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ORIGINAL ARTICLE – CANCER RESEARCH

Individual patient risk of progression of urinary bladder papillary tumors estimated from biomarkers at initial transurethral resection of bladder tumor

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Abstract

Objective To determine if individual, instead of group, patient progression risk could be predicted using p53, Ki67 and CK20 biomarker percentage values at initial transurethral resection of bladder tumor specimens.

Methods This was an observational study where biomarkers were measured with no knowledge of tumor outcome. Initial bladder tumor specimens were classified as non-invasive and invasive to sub-epithelium (pT1). Percentages of stained biomarker cells were tested as progression predictors from non-invasive to pT1 and pT1 to pT2. Progression probability was correlated with biomarker percentages resulting in a regression equation.

Results We studied 112 patients (median age = 67, range 37–91, males 83/112 (73%), with median follow-up of 39 months (range 1.7–140). Mean biomarker values were higher in stage pT1 than in non-invasive (all $p < 0.001$). Cut-off points separating progression from non-progression groups in stage pT1 were higher than in non-invasive for all biomarkers. Correlation R values for progression probability vs. biomarker percentages varied from 0.7 to 0.9 (all $p < 0.001$), regression slopes from 0.1 to 0.8 and intercepts from 11 to 35. A novel individual progression probability was calculated as the product of biomarker percentage of stained cells and slope, plus the prevalence-adjusted intercept.

Conclusions Identification of individual risk of progression in patients with non-muscle-invasive bladder tumors was possible using p53- and Ki67-derived progression probability using a regression equation. Combining biomarker-derived progression probability to tumor stage pT1 improves progression to pT2 predictive accuracy.

Keywords Biomarkers · Bladder tumors · Individual prediction of progression

Background

Urinary bladder tumors include heterogeneous groups with unpredictable clinical course. Most are papillary carcinomas, of which 80% are non-invasive neoplasms and 20%, invasive carcinomas (Quintero et al. 2006). Non-invasive tumors may remain stable, recur or progress, invading the sub-epithelium (pT1) or detrusor muscle (pT2). Since invasive forms have poor prognosis, accurate risk stratification at the time of the first transurethral resection of a bladder tumor (TURBT) would be of immense value for the patient. Those at high risk will benefit from more aggressive treatment such as radical cystectomy, and those with low risk may be cured by more conservative therapy (Kulkarni et al. 2010).

Tumor progression prediction criteria such as grade, stage, size, trigone and bladder neck locations, number of lesions and the presence of *carcinoma* in situ (CIS) do not always predict clinical outcomes of bladder papillary tumors, although it has been suggested that accuracy could be improved by molecular and biomarker studies (Patterson et al. 2016; Kojima et al. 2017; van Kessel et al. 2018; Kutwin et al. 2018).

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Clinical and pathological parameters used for grading and staging are still being used (Humpfrey et al. 2016), although staging was challenged due to the complexity of bladder histoanatomy, such as *lamina propria* variations in depth, *muscularis propria* being confused with hyperplasic *muscularis mucosa* leading to staging implications (diagnosing pT2 instead of pT1) (Paner et al. 2017; Poletajew et al. 2017).

There are numerous studies reporting the use of immunohistochemical markers to establish prognosis at initial trans-urethral resection of bladder tumor (TURBT) specimens (Quintero et al. 2006; Grossman et al. 1998; Serth et al. 1995; Vetterlein et al. 2017; Hitchings et al. 2004), but in most cases, threshold values are used to separate low- from high-progression-risk groups (Vetterlein et al. 2017; Hitchings et al. 2004; Shariat et al. 2009). These thresholds result from optimal statistical cut-off points that separate groups with balanced sensitivity and specificity (Vetterlein et al. 2017), but the actual percentage of cells expressing a biomarker mutation has not been suggested as a predictor. Some authors have shown an association between risk group and progression, based on p53 and retinoblastoma (RB) (Grossman et al. 1998; Serth et al. 1995; Hitchings et al. 2004), while for others, Ki67 was superior to p53 (Vetterlein et al. 2017).

A limited individual patient progression risk assessment can be done with nomograms, such as EORTC or CUETO (Shariat et al. 2008; Sylvester et al. 2006; Seo et al. 2010; D'Andrea et al. 2018; Ravvaz et al. 2017; Xylinas et al. 2013) using weighed discrete binary parameters, (presence or absence of given clinical/histopathological features), resulting in a score. In spite of the addition of lymphovascular invasion and variant histology (D'Andrea et al. 2018), these scoring systems differ significantly between them on their predictive ability (Shariat et al. 2008; Ravvaz et al. 2017) and have been challenged due to low positive predictive value in high-risk patients, namely 21% for CUETO and 24% for EORTC (Sylvester et al. 2006; Xylinas et al. 2013). This results in a considerable number of patients without the benefit of an accurate prognosis. An accurate prediction of progression risk for the individual patient is, therefore, critical for non-muscle-invasive bladder cancer, since patients who progress to muscle-invasive cancer have significantly shorter life expectancy, unless they have early radical cystectomy (May et al. 2004; Fujii 2018).

Importance of biomarkers

Low-grade tumors are associated with fibroblast growth factor receptor 3 and *RAS* proto-oncogene mutations, and high-grade tumors and *carcinoma* in situ (CIS), with mutant protein p53 and retinoblastoma protein (Ahmad et al. 2012; Al-Hussain and Akhtar 2013). Wild-type p53 tumor suppressor protein encoded by *Tp53* gene controls cell proliferation, and upon damage to DNA, this protein responds with apoptosis, cell cycle arrest and replication block. However, after severe DNA damage, gene *Tp53* suffers numerous mutations, leading to altered or mutated p53 protein, and loss of its tumor suppression function (Goldstein et al. 2011). The normal urothelium has wild-type p53 status, but altered expression of mutated p53 with loss of function may turn to a more aggressive epithelial phenotype associated with tumor progression (Serth et al. 1995; Hitchings et al. 2004). Ki67 is a marker of tumor cell proliferation (expressed in G1, S, M and G2 of cell cycle). Cytokeratin 20 (CK20) is an intermediate filament protein normally expressed in superficial and some intermediate cells, and in aberrant diffuse form in urothelial carcinoma (Margulis et al. 2009; Mumtaz et al. 2014). Therefore, in view of above, it seems reasonable to expect biomarkers to provide not only tumor assessment but also prognostic value. Furthermore, immunohistochemistry is cost effective and widely available molecular biomarkers such as miRNA, mRNA, using next-generation sequencing and PCR are very promising for early, noninvasive diagnosis and possibly for prognosis, (Butterfield and Gupta 2017; Pietzak et al. 2017; Kutwin et al. 2018), but they are expensive and not yet widely available.

Hypothesis

Since biomarkers are known to be highly expressed in higher-grade and -stage tumors and in those eventually progressing (Agarwal et al. 2018), we hypothesized that progression risk is a continuous, not binary variable, and there may be an association between the actual biomarker percentage value and the individual patient progression risk. In other words, the higher the biomarker percentage, the higher the likelihood of progression. This association, via a regression equation, could estimate the individual patient risk of progression to a higher stage.

Objective

To explore the possible usefulness of p53, Ki67 and CK20 at initial TURBT specimens to predict the individual patient progression risk from non-muscle-invasive bladder cancer (NMIBC) to muscle-invasive bladder cancer (MIBC).

Methods

This was an observational study where histological grade and stage were made by three observers for routine diagnostic purposes, independently from immunohistochemistry analysis, and previously to this investigation design. Data collection started January, 2003, and patients were followed up for a maximum of 11.7 years.

The study was approved by the Hospital Ethics Committee (Head, Dr. Haydee Wimmers) and Research/Education Committee (Prof. Dr. Jorge Gori). Patient privacy was carefully guarded.

Full TURBT specimens were fixed in 10% buffered formalin, embedded in paraffin blocks, sectioned in 4-μ slices and stained with hematoxylin and eosin. UBPTs were initially classified following World Health Organization (WHO) 2004, ISUP 1998, Tumor Node Metastasis (TNM) 2010 for grade and stage (Lopez Beltran and Montironi 2004) and re-classified for this study according to WHO2016/ISUP/TNM 2010 (Grignon et al. 2016).

All patients staged pT1 underwent a repeat TURBT to look for residual tumor and confirm original diagnosis. Recurrence was defined as tumor evidence of the same or lesser stage after at least 3 months post treatment, and progression, as the finding of a higher stage in TURBT specimens in recurring tumors (Shariat et al. 2007). Non-progression was defined as the absence of stage upgrade after at least 1 year. For progression evaluation, all TURBT specimens were classified as non-invasive, pT1 or invasive to muscle (pT2). Non-Invasive group included papillary urothelial neoplasm of low malignant potential (PUNLMP) and non-invasive papillary carcinoma (pTa).

Inclusion and exclusion criteria at initial TURBT

We included specimens with PUNLMP, low- and high-grade pTa and usual-type urothelial carcinoma invading sub-epithelium (pT1). Not included were specimens with flat lesions, papillomas, inverted papillomas, variant of invasive carcinomas with divergent differentiation, pT2 and CIS. Other parameters such as tumor size and location were not part of this study. Since the response to BCG treatment on tumor progression is variable (Fujii 2018), treated patients were not considered separately.

Biomarkers

Biomarker expression was evaluated in the first TURBT specimen. Representative sections were stained with an automated immunohistochemistry platform (Roche-Ventana Benchmark XT, Roche Diagnostics, USA) for immunohistochemistry testing with monoclonal antibodies p53, Ki67 and CK20. Primary antibody p53 (clone DO-7, Ventana) is a mouse monoclonal antibody (IgG1, kappa) directed against mutant and wild type of nuclear phosphoprotein p53. Ki67 is a marker of cell proliferation (clone MIB-1, Ventana). CK20 (Clone SP33, Ventana) is a rabbit primary monoclonal antibody directed against a cytokeratin protein type I of enterocytes and superficial urothelial cell. Cells were considered positive when nuclear p53 and Ki67 or cytoplasmic CK20 staining was present with any intensity. Values of biomarkers were expressed as a percentage after counting 400 urothelial cells with a light microscope at 400× in four fields, with an estimated error of 5–10%, using a mechanical counter, in well-preserved, representative tissue areas. Results were tested against known positive and negative controls. Figure 1a shows an example of a papillary tumor at low-power field (10×), and Fig. 1b, at 400× exemplifying immunohistochemistry determination of mutated p53 gene.

Patients were followed up every 3 months by urine cytology and cystoscopy. They underwent either clinical surveillance or treatment with intra-vesical chemotherapy or BCG according to current guides (Spiess et al. 2017). If papillary lesions were detected by cystoscopy, TURBT was performed.

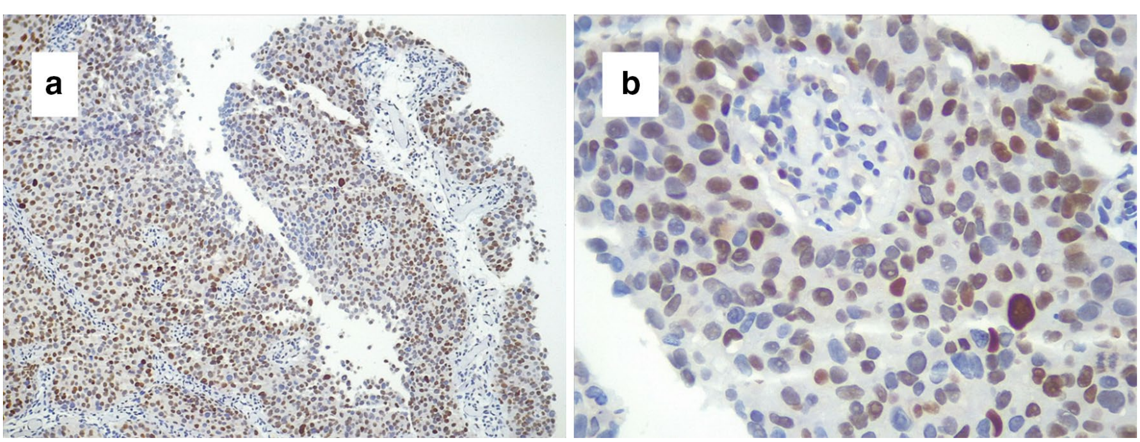

Fig. 1 Example of mutant, overexpressed p53 indicated as dark brown nuclear staining. Patient with papillary bladder cancer. **a** 10×, **b** 400×

Statistics

All data were included in Excel spreadsheet, using Data Analysis for basic statistical analyses. Mean biomarker values obtained in non-invasive and pT1 groups and in patients with and without progression were compared using Student's *t* test. Progression sensitivity and specificity curves for each biomarker were plotted using biomarker percentages from 0 to 100% at 5% steps. The optimal cut-off points, defined as the point of crossing of sensitivity and specificity curves, separates progression vs. non-progression groups at each step. Group progression probability was analyzed using 2 × 2 contingency tables and two-tailed Fisher's exact test for significance and whenever appropriate, 95% confidence intervals (CI) were included (MacEneaney and Malone 2000). Odds ratios (OR) were calculated as (TP/FN)/(FP/TN), where TP = true +, FN = false −, FP = false + and TN = true −.

For biomarker values between 0 and 100%, Progression Probability (PProb) was calculated as the ratio TP/(TP + FP), and correlation coefficients and regression equations were calculated between biomarker values and PProb. Individual patient PProb for a given biomarker value was calculated by multiplying the regression slope by the biomarker value and then adding the intercept. PProb was adjusted for prevalence with Bayes' approach. This method resulted in progression probabilities ranging from prevalence values to 100%.

Results

We studied 112 patients with a median age of 67 years, range 37–91, with 85/113 (75%) males, followed up for a median time of 30 months (range 1.7–140 months).

At initial TURBT, there were 6 patients with PUNLMP, 60 of low-grade pTa, 5 high-grade pTa, and 41 patients with Stage pT1. Overall, recurrence occurred in 92/112 patients (82%), with 24/92 (26%) of them within the first year. The overall progression risk to pT2 in our cohort was 11/112, (9.8%, CI 5.6–16%). Of these, two progressed from NonInv and nine from pT1.

There were 78/112 (70%) single and 34/112 (30%) multiple lesions. In patients with single lesion, progression from non-invasive to invasive was present in 20/78 (26%). In multiple lesions, 20/34 (59%) progressed ($p = 0.001$).

Biomarker values, stage and progression

In the non-invasive group, PUNLMP patients had no progression to pT1. In low-grade pTa, 3/60 progressed to high-grade pTa (5%, CI 2–15%), and 14/60 to pT1 (23%, CI 14–35%). In patients staged pT1, there was progression to pT2 in 9/41 (22%, CI = 12–37%). Figure 2a shows the association between PProb and biomarker values for patients staged NonInv progressing to pT1, using p53; Fig. 2b is for patients staged pT1 progressing to pT2, with the same biomarker. The intercept in both cases represents the progression prevalence of the cohort. Table 1 shows tumor stage, correlation coefficients, regression equations, *R* confidence intervals and *p* values for the association between PProb and biomarker value. Given tumor stage, individual patient

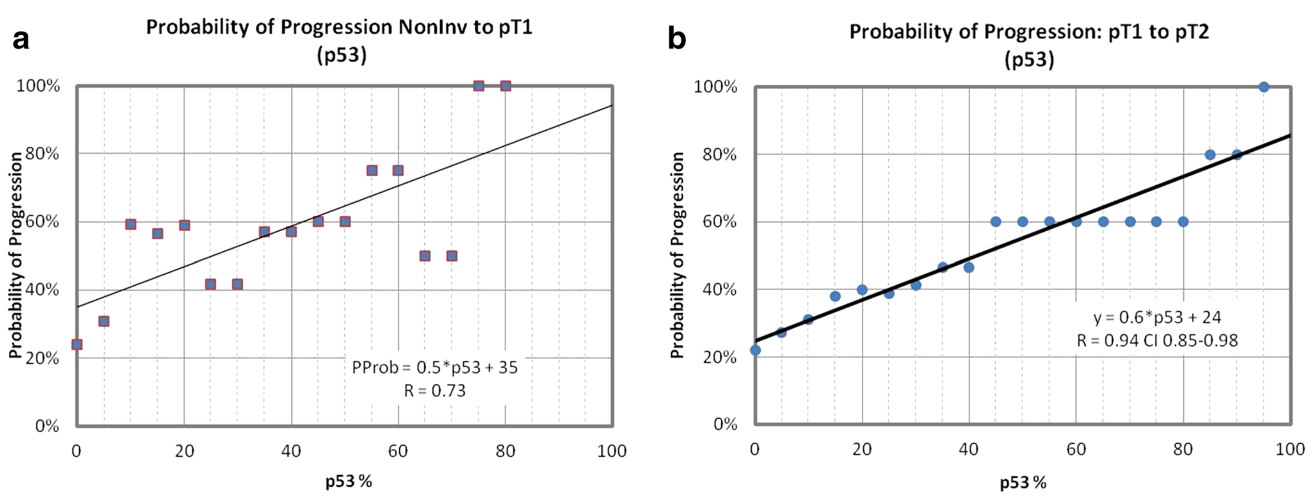

Fig. 2 Association of probability of individual progression risk and percent value of p53. Regression equations allow calculation of individual patient progression probability given the biomarker value, in patients with non-invasive stage (**a**) progressing to pT1 and in the group of patients staged pT1 (**b**) progressing to pT2. Of note is that predictive ability of p53 is better for patients staged pT1 at initial TURBT

Table 1 Regression equations to predict progression

	Stage	Correlation coefficients	Regression equation PProb	95% CI	*p* value
p53	NonInv to pT1	$R=0.73$	$0.59\times p53+39$	0.4–0.89	$p<0.001$
	pT1 to pT2	$R=0.94$	$0.65\times p53+23$	0.84–0.98	$p<0.001$
Ki67	NonInv to pT1	$R=0.59$	$0.4\times Ki67+38$	0.17–0.83	$P=0.04$
	pT1 to pT2	$R=0.89$	$0.8\times Ki67+8.4$	0.72–0.96	$P<0.001$
P53+Ki67	pT1 to pT2	$R=0.91$	$0.36\times(p53+Ki67)+17$	0.77–0.97	$P<0.001$
CK20	NonInv to pT1	$R=0.44$	$0.1\times CK20+31$	−0.03 to 0.75	$P=0.012$
	pT1 to pT2	$R=0.57$	$0.07\times CK20+31$	0.2–0.8	$P=0.007$

Table shows the tumor stage at the time of initial TURBT and regression equations to estimate individual progression probability (PProb) to a higher stage (NonInv to pT1 and pT1–pT2) in subsequent TURBT. The combination of p53 and Ki67 was also highly predictable of progression, and similar to single markers

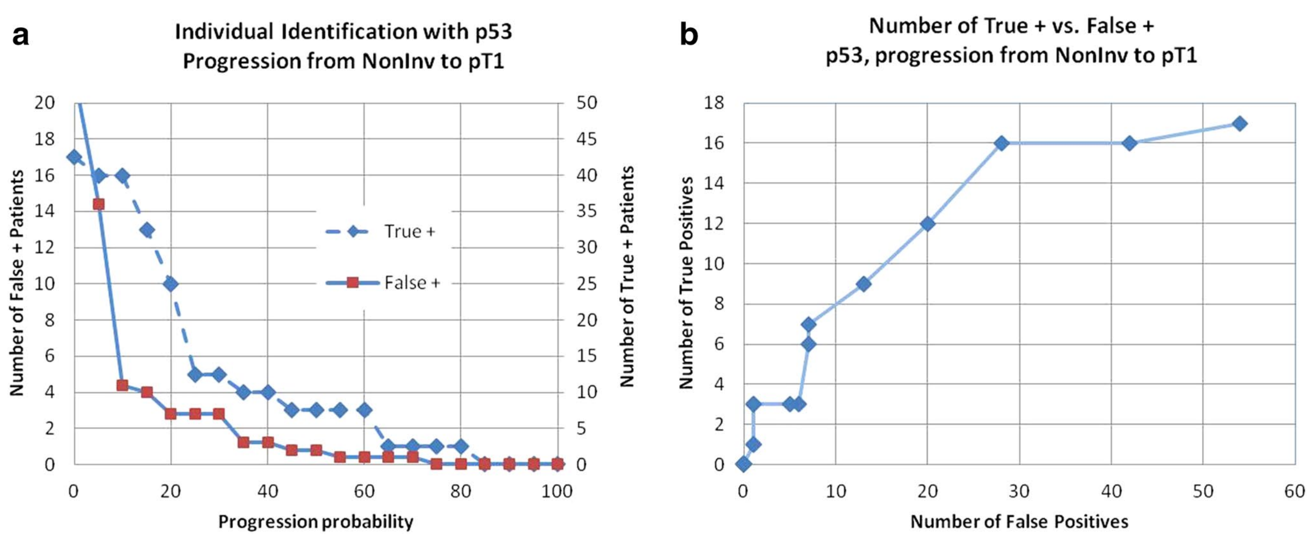

Fig. 3 Identification of individual patient risk. Number of true-positive and false-positive progression from NonInv to pT1 using p53. Panel **a**: For a given progression probability (x-axis) the primary y-axis shows false-positive results, and secondary y-axis, true + results, that is, patients who had progression from NonInv to pT1. Panel **b**: Shows the trade-off between numbers of true positives vs. false positives in a ROC-type curve for the same marker

progression probability results from multiplying biomarker value by regression slope and adding intercept. Only p53 and Ki67 had statistically and clinically relevant correlations. The combination of both (p53 + Ki67) had also an important correlation although not superior to single markers. Progression from NonInv to pT2 was present in only two patients, precluding statistical analysis.

Figure 3a shows the number of patients with false + (primary y-axis) and true + (secondary y-axis) progression from NonInv to pT1, for PProb from 0 to 100%, using p53. Panel B shows the trade-off between true + and false + progression number of patients observed in our cohort.

Figure 4 shows the number of patients with true + and false + prediction of progression from pT1 to pT2 using p53 (Panel A) and Ki67 (Panel B). Both x-axes (Panels A and B) show PProb; primary y-axes show the number of patients with no progression to pT2 (false +), and the secondary y-axes, the number of patients with progression (true +). From 41 patients staged pT1, there were 9 who progressed to pT2 and 32 who did not.

Progression prediction of patient groups and cut-offs

Figure 5a shows sensitivity and specificity curves of p53 for progression from Non-Inv to pT1, where the crossing of both curves determines an optimal p53 cut-off point of 12%, separating patients into groups with lower and higher progression risk. At this cut-off, sensitivity and specificity are balanced, with values of 0.87 ($p<0.001$), OR = 48. Figure 5b shows p53 curves for progression from pT1 to pT2, with an optimal cut-off of 40%, representing sensitivity and specificity

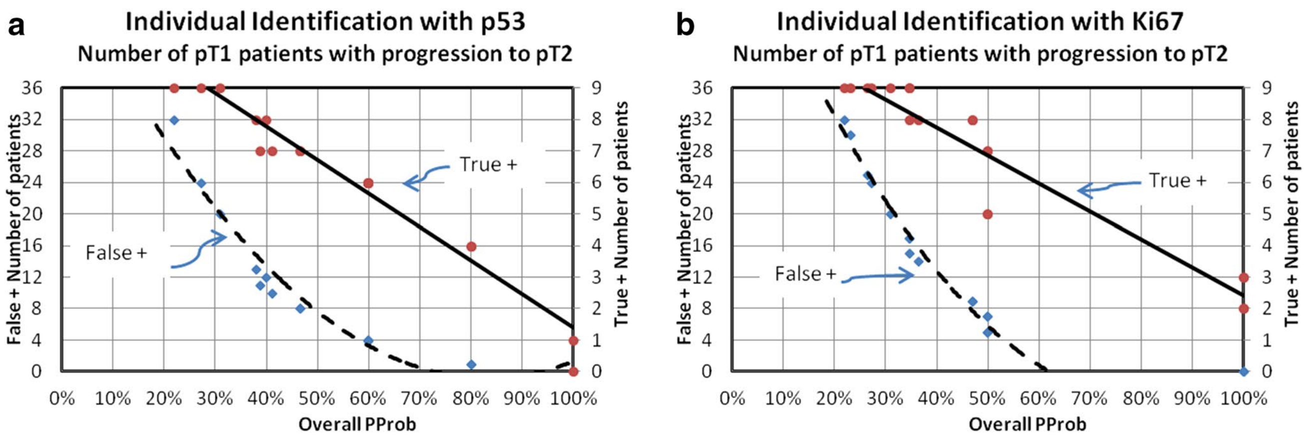

Fig. 4 Identification of individual patient risk. Number of patients with true-positive and false-positive results for progression probability from pT1 in the initial TURBT to pT2, using p53 (**a**) and Ki67 (**b**)

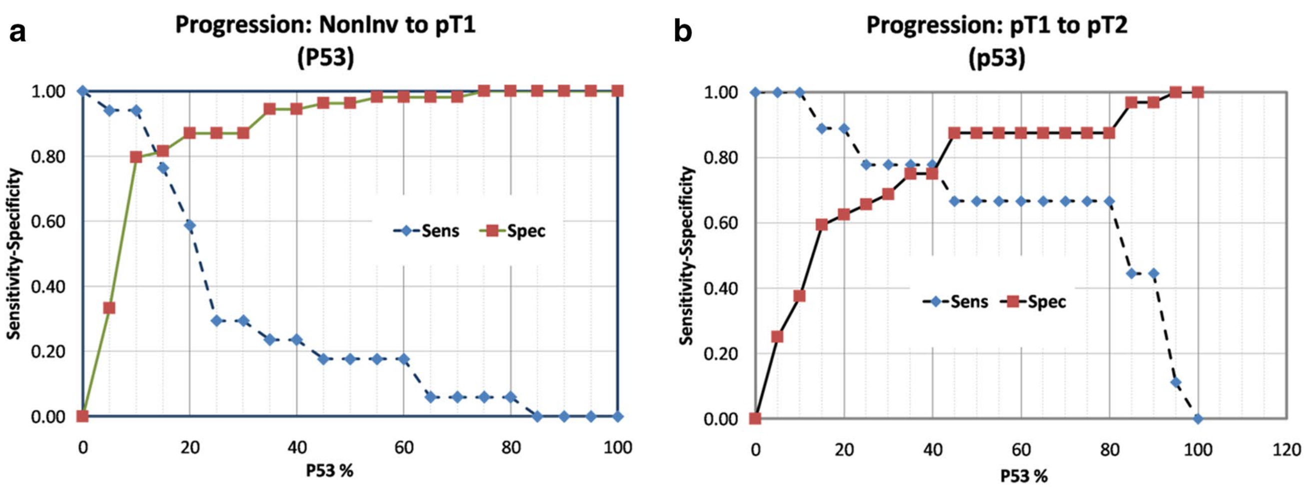

Fig. 5 Sensitivity and specificity plots. Sensitivity and specificity of p53 to predict progression from non-invasive to pT1 (**a**) and from pT1 to pT2 (**b**). The optimal cut-off (crossing of sensitivity and specificity) serves to separate risk groups. Cut-off is lower in patients initially staged non-invasive (**a**, 12%) as compared to stage pT1 (**b**, 40%)

values of 0.77 ($p = 0.003$), OR = 11. Ki67 had sensitivity and specificity curves for progression from Non-Inv to pT1, with optimal Ki67 cut-off point of 14%, separating patients with higher- from lower-progression-risk groups, with optimal sensitivity and specificity of 0.6 ($p < 0.001$). For progression from pT1 to pT2, Ki67 (Fig. 6a), revealed an optimal cut-off of 55%, and balanced sensitivity and specificity of 0.78 ($p = 0.014$), OR = 13. Figure 6b shows that the combination of p53 and Ki67 does not improve prognostic value, as compared to single markers. Individual patient progression risk (sensitivity and specificity) can be estimated from Figs. 5 and 6 given the patient's actual biomarker values.

Mean biomarker values of patients with non-invasive tumors were 14% ± 17, 16% ± 14 and 30% ± 37 for p53, ki67 and CK20, respectively, as compared to 32% ± 33, 39% ± 33, 49% ± 42, respectively, in patients staged pT1 (p values = 0.002, 0.001, 0.014).

Mean biomarker values of patients with no progression were 8% ± 10, 13% ± 12 and 23% ± 33 for p53, ki67 and CK20, respectively, as compared to 29% ± 22, 24% ± 16, 47% ± 41, respectively, in patients who showed tumor progression (p values < 0.002, = 0.006, = 0.006). CK20 showed very weak predicting ability and is not further considered.

Prediction of individual progression risk

For all biomarkers, the individual patient PProb results from applying the corresponding regression equations shown in Table 1. In all cases, the slope of regression ranged from 0.1 and 0.8, and intercepts between 11 and

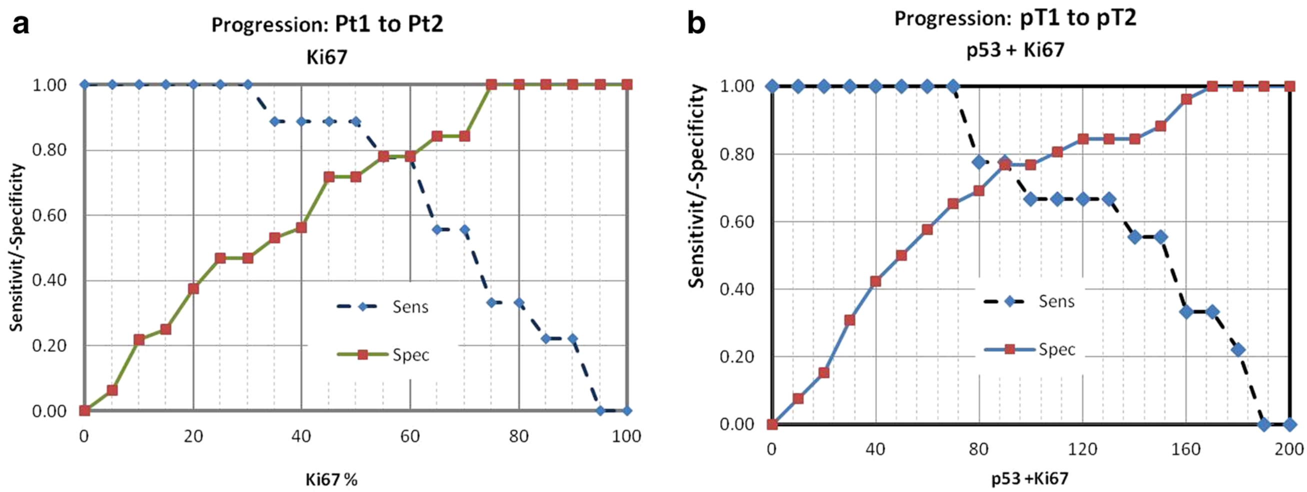

Fig. 6 Sensitivity and specificity plots: Single vs. combined biomarkers. Sensitivity and specificity to predict progression from pT1 to pT2 for Ki67 alone (**a**) and for the combination (algebraic sum) of p53 + Ki67 (**b**). No significant improvement is noted for the combination of biomarkers for prediction of progression to MIBC as compared to single Ki67. Findings are also similar to Fig. 5b, depicting results of p53 alone

35, depending on the stage progression prevalence. To calculate PProb at a given tumor stage at initial TURBT (non-invasive or pT1), the patient's biomarker value is multiplied by the regression slope and then adding the intercept, which represents the progression prevalence of the stage cohort. Figure 2a shows the linear association between p53 value and PProb in patients of our cohort with NonInv stage progressing to pT1, and Fig. 2b, a similar association but for progression from pT1 to pT2.

Table 2 shows biomarker sensitivity and specificity for progression from pT1 to pT2, using optimal cut-off points to separate risk groups. Values for progression from non-invasive to pT2 were not statistically tested because there were only two patients who progressed.

Biomarkers were not tested to predict recurrences in view of high recurrence prevalence (82%), which would falsely increase sensitivity of any predictor used.

Discussion

Our aim was to explore the possible usefulness of p53, Ki67 and CK20 at initial TURBT specimens to predict the individual patient progression risk from NMIBT to MIBC. This would allow urologists to more precisely assess progression risk at initial TURBT and to select the appropriate therapy, especially in stage pT1 where progression is associated with reduced life expectancy (Sanli and Lotan 2018). In our study, grade sub-classification of stage pT1 was not included, since many researchers consider that the vast majority of pT1 is high grade because a low-grade pT1 is either high grade or pTa (Grignon 2014).

The hallmark result of this study was the high association we found between percentage values of p53 and Ki67 and PProb at the time of first TURBT, revealing that the higher the biomarker value, the higher the patient's progression probability (Fig. 2a and b). Although existing nomograms have slightly improved individual patient progression risk assessment, they are limited by a low positive predictive

Table 2 Sensitivity and specificity of biomarkers

IHCM	Progression from pT1 to pT2							
	Cut-off %	True +	False +	False –	True –	Sens	Spec	*p* value
p53	40	7	8	2	24	0.78	0.75	0.004
Ki67	55	7	7	2	25	0.78	0.78	0.002
CK20	40	6	16	7	15	0.46	0.48	0.311

Prediction of group progression from pT1 to pT2, using optimal cut-off values. IHCM stands for immunohistochemical marker, and Cut-off is the biomarker value that best separates non-progression vs. progression groups value for progression, especially in patients with high-grade disease (EORTC 21%, CUETO 24%) (Kluth et al. 2015).

Given the high correlation values between percentages of p53 and KI67 and PProb, it seemed reasonable to expect that the use of regression equations could allow individual patient progression risk assessment (Table 1). Knowledge of individual progression risk using biomarkers could be of help when conventional histological criteria are not sufficient for a given patient, and could be added to existing nomograms as a weighed variable, thus facilitating therapeutic decisions. As shown in Results, the overall, regardless of stage, progression risk to muscle-invasive cancer in our cohort was 9.8%, but in patients staged pT1, progression prevalence was 22%. As compared to the overall progression risk, this represents a modest improvement in positive predictive value using stage as a predictor. This value, however, is too low and insufficient to plan an aggressive treatment such as radical cystectomy, unless a more convincing positive predictive value is added. Progression probability to pT1 in NonInv stage at initial TURBT is shown in Fig. 2a. It increases linearly starting at the progression prevalence. However, the association is much stronger when assessing progression risk to pT2 in patients staged pT1 (Fig. 2b).

Given these strong associations, accurate identification of the individual patient with high progression risk is possible. For example, using p53 with PProb of 60% at initial TURBT, and assuming that radical cystectomy may be indicated for a high progression risk, in 6/9 patients (67%), surgery would have been correctly indicated (true +); while 4/32 (12.5%, false +) would have not progressed to pT2. Therefore, the total number of pT1 patients who might benefit from p53-derived PProb in this example from our cohort is the sum of true positives plus true negatives, which is (28 + 6)/41 (83%, a measure of accuracy). In practical terms, calculation of PProb in the context of pT1 could benefit a considerable number of patients by the proper identification of those at higher progression risk. A similar hypothetical patient example with large (> 3 cm), high-grade, stage pT1, multiple tumor, with CIS, but using EORTC nomogram software (Sylvester et al. 2006), but with no biomarkers calculated 17% probability of progression, an insufficient value to make a therapeutic decision in a critical stage as pT1 (Sylvester et al. 2006). The PProb based on Ki67 was also high and statistically significant as shown in Fig. 4b.

We also used actual biomarker percentage of stained cells as a quantitative value to determine crossing of sensitivity and specificity curves, corresponding to optimal cut-off points for risk-group separation. This resulted in higher cut-off values in patients with higher tumor stages, as shown in Fig. 5a, b, where optimal cut-off for p53 is 12% for non-invasive stage, and 40% for pT1 patients, a value also found by other authors (Vetterlein et al. 2017). Using the wrong cut-off point to assess group progression risk could result in decreased accuracy and lower odds ratio when trying to group-stratify progression risk.

The prognostic value of biomarkers in UBPT has been previously shown in numerous studies for group stratification (Quintero et al. 2006; van Kessel et al. 2018; Grossman et al. 1998; Serth et al. 1995; Hitchings et al. 2004). However, there is no strong evidence in the literature supporting the use of biomarker values to predict individual patient progression from stage pT1 to pT2. The current recommendation in the literature is to use grade and stage as prognostic markers (Sylvester et al. 2006; Fujii 2018; Lopez Beltran and Montironi 2004) in spite of their prognostic limitations.

We believe that biomarkers p53 and Ki67 represent an important cost-effective, widely available diagnostic tool, particularly in combination with clinical/histologic parameters, especially, stage pT1. Molecular biomarkers using NGS, PCR miRNA, mRNA and others have shown to be of value for early, noninvasive diagnosis, but their value to predict progression from NMIBT to MIBC is not well established. Furthermore, due to high cost and non-availability in many centers, we elected not to consider them in this study. Accurate patient risk stratification is of value to urologists for therapeutic decisions such as radical cystectomy, since early surgical intervention may improve survival (Sanli and Lotan 2018).

Limitations

Although we tried to overcome some of the possible causes of controversy of previous biomarker studies, several limitations remain: possible errors due to visual estimation of biomarker percentages, possible tumor staging errors, and need for prospective, multicenter studies to validate equations. Furthermore, given that progression prevalence affects further progression probability, this needs to be accounted for geographically different locations.

Conclusions

Identification of individual risk of progression in patients with non-muscle-invasive bladder tumors was possible using p53- and Ki67-derived progression probability using a regression equation. Combining biomarker-derived progression probability to tumor stage pT1 improves progression to pT2 predictive accuracy.

Compliance with ethical standards

Conflict of interest Authors reported no conflicts of interest.

Ethical approval This study complies with WHO ethical standards. Patients were not subject to intervention or exposed to manipulation, intervention, or other interaction with investigators either directly or through alteration of their environment; patients in this study cannot be individually identified through investigator's collection, preparation, or use of biological material or medical or other records.

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