The Search for the Optimal cut-off Value of p53-Immunohistochemistry to Predict Prognosis of Invasive Bladder Cancer: A Multi-Center, Multi-Laboratory Analysis

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Abstract

Introduction: Mutations in the *TP53* gene are indicative of worse outcome in bladder cancer and are usually assessed by immunohistochemistry. To define p53-overexpression, a threshold of >10% is most commonly used (cut-off1). Recently, a novel cut-off (aberrant = 0% or ≥50%) (cut-off2) showed better correlation to clinical outcome. In this study, we evaluate the association between p53-immunohistochemistry cut-offs, clinico-pathological variables and disease-specific survival (DSS). **Methods:** Seven-hundred-fifty chemotherapy-naïve patients who underwent radical cystectomy were included (92% muscle-invasive bladder cancer. In addition to cut-off1 and cut-off2, a third cut-off (cut-off3) was determined based on the highest Youden-index value. Cut-off values were associated with clinico-pathological variables and *FGFR3* mutation status. The Kaplan-Meier method was used to estimate DSS. **Results:** Aberrant p53-expression was found in 489 (65%) (cut-off1) and 466 (62%) (cut-off2) tumors. Cut-off3 was determined at 25% and aberrant p53-expression in 410 cases (55%) (cutoff3). p53-expression levels were significantly associated with higher pT-stage (cut-off1/2/3: P = 0.047, P = 0.006 and P = 0.0002, respectively), higher grade (all, P < 0.0001), and *FGFR3* wild-type (cut-off1: P = 0.02, cut-offs2&3: P = 0.001). Median follow-up was 5.3 years (interquartile range, 4.0-6.0 years). p53-expression was not associated with DSS for any of the three cut-offs (cut-off1/2/3: P-log-rank = 0.566, 0.77 and 0.50, respectively). If

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we only considered locally advanced bladder cancer, results on DSS remained non-significant. **Conclusion:** This multi-center, multi-laboratory study showed that, regardless of the cut-off used, p53-immunohistochemistry did not enable selection of patients with worse outcome. Our results suggest that p53-immunohistochemistry alone is not suitable to guide clinical decision making after radical cystectomy.

Keywords
bladder cancer, radical cystectomy, p53, immunohistochemistry, prognosis

Introduction

Bladder cancer is one of the most common malignancies worldwide.[1] More than half of bladder cancers are non-invasive at diagnosis, while approximately one in three patients have muscle-invasive bladder cancer. Standard treatment for muscle-invasive bladder cancer involves radical cystectomy.[2] Nevertheless, recurrence-rates remain high and disease-specific survival (DSS) is around 50% at two years.[3] This highlights the need for reliable biomarkers for risk-stratification and guidance of management decisions such as (neo)adjuvant therapy and follow-up strategies.

Multiple studies demonstrated that *TP53* mutations were associated with a higher chance of progression and worse survival after radical cystectomy.[4,5] In over 90% of studies, p53 immunohistochemistry was used as a surrogate for *TP53* mutations.[6] The most common cut-off to define aberrant p53 expression is >10% and the vast majority of reports concerned single center, single laboratory studies.[6] Recently, a multi-center, single laboratory study reassessed p53-immunohistochemistry thresholds using an adapted cut-off value (aberrant = 0% or ≥50%) in invasive bladder cancer and showed a better correlation with *TP53* mutations and oncological outcome.[7] The aim of the present study was to validate several p53-immunohistochemistry thresholds among radical cystectomy patients in a multi-center, multi-laboratory setