

Original Article

Nomograms including the UBC[®] Rapid test to detect primary bladder cancer based on a multicentre dataset

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Objectives

To evaluate the clinical utility of the urinary bladder cancer antigen test UBC[®] Rapid for the diagnosis of bladder cancer (BC) and to develop and validate nomograms to identify patients at high risk of primary BC.

Patients and Methods

Data from 1787 patients from 13 participating centres, who were tested between 2012 and 2020, including 763 patients with BC, were analysed. Urine samples were analysed with the UBC[®] Rapid test. The nomograms were developed using data from 320 patients and externally validated using data from 274 patients. The diagnostic accuracy of the UBC[®] Rapid test was evaluated using receiver-operating characteristic curve analysis. Brier scores and calibration curves were chosen for the validation. Biopsy-proven BC was predicted using multivariate logistic regression.

Results

The sensitivity, specificity, and area under the curve for the UBC[®] Rapid test were 46.4%, 75.5% and 0.61 (95% confidence interval [CI] 0.58–0.64) for low-grade (LG) BC, and 70.5%, 75.5% and 0.73 (95% CI 0.70–0.76) for high-grade (HG) BC, respectively. Age, UBC[®] Rapid test results, smoking status and haematuria were identified as independent predictors of primary BC. After external validation, nomograms based on these predictors resulted in areas under the curve of 0.79 (95% CI 0.72–0.87) and 0.95 (95% CI: 0.92–0.98) for predicting LG-BC and HG-BC, respectively, showing excellent calibration associated with a higher net benefit than the UBC[®] Rapid test alone for low and medium risk levels in decision curve analysis. The R Shiny app allows the results to be explored interactively and can be accessed at www.blucab-index.net.

Conclusion

The UBC[®] Rapid test alone has limited clinical utility for predicting the presence of BC. However, its combined use with BC risk factors including age, smoking status and haematuria provides a fast, highly accurate and non-invasive tool for screening patients for primary LG-BC and especially primary HG-BC.

Keywords

bladder cancer, diagnosis, logistic model, nomogram, UBC[®] Rapid test, urothelial cancer, #uroonc, #BladderCancer, #blcsm

Introduction

Urinary bladder cancer (BC) is the tenth most prevalent cancer, affecting mainly the elderly and men [1]. The global incidence and mortality rates for BC are fourfold higher in men than in women (9.6 and 3.2 per 100 000 in men vs. 2.4 and 0.9 per 100 000 in women, respectively) and it shows a peak incidence at the age of 70–79 years [1,2]. Tobacco smoking is the most relevant risk factor for BC, with summary odds ratios of 3.14 for current smokers and 1.83 for former smokers [3]. Even 20 years after quitting, ex-smokers have a 50% higher risk of developing BC than non-smokers [3].

The suspicion of BC often arises after the detection of microscopic or macroscopic (gross) haematuria during a patient's examination [4]. Although the need for examination of all patients with macroscopic haematuria for the presence of BC is indisputable, this evaluation in patients with microscopic haematuria is debatable [5]. A multicentre study including patients with newly diagnosed BC found that, although patients with macroscopic haematuria presented with a higher stage of disease than patients with microscopic haematuria, the groups had a similar tumour grade distribution [4]. BC is typically diagnosed by cystoscopy and treated by complete transurethral resection of all visible lesions and pathological examination. The current guidelines define cystoscopy and cytology as the standard procedures for the detection and surveillance of BC [6]. Cystoscopy is a minimally traumatic and reliable procedure that can detect most cases of BC; however, it is costly and invasive, and is associated with complications such as UTI [7]. Furthermore, cystoscopy has limited sensitivity for flat lesions such as carcinoma *in situ* (CIS), and this invasive procedure may lead to patient discomfort [8]. Urine cytology is, in combination with cystoscopy, the most commonly used method for the early detection of BC, and has a high specificity of 83%–100% [9]. However, it has a low sensitivity, especially for low-grade (LG) BC, where it is in the range of 4%–31% compared with 20%–53% for all patients [9].

An accurate urinary marker for BC could be valuable in identifying high-risk patients and reducing the number of control cystoscopies. The broad spectrum of point-of-care (POC) urinary analysis tests currently available facilitates the rapid, non-invasive and cost-efficient determination of urinary markers. However, because these tests have a high

rate of false-positive cases, their diagnostic accuracy is controversial and their use is not recommended in the current guidelines [9–11]. The UBC[®] Rapid test (IDL Biotech, Bromma, Sweden) is a quantitative immunochromatographic POC assay that measures soluble fragments of cytokeratin-8 and -18 in urine, and achieves a sensitivity of 53%–71% and a specificity of 61%–96% [12–19]. The UBC[®] Rapid test shows particularly good results in the detection of high-grade (HG) BC and CIS [12–19]. Despite efforts to quantify the influence of known risk factors on BC and to assess the quality of diagnostic tools, their combined contribution, in which confounding effects are eliminated, is more difficult to measure. This study investigated the diagnostic accuracy of the UBC[®] Rapid test for BC and proposes models for the prediction of primary BC based on the UBC[®] Rapid test in combination with established predictors of BC. The findings were validated in an independent external validation cohort.

Patients and Methods

Patients

This retrospective study included 1877 patients recruited from 13 study centres. Of these, 90 were excluded because of lack of data or unclear tumour status. The patient population used to determine the overall diagnostic accuracy of the UBC[®] Rapid test comprised tumour patients with confirmed newly diagnosed BC and patients with recurrent BC. The control group included patients with no history of BC (healthy controls) and patients with a history of BC but no evidence of disease (NED). For the development of the risk assessment nomograms for primary BC, the patient population was narrowed down to patients with primary BC and the control group to healthy controls. Patient data were collected independently at each study centre between 2012 and 2020 and pooled in October 2020.

The protocol included cystoscopy as the 'gold standard' for detecting BC in patients with tumours and patients with NED. In cases of cystoscopy-verified tumours, transurethral resection of the bladder or biopsies were performed; samples were assessed for tumour grade according to the 2004 WHO classification [20] and for clinical stage according to the 2009 TNM classification [21]. Exclusion criteria were any kind of mechanical manipulation (surgery, biopsy, instillation, catheterization) within 10 days before urine

sampling, UTIs, kidney and bladder stones, neobladder, and pregnancy.

Urine samples were analysed with the POC UBC[®] Rapid test (IDL Biotech). The results were quantified by the Concile[®] Ω 100 POC reader (Concile, Freiburg/Breisgau, Germany). The tests were performed according to the manufacturer's protocols. In several study centres the UBC[®] Rapid test was performed several times simultaneously for each patient. This was due to varying study protocols. The results from these centres were then averaged. Table 1 provides an overview of patient numbers and study approvals by the local ethics committees of the respective study centres. Written consent was obtained from each patient prior to study enrolment.

Statistical analysis

The mean concentrations of the UBC[®] Rapid test were compared using Welch's two-sided *t*-test. The diagnostic accuracy of the UBC[®] Rapid test was evaluated by receiver-operating characteristic (ROC) curve analysis. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the area under the curve (AUC) were calculated at the optimal cut-off identified using the maximal Youden index.

For a subgroup of 291 patients with primary bladder cancer and 303 healthy controls from HELIOS Hospital Bad Saarow, University Hospital Schleswig-Holstein Campus Lübeck and Lukas Hospital Neuss (all Germany), data on established risk factors were available. This subgroup was divided randomly into a development cohort of 320 patients and a validation cohort of 274 patients. Exclusion of patients with HG-BC from the development and validation group of the LG model and vice versa resulted in group sizes of $n = 244$ and $n = 200$ for the development and validation of the LG model, respectively, and group sizes of $n = 237$ and $n = 216$ for the development and validation of the HG model, respectively. Patients with CIS were included in the HG group according to the 2004 WHO classification [20].

The cohorts were compared using analysis of variance (ANOVA) and chi-squared tests. Multivariate logistic regression models were fitted within the development cohort. The association of potential risk factors, including UBC[®] Rapid test results, age, smoking status and haematuria, with the presence of BC was investigated, and the results were reported with odds ratios and 95% CIs. A backward stepwise selection based on the lowest Akaike information criterion was used to develop the most parsimonious and accurate model. Two separate models to predict primary LG-BC and HG-BC were developed and depicted in the nomogram format. Both models were externally validated in a validation cohort. External validation focused on AUC, calibration

curves, Brier scores, decision curve analysis and cut-off analysis.

A significance level of $\alpha = 0.05$ (two-tailed) was applied to all *P* values. Statistical analysis was performed using R (version 4.0.2 R Project for Statistical Computing) within Rstudio Inc. (Boston, MA, USA).

Results

Regarding the clinical characteristics of patients, the cohort reflected the population of patients suspected of having BC, with a high proportion of men (76%) and senior patients overall (Table 2). The optimal threshold for the quantitative UBC[®] Rapid test, according to the Youden index, was a cut-off ≥ 9.88 $\mu\text{g/L}$. The results of the UBC[®] Rapid test were dichotomized for further analysis.

The median cytokeratin concentrations of the UBC[®] Rapid test were 8.7 $\mu\text{g/L}$ in LG-BC, and 32.1 $\mu\text{g/L}$ in HG-BC excluding CIS, 29.3 $\mu\text{g/L}$ in patients with concomitant CIS and 19.4 $\mu\text{g/L}$ in patients with solitary CIS, respectively. The mean cytokeratin concentration of the UBC[®] Rapid test was significantly higher in tumour patients (56.1 $\mu\text{g/L}$) than in non-tumour patients (14.8 $\mu\text{g/L}$; $P < 0.001$). Furthermore, the mean cytokeratin concentration was lower in LG-BC patients (35.1 $\mu\text{g/L}$) than in patients with HG-BC excluding CIS (76.2 $\mu\text{g/L}$; $P < 0.001$) and lower than in patients with concomitant CIS (87.8 $\mu\text{g/L}$; $P < 0.001$), but not significantly different from the concentration in patients with solitary CIS (34.7 $\mu\text{g/L}$; $P = 0.972$). The mean cytokeratin concentrations in patients with primary BC (61.1 $\mu\text{g/L}$; $P = 0.395$) were not significantly different from those in patients with recurrent BC (54.8 $\mu\text{g/L}$). The median cytokeratin concentrations were at 11.1 $\mu\text{g/L}$ and 18.3 $\mu\text{g/L}$, respectively.

The overall sensitivity, specificity and AUC for the UBC[®] Rapid test alone were 60.4%, 75.5% and 0.68 (95% CI 0.66–0.70), respectively. Both sensitivity and AUC were higher for HG-BC excluding CIS (70.5% and 0.73, 95% CI 0.70–0.76) than for LG-BC (46.4% and 0.61, 95% CI 0.58–0.64). Concomitant CIS was detected with a sensitivity of 85.2%, with an AUC of 0.80 (95% CI 0.76–0.85), while solitary CIS was detected with a sensitivity of 68.0% and an AUC of 0.72 (95% CI 0.62–0.81). The PPVs for all patients, and those with LG-BC, HG-BC, concomitant and solitary CIS were 64.7%, 36.1%, 46.4%, 17.2% and 6.3%, respectively. The NPVs were 71.9%, 82.5%, 89.5%, 98.8% and 99.0%, respectively. The UBC[®] Rapid test detected primary BC with a higher sensitivity (65.3%), specificity (79.0%) and AUC (0.72; 95% CI 0.69–0.75) than it did recurrent BC, for which the sensitivity, specificity and AUC were 54.1%, 71.1% and 0.63 (95% CI 0.58–0.67), respectively. There was no significant difference in tumour grade distribution between the screening group, which consisted of patients with primary BC and healthy controls, and the surveillance group, which

Table 1 Overview of study approvals and patient numbers in the respective study centres.

Study centre	Regional ethics committee	Reference number	n	Tumour, n (%)	Control, n (%)	Excluded, n (%)
University Hospital Innsbruck, Austria	Local ethics committee of the Medical University of Innsbruck	AN2016-0056; 360/4.7	75	31 (41.3)	44 (58.7)	0 (0.0)
University Hospital Tübingen, Germany	Local ethics committee of the Medical University of Tübingen	032/2013BO2	198	61 (30.8)	137 (69.2)	0 (0.0)
University Hospital of Umeå, Sweden	Regional ethical board of the Umeå University	2012-478-31M/2013-364-32M	112	35 (31.2)	66 (58.9)	11 (9.8)
Danderyd Hospital, Sweden	Regional ethical board of the Umeå University	2012-478-31M/2013-364-32M	31	31 (100)	0 (0.0)	0 (0.0)
Enköping Hospital, Sweden	Regional ethical board of the Umeå University	2012-478-31M/2013-364-32M	13	12 (92.3)	0 (0.0)	1 (7.7)
Uppsala University Hospital, Sweden	Regional ethical board of the Umeå University	2012-478-31M/2013-364-32M	120	37 (30.8)	68 (56.7)	15 (12.5)
Uppsala University Hospital, Sweden	Regional ethics committee of Uppsala	2017/127	96	9 (9.4)	84 (87.5)	3 (3.1)
Enköping Hospital, Sweden	Regional ethics committee of Uppsala	2017/127	85	11 (12.9)	72 (84.7)	2 (2.4)
Fundació Puigvert, Universitat Autònoma de Barcelona, Spain	Ethics committee for investigation with medicinal products (CEIm) of Fundació Puigvert	2017/00486	30	6 (20.0)	23 (76.7)	1 (3.3)
National University Health System, Singapore	National Healthcare Group Domain Specific Review Board	AS147(bb)/2013	460	242 (52.6)	197 (42.8)	21 (4.6)
HELIOS Hospital Bad Saarow, Germany	Local ethics committee of the Landesärztekammer Brandenburg	AS147(bb)/2013	113	47 (41.6)	59 (52.2)	7 (6.2)
Charité – Universitätsmedizin Berlin, Germany	Local ethics committee of the Landesärztekammer Brandenburg	AS147(bb)/2013	313	111 (35.5)	178 (56.9)	24 (7.7)
Rheinland Klinikum Neuss, Germany	Local ethics committee of the Landesärztekammer Brandenburg	2019071	64	36 (56.2)	26 (40.6)	2 (3.2)
Rheinland Klinikum Neuss, Germany	Landesärztekammer Nordrhein	2019024992; 6260R (MPG §23b)	25	13 (52.0)	11 (44.0)	1 (4.0)
University Hospital Düsseldorf, Germany	Local ethics committee of the Heinrich Heine University Düsseldorf	19-214	30	30 (100)	0 (0.0)	0 (0.0)
University Hospital Schleswig-Holstein, Campus Lübeck, Germany	Local ethics committee of the University Lübeck					

Table 2 Clinical characteristics of the patient cohort and results of the UBC[®] Rapid test with a cut-off of 9.88 µg/L.

	All patients	Tumour	Primary tumour	NED	Healthy control	Development	Validation	P ^a
N	1787	763	453	456	568	320	274	
Age, years								
Mean (SD) [†]	69.0 (12.8)	71.8 (10.7)	71.1 (11.0)	70.6 (10.5)	64.2 (15.5)	69.5 (12.2)	69.6 (12.5)	0.95
Range	20–98	26–98	26–95	22–92	20–93	26–92	20–94	
Gender, n (%)								
Male	1355 (76)	597 (78)	346 (76)	353 (77)	405 (71)	238 (74)	192 (70)	0.28
Female	432 (24)	166 (22)	107 (24)	103 (23)	163 (29)	82 (26)	82 (30)	
UBC[®] Rapid								
Positive, n (%)	712 (40)	461 (60)	296 (65)	132 (29)	119 (21)	113 (35)	105 (38)	0.50
Negative, n (%)	1075 (60)	302 (40)	157 (35)	324 (71)	449 (79)	207 (65)	169 (62)	
Mean (SD), µg/L	32.4 (64.8)	56.1 (85.9)	61.1 (89.2)	15.3 (32.1)	14.4 (33.9)	36.8 (75.9)	30.7 (59.2)	0.28
Median, µg/L	7.0	15.5	18.3	5.8	5.0	6.5	5.6	
Smoking status, n (%)								
Active	230 (13)	133 (17)	90 (20)	39 (9)	58 (10)	82 (26)	66 (24)	0.77
Former	368 (21)	208 (27)	127 (28)	61 (13)	99 (17)	116 (36)	107 (39)	
Never	358 (20)	133 (17)	78 (17)	77 (17)	148 (26)	122 (38)	101 (37)	
Missing	831 (47)	289 (38)	158 (35)	279 (61)	263 (46)	0 (0)	0 (0)	
Haematuria, n (%)								
Positive	722 (40)	447 (59)	324 (72)	91 (20)	184 (32)	170 (53)	157 (57)	0.35
Negative	665 (37)	178 (23)	96 (21)	106 (23)	381 (67)	150 (47)	117 (43)	
Missing	400 (22)	138 (18)	33 (7)	259 (57)	3 (1)	0 (0)	0 (0)	
Group, n (%)								
Tumour	763 (43)		453 (100)			159 (50)	132 (48)	0.78
LG	306 (17)		184 (41)			83 (26)	58 (21)	0.31
HG	308 (17)		198 (44)			62 (19)	63 (23)	
Concomitant CIS	61 (3)		38 (8)			14 (4)	11 (4)	
Solitary CIS	25 (1)		2 (0.4)			0 (0)	0 (0)	
Missing	63 (4)		31 (7)			0 (0)	0 (0)	
NED	456 (26)		0 (0)			0 (0)	0 (0)	
Healthy control	568 (32)		0 (0)			161 (50)	142 (52)	
TNM								
Tumour	763 (43)		453 (100)			159 (50)	132 (48)	0.60
T _a	431 (24)		234 (52)			88 (28)	62 (23)	
T ₁	158 (9)		102 (23)			31 (10)	35 (13)	
T ₂	126 (7)		96 (21)			34 (11)	30 (11)	
T ₃	17 (1)		14 (3)			6 (2)	5 (2)	
Solitary CIS	25 (1)		2 (0)			0 (0)	0 (0)	
Missing	6 (0)		5 (1)			0 (0)	0 (0)	

CIS, carcinoma in situ; HG, high-grade; LG, low-grade; NED, no evidence of disease. ^aP values regarding the comparison of the development and validation subgroup using analysis of variance (ANOVA) and chi-squared tests. [†]Standard deviation.

consisted of patients with recurrent BC and patients with NED ($P < 0.001$). The PPV and NPV were 71.3% and 74.1%, in the screening group, and 44.5% and 78.3% in the surveillance group, respectively.

To identify independent risk factors for the development of a predictive model, univariate logistic regression analysis was performed for the predictors age, gender, UBC[®] Rapid test results, smoking status and haematuria in a cohort of 320 patients with documented risk factors (Table 3). Gender and age showed no significant association with the presence of BC in the LG model, and gender showed no association in the HG model. Backward selection showed that multivariate models that included age, UBC[®] Rapid test results, smoking and haematuria had the lowest Akaike information criteria (Table 3). Cytology is a highly specific predictor of the presence of bladder cancer [9]. However, due to the heterogeneity in study protocols across study centres, data on this risk factor were only available for a

small number of patients and this was therefore excluded as a predictor for the model.

Based on the multivariate logistic regression models, we developed two nomograms to predict the risk of primary LG-BC and primary HG-BC (Fig. 1). The nomograms showed robust discrimination after external validation, with an AUC of 0.79 (95% CI 0.72–0.87) for the LG model and 0.95 (95% CI 0.92–0.98) for the HG model. Brier scores of 0.15 for the model for LG-BC and 0.09 for the model for HG-BC proved that both models were well calibrated. The calibration plots shown in Fig. 2 indicate a constant overestimation of the risk for BC in both models.

The decision curve analysis results (Fig. 3) showed that the decision to investigate further based on the nomograms provided a net benefit over a decision based solely on the results of the UBC[®] Rapid test across all low- and

intermediate-risk thresholds, which are the most relevant to the decision on further treatment. This was true for thresholds below 30% for the LG model and for risk thresholds below 35% for the HG model.

The results of the cut-off analysis including sensitivity, specificity, PPV and NPV for various cut-off values are presented in Table 4. The use of a threshold risk of 15% would result in no recommendation to pursue further diagnostics for LG-BC and HG-BC in 51 (25.5%) and 104 (48.1%) patients in the validation group, respectively, and BC would be missed in five cases (2.5%) and three cases (1.4%), respectively. Conversely, further diagnostics for LG-BC and HG-BC would be recommended in 149 (74.5%) and 112 patients (51.9%), of whom 53 (26.5%) and 71 (32.9%) would be correctly identified as tumour patients, respectively. For patients with HG non-muscle-invasive BC (NMIBC) and HG muscle-invasive BC (MIBC), no further diagnostics would be recommended in 102 (56.4%) and 103 patients (58.2%), among which one case (0.6%) and two cases (1.1%) would falsely be classified as negative, respectively. Further diagnostics would be advised in 79 (43.6%) and 74 patients (41.8%), of whom 38 (21.0%) and 33 (18.6%) would have BC, respectively. This equals a sensitivity of 97.4% and 94.3%, respectively, and a specificity of 71.1%.

Discussion

Decisions regarding the best clinical management of patients at risk of BC can be challenging. This analysis aimed to evaluate the clinical usefulness of the quantitative UBC[®] Rapid test for the detection of BC. We developed two nomograms that describe the patient-specific risk for primary LG-BC and HG-BC.

The cytokeratin concentration measured with the UBC[®] Rapid test was significantly higher in patients with BC than

in patients without tumours (56.1 µg/L in patients with tumours, 15.3 µg/L in patients with NED, and 14.4 µg/L in the healthy control group). Studies analysing the UBC[®] Rapid test reported a cut-off of 6.7–12.0 µg/L, with a sensitivity of 56%–71% and a specificity of 61%–96% [13–19]. These data are consistent with the sensitivity of 60.4% and the specificity of 75.5% calculated in this study. The sensitivity was 70.5% for the detection of HG tumours, 85.2% for concomitant CIS and 68.0% percent for solitary CIS, whereas it was 46.4% for LG-BC. As a parameter of diagnostic accuracy, the AUC of 0.68 (95% CI 0.66–0.70) determined in this study is at the lower end of the range of 0.68–0.84 previously reported [13–19]. With a cut-off value of 9.88 µg/L, the UBC[®] Rapid test is better suited to rule out BC than to detect it.

The cytokeratin concentrations determined by the UBC[®] Rapid test were significantly higher in patients with concomitant CIS ($P < 0.001$) and HG-BC ($P < 0.001$) than in patients with LG-BC, which is consistent with previous reports [13,18]. Although the UBC[®] Rapid test can predict HG-BC and CIS reliably, similar to other POC tests for BC such as the bladder tumour antigen (BTA) and nuclear matrix protein 22 (NMP22) assays, it is of limited diagnostic value for LG-BC and lacks the specificity of urine cytology [11]. With an AUC of 0.72 (95% CI 0.69–0.75) for the detection of primary BC found in this study, the UBC[®] Rapid test is better suited for the screening of healthy patients than for the surveillance of patients at risk of recurrence, where the AUC was 0.65 (95% CI 0.60–0.70). Due to a similar tumour grade distribution ($P < 0.001$) between the screening group and the surveillance group, tumour grade was excluded as a confounding factor for this effect.

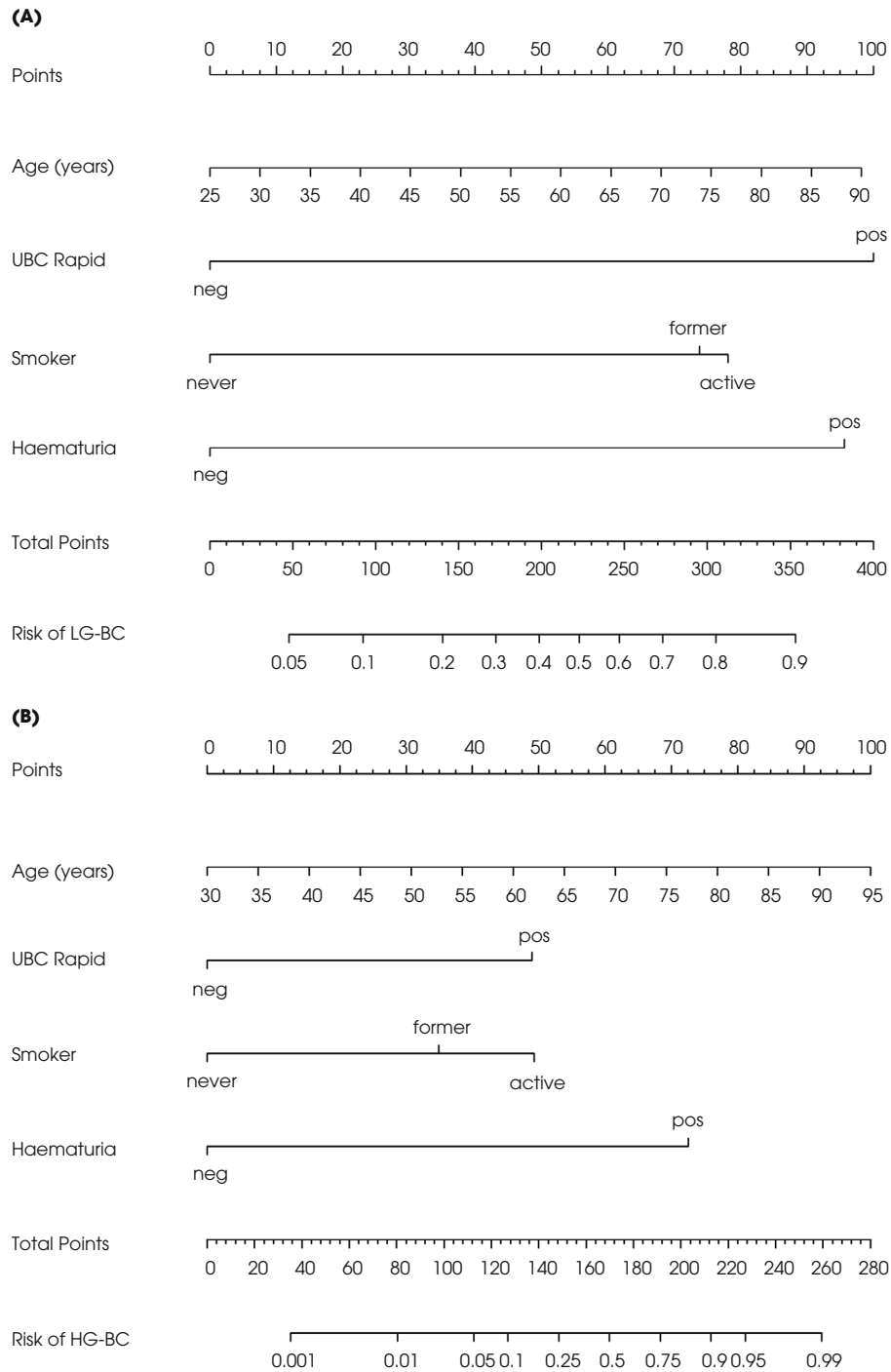
Based on these findings, we have developed what is, to our knowledge, the first nomogram for assessing bladder cancer risk in healthy patients. In addition to the UBC[®] Rapid test

Table 3 Univariate and multivariate logistic regression analyses for the nomogram development subgroup analysing associations with primary low-grade bladder cancer (BC; $n = 245$) and primary high-grade BC ($n = 236$).

Factor	Univariate model			Multivariate model		
	OR	95% CI	P	OR	95% CI	P
LG-BC						
Age	1.02	0.99–1.04	0.177	1.03	1.00–1.05	0.066
Gender (female)	1.09	0.59–1.96	0.786			
UBC [®] Rapid	7.39	3.91–14.47	0.000	5.39	2.66–11.25	0.000
Smoking (active)	2.71	1.31–5.62	0.007	3.73	1.53–9.38	0.004
Smoking (former)	2.09	1.14–3.91	0.018	3.46	1.64–7.69	0.002
Haematuria	5.06	2.89–9.06	0.000	5.01	2.61–9.91	0.000
HG-BC						
Age	1.04	1.01–1.07	0.003	1.08	1.04–1.14	0.001
Gender (female)	0.82	0.42–1.53	0.537			
UBC [®] Rapid	20.81	10.55–43.14	0.000	12.36	5.01–33.34	0.000
Smoking (active)	6.15	3.01–13.05	0.000	12.57	3.75–48.62	0.000
Smoking (former)	2.14	1.06–4.39	0.035	5.99	1.91–21.03	0.003
Haematuria	30.07	13.12–81.97	0.000	41.31	13.56–157.78	0.000

BC, bladder cancer; HG, high-grade; LG, low-grade; OR, odds ratio.

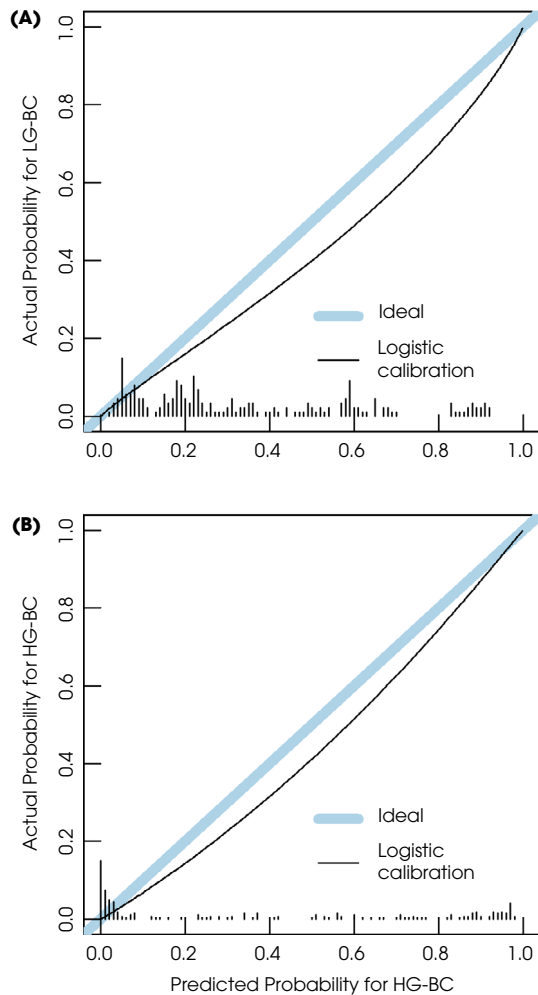
Fig. 1 Nomograms predicting the probability of low-grade bladder cancer (LG-BC) **(A)** and high-grade bladder cancer (HG-BC) **(B)** using age, UBC® Rapid test, smoking status and haematuria. To calculate the likelihood of each type of bladder cancer (BC) in a patient, the score for each risk factor is measured on the point scale axis. The total score is calculated by adding up each individual score. The probability of BC is estimated by projecting the total score onto the lower total score scale.



results, the logistic regression models identified age, smoking status and haematuria as factors predicting the risk of primary BC. Although BC is four times more common in men [1], gender did not play a crucial role in our

nomograms. In the models developed in this study, patient age was the biggest contributor to the risk of BC. The role of the factor age in risk assessment was less important in LG-BC than in HG-BC.

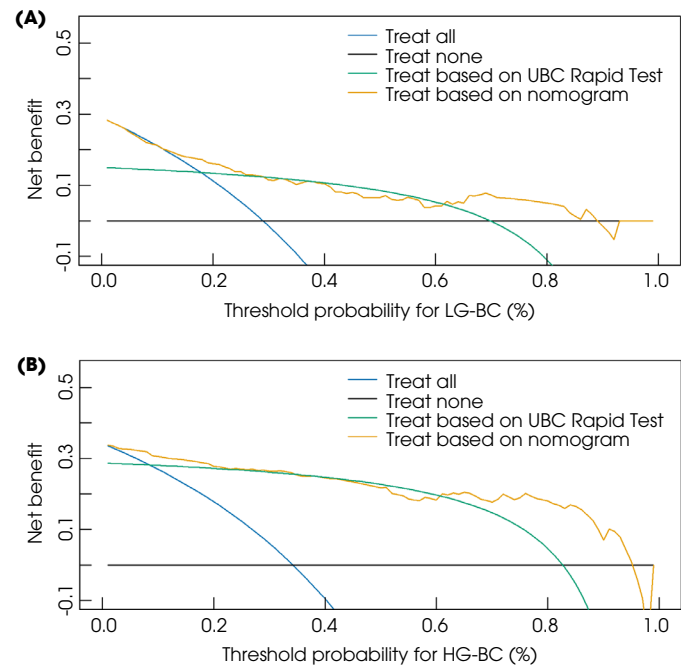
Fig. 2 Calibration plot to estimate the performance of the nomogram for the prediction of low-grade bladder cancer (LG-BC) (A) and high-grade bladder cancer (HG-BC) (B). Ideal prediction corresponds to a slope of 1. The solid line represents the logistic calibration curve. The vertical lines at the bottom indicate the risk distribution of the patients in the validation cohort.



To assess the overall probability of BC, multiple models have been developed [22–24]. A multivariate logistic regression model, including age, gender, smoking status and degree of haematuria in 1182 patients showed an AUC of 0.74, while another nomogram including age, sex, race, degree of haematuria and smoking status in 4178 patients was able to predict the presence of BC with an AUC of 0.79 [22,23]. The present results support the notion that asymptomatic haematuria in combination with demographic markers plays an important role in BC risk assessment.

Huang et al. [24] proposed another nomogram combining demographic factors (age, race and smoking habits) with biomarker data (matrix metalloproteinase [MMP]-9, MMP-

Fig. 3 Decision curve analysis comparing the net benefit of using the developed nomogram for low-grade bladder cancer (LG-BC) (A) and high-grade bladder cancer (HG-BC) (B) for decision making (yellow line) with using exclusively the results of UBC[®] Rapid test (green line) and with the strategy of assuming no patients have LG-BC or HG-BC (black line) or all patients have LG-BC or HG-BC (blue line), respectively.



10, interleukin-8, vascular endothelial growth factor, angiogenin and syndecan) from 686 patients to obtain a model for BC prediction with an AUC of 0.89. The AUC values of the demographic model and the biomarker model alone were 0.81 and 0.84, respectively. Their observations suggest that including a large variety of factors in a model can increase the predictive power of a nomogram. The major advantage of our nomogram, which focuses on established and readily available risk factors, is its clinical applicability.

New laboratory techniques have led to significant advances in the evaluation of urinary tumour RNA as diagnostic markers. This allowed for the development of a nomogram based on the expression of a cluster of urinary microRNA consisting of let-7c-5p miRNA, miR-99a-5p, and -125b-5p and the European Organization for Research and Treatment of Cancer (EORTC) score for progression to predict the probability of progression-free survival. The nomogram helps to identify patients at risk of progression with an AUC of 0.87 [25]. In addition to RNA, the analysis of epigenetic and genetic alterations such as DNA methylation, histone tail modifications, and DNA mutations offers a promising new approach for the diagnosis of bladder cancer. However, novel markers, such as the established tests, are often limited by low specificity [11].

Table 4 Sensitivity, specificity, and positive and negative predictive values for the prediction of low-grade bladder cancer (LG-BC) and high-grade bladder cancer (HG-BC) at different thresholds for the nomogram-derived risk value for LG-BC and HG-BC.

Threshold (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
LG-BC				
5	98.3	4.9	29.7	87.5
10	93.1	26.8	34.2	90.5
15	91.4	32.4	35.6	90.2
20	86.2	49.3	41.0	89.7
25	77.6	63.4	46.4	87.4
30	74.1	66.9	47.8	86.4
35	70.7	73.2	51.9	86.0
40	70.7	78.9	57.7	86.8
HG-BC				
5	98.6	62.7	57.9	98.9
10	95.9	68.3	61.2	97.0
15	95.9	71.1	63.4	97.1
20	94.6	71.8	63.6	96.2
25	94.6	75.4	66.7	96.4
30	94.6	78.9	70.0	96.6
35	91.9	82.4	73.1	95.1
40	90.5	85.2	76.1	94.5

BC, bladder cancer; HG, high-grade; LG, low-grade; NPV, negative predictive value; PPV, positive predictive value.

Decision curve analysis indicated the clinical usefulness of the proposed nomograms. At a risk threshold of 15%, the LG and HG models showed a sensitivity of 91.4% and 95.9%, and a specificity of 32.4% and 71.1%, respectively, for the detection of LG-BC and HG-BC. In addition, the HG model was highly accurate in detecting HG-NMIBC as well as HG-MIBC. Due to the high sensitivity, at this risk threshold the nomograms could also be used for so-called 'reflex testing' to increase the accuracy of a previous test, for example, urine cytology [26]. However, validation of the potential benefits of this procedure is still required. Other single urine biomarkers such as BTastat[®], NMP22[®] BladderChek[®] and ImmunoCyt achieve sensitivity values of 64%, 58% and 78%, respectively, and specificity values of 77%, 88% and 78%, respectively [27].

Applying a risk threshold of 15% to our models, BC was not detected in 0.6% and 1.1% of patients in the validation group, respectively. In comparison, patients with LG-NMIBC were classified as false-negative in 2.5% of cases. Although 75% percent of patients present with NMIBC at time of diagnosis, patients who are diagnosed with MIBC have a much higher mortality risk due to rapid tumour progression, which is why early diagnosis is particularly important [4].

The models presented here offer a good complement to the EORTC risk tables and CUETO (*Club Urológico Español de Tratamiento Oncológico*) scoring models, which can be used to assess the risk of tumour recurrence and progression of NMIBC [28,29]. In an external validation, the EORTC model showed an AUC for disease recurrence and progression of 0.60 and 0.66, respectively. The CUETO model could discriminate disease recurrence and progression with an AUC

of 0.52 and 0.62, respectively [30]. The development of models with high accuracy for the prediction of the risk of tumour recurrence was not possible based on our dataset.

The main limitation of this study was the small number of patients involved in the development of the nomograms, which limited both the statistical interpretation and the quality of the nomograms. This can be attributed to the heterogeneity of the data from the different study centres, which were collected retrospectively. Therefore, data on significant risk factors as well as urine cytology results were only available in some of the study centres. However, given its high specificity, the inclusion of urine cytology as a risk factor in the models might further improve their performance. This was shown by Cha et al. [22] in their above-mentioned BC risk model, where adding cytology results to the model significantly increased the AUC from 0.74 to 0.83. In addition, the influence of haematuria could not be assessed separately for microscopic and macroscopic haematuria.

Because we focused on the clinical applicability of the models and because data for all risk factors may not be available for all patients, we developed the BLUCAB Index[®], a web-based calculator based on our dataset. The BLUCAB Index[®] can predict the probability of primary and recurrent LG-BC, HG-BC and BC in general for any combination of risk factors. The BLUCAB Index[®] is available for free at www.blucab-index.net.

In conclusion, the UBC[®] Rapid test alone has limited clinical utility for predicting primary or recurrent BC. Two nomograms were developed to estimate the risk of primary BC in patients who have undergone the UBC[®] Rapid test, including the predictors age, smoking status and haematuria. The nomograms provide a highly accurate tool for the prediction of primary LG-BC and especially primary HG-BC, and have good discrimination and calibration. The UBC[®] Rapid test in combination with these predictors might facilitate clinical decision making for patients at risk of primary BC.

Conflicts of Interest

Johan Styrke and Amir Sherif received grants from IDL Biotech, Bromma, Sweden in 2014. Thorsten H. Ecke received grants from IDL Biotech, Bromma, Sweden in 2019–2021. Roland Einarsson and Per-Uno Malmström are scientific advisors to IDL Biotech. Arnulf Stenzl, René Ritter and Jörg Hennenlotter received grants from IDL Biotech, Bromma, Sweden in 2013. The test systems were sponsored by Concile GmbH, Freiburg/Breisgau, Germany, and IDL Biotech, Bromma, Sweden.

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Abbreviations: AUC, area under the curve; BC, bladder cancer; CIS, carcinoma in situ; CUETO, *Club Urológico Español de Tratamiento Oncológico*; EORTC, European Organization for Research and Treatment of Cancer; HG, high-grade; LG, low-grade; MIBC, muscle-invasive bladder cancer; MMP, matrix metalloproteinase; NED, no evidence of disease; NMIBC, non-muscle-invasive bladder cancer; NPV, negative predictive value; POC, point-of-care; PPV, positive predictive value.