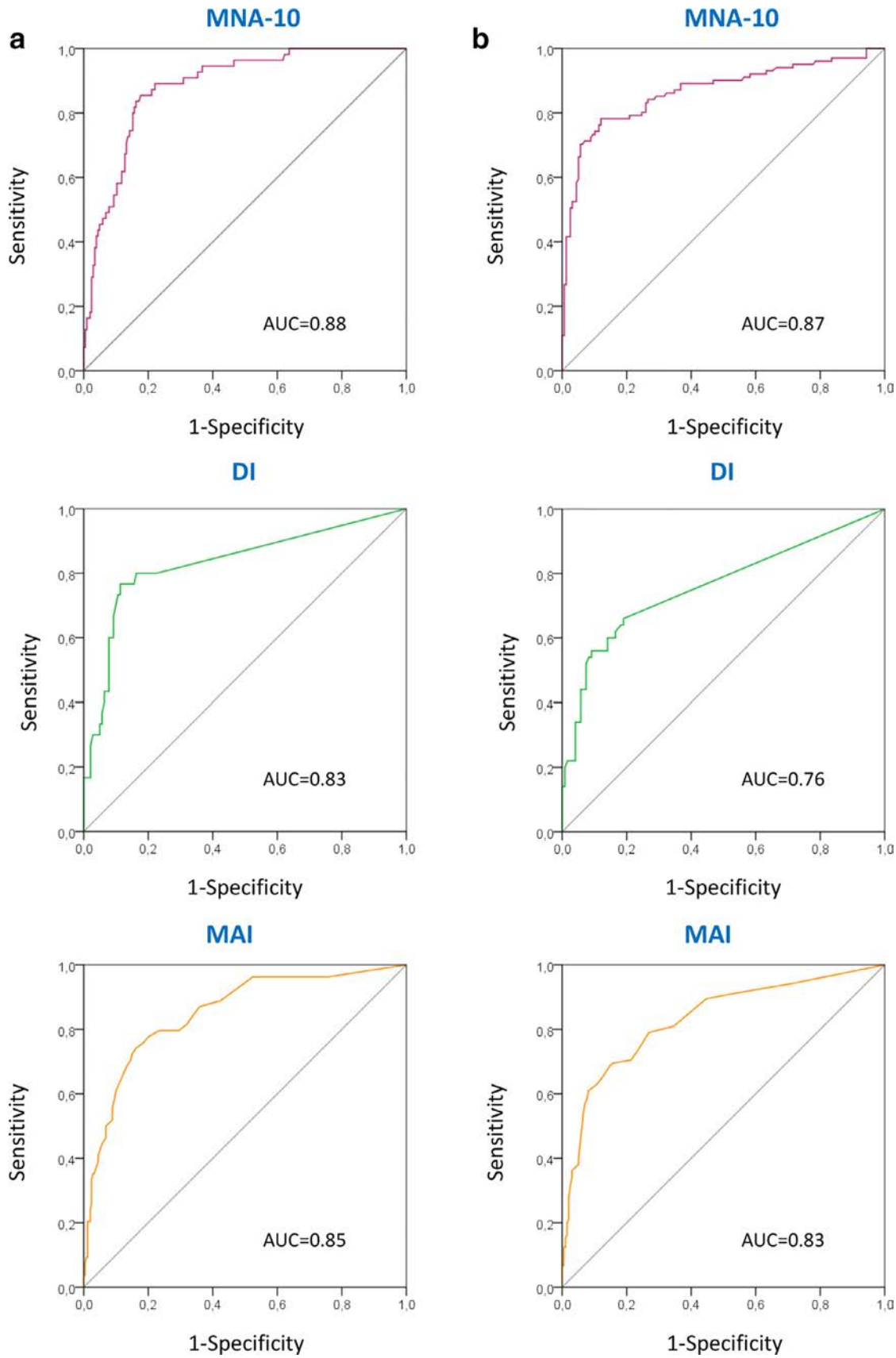

Fig. 2. Receiver operator characteristic (ROC) curves and area under the curve (AUC) of the measures MNA-10, DI, and MAI for the diagnosis of grade 3 tumors of the World Health Organization (WHO) 1973 (a) and high-grade tumors (WHO2004) (b). DI = DNA index; MAI = mitotic activity index; MNA-10 = mean nuclear area of the 10 largest nuclei.

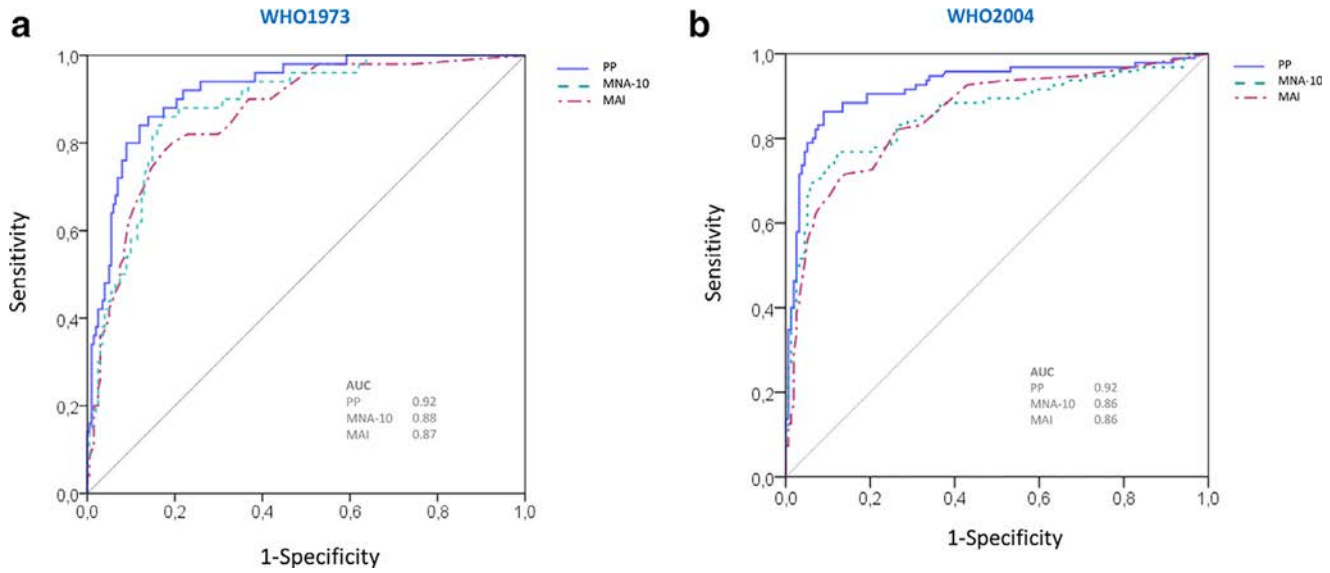

Fig. 3. Receiver operator characteristic (ROC) curves and area under the curve (AUC) of the measures MNA-10 and MAI and the accompanying predicted probabilities (PP) for the diagnosis of grade 3 tumors of the World Health Organization (WHO) 1973 (a) and high-grade tumors (WHO2004) (b). MAI = mitotic activity index; MNA-10 = mean nuclear area of the 10 largest nuclei.

MNA-10 and MAI ("believe-the-positive") was 94% (95% CI: 87−100) at a specificity of 72% (95% CI: 66−78). For the diagnosis of HG tumors, sensitivity was 92% (95% CI: 86−97) at a specificity of 76% (95% CI: 70−93).

3.4. Association and discriminative value of the three measures and G2-HG vs. G2-LG tumors

MNA-10 and MAI were significantly associated with G2-HG vs. G2-LG tumors ($P < 0.001$). There was no association between DI and G2-HG vs. G2-LG tumors ($P = 0.076$). The combination of MNA-10 and MAI was selected by forward selection. For patients in whom both MNA-10 and MAI were known, the ROC-curves of the predicted probabilities of the combined measures are shown in Supplementary Fig. 1. The AUC for MNA-10 and MAI was 0.85 (95% CI: 0.77−0.93) for the diagnosis of G2-HG tumors. For the discrimination of G2-HG from G2-LG tumors, sensitivity was 83% (95% CI: 72−93) at a specificity of 71% (95% CI: 63−78).

3.5. Prognostic performance

3.5.1. Recurrence

After a median follow-up of 60 months (interquartile range: 32−84), 97 patients developed a recurrence. None of the measures influenced recurrence in univariable or multivariable analysis (Table 3). Variables that predicted recurrence in the multivariable model were prior recurrence rate and tumor size.

3.5.2. Progression

After a median follow-up of 60 months (interquartile range: 32−84), 30 patients developed progression (muscle-invasion $n = 26$, metastasis $n = 4$). In univariable analysis, both grades (WHO1973 and WHO2004), MAI and MNA-10 influenced progression (Table 3). Using forward selection, T-category and recurrence rate were added to the model. In this multivariable model, only WHO2004 predicted progression.

4. Discussion

In this study, we analyzed the diagnostic value of the quantification of 3 BC features for BC grade and found that the combination of MNA-10 and MAI resulted in a sensitivity of 94% at a specificity of 72% for G3 vs. G1/G2 tumors. For HG tumors, sensitivity was 92% at a specificity of 76%. Also, G2-HG tumors could be discriminated from G2-LG tumors with a sensitivity of 83% at a specificity of 71%. These findings show that quantification of histopathological features holds promise as a diagnostic tool for BC grade.

Determining grade by quantifying BC features could especially be useful for cases in which BC grade is decisive in the eventual treatment plan and/or the pathologist doubts between G2 and G3. Given the high sensitivity found (94%) with MNA-10 and MAI, a negative result would then lead to a G2 diagnosis whereas a positive results could be reason to consult an expert panel.

Both MNA-10 and MAI have been previously described to be of value in BC in terms of predicting progression

Table 3
Prediction of time to recurrence and time to progression for both grades (WHO1973 and WHO2004) and 3 morphometric measures (MNA-10, DI, and MAI).

	Recurrence			Progression		
Uncorrected analysis	HR	95% CI	*P* value	HR	95% CI	*P* value
MAI			0.07			0.037
0	1.00	–		1.00	–	
1–5	0.89	0.47–1.68		0.42	0.09-1.86	
6–10	2.01	1.01–4.03		1.67	0.42-6.69	
11–20	1.47	0.74–2.92		1.88	0.53-6.65	
>20	1.63	0.83–3.21		3.07	0.94-9.99	
DI			0.9			0.058
Diploid	1.00	–		1.00	–	
Near diploid	0.74	0.27–2.06		2.97	0.60-14.8	
Tetraploid	1.15	0.54–2.45		1.88	0.38-9.33	
High aneuploid	0.94	0.34–2.62		6.64	1.65-26.6	
MNA-10 (per 10 μm^2)	0.99	0.97–1.02	0.7	1.05	1.01-1.09	0.012
WHO1973 (G3)	1.24	0.79–1.96	0.3	2.48	1.19-5.16	0.015
WHO2004	1.16	0.78–1.74	0.5	3.47	1.59-7.60	0.002
Corrected analysis*	HR	95% CI	*P* value	HR	95% CI	*P* value
MAI			0.7			0.2
0	1.00	–		1.00	–	
1–5	0.86	0.43–1.69		0.43	0.10–1.91	
6–10	1.85	0.85–4.01		1.40	0.35–5.67	
11–20	1.52	0.71–3.28		1.56	0.42–5.80	
>20	1.46	0.66–3.22		2.50	0.69–9.05	
DI			0.9			0.2
Diploid	1.00	–		1.00	–	
Near diploid	0.74	0.23–2.43		2.97	0.56–15.6	
Tetraploid	1.22	0.52–2.88		1.15	0.21–6.46	
High aneuploid	0.88	0.26–3.04		4.24	0.86–20.8	
MNA-10 (per 10 μm^2)	0.97	0.93–1.01	0.2	1.02	0.98–1.07	0.3
WHO1973 (G3)	0.87	0.49–1.57	0.7	1.37	0.55–3.41	0.5
WHO2004	0.93	0.57–1.51	0.8	2.69	1.14–6.33	0.023

CI = confidence interval; DI = DNA index; HR = hazard ratio; MAI = mitotic activity index; MNA-10 = mean nuclear area of the 10 largest nuclei; WHO = World Health Organization.

* Corrected for T-category, tumor size, number of tumors, and prior recurrence rate (recurrence); corrected for prior recurrence rate and T-category (progression).

[9,10]. However, development of progression depends on many factors, such as the number of tumors, tumor size, prior recurrence rate, T-category, and the presence of carcinoma in situ [3]. Furthermore, patients may have already received additional treatment based on the initially assigned grade and it is known that treatment may reduce risk of progression [16,17]. Correcting for all of these confounders complicates the statistical model, especially in case of a limited sample size. For these reasons, we have used BC grade as the primary outcome instead. To determine the "true" pathological grade, 3 uropathologists held consensus meetings in which the diagnosis was adjusted to the consensus grade. With this approach, BC grade is still not fully objective, but the diagnosis of 3 experienced uropathologists does strengthen the final diagnosis.

To further optimize the diagnostic value of the quantification of BC features, complementary measures or markers should be investigated. Furthermore, digitalization of pathological slides and computerized measures could aid in making these measures practical for everyday use. Studies on other cancer types have shown that algorithms developed for pathologic slides may even predict patients' outcome [18,19].

Although rare, there are histological variants of urothelial carcinomas of the bladder, such as the nested and plasmacytoid variant. Based on their histologic features, e.g., nuclear size, these tumors may appear mild, but they have the potential to act aggressively [20]. We did not include these variants in our study, but the measures MAI, MNA-10, and DI might be of limited value in these particular cases of urothelial cancer.

Strengths of our study include the large sample size, the use of a consensus grade, and the lack of intra-observer variability. The last was avoided by assigning WHO1973 and WHO2004 grades simultaneously in 1 session. It is important to note that our study also has several limitations. The number of patients with progression was small and therefore we could not correct for all known prognosticators. Furthermore, our study was retrospective and therefore these findings

should be confirmed in a prospective setting. Also, the majority of patients were diagnosed with a grade 2 tumor, which is in accordance with standard clinical practice.

5. Conclusions

MNA-10 and MAI are promising aids to determine histological grade in NMIBC in an objective way. These findings warrant further studies on the diagnostic and prognostic value of proliferative and quantitative features in NMIBC.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2019.03.002.

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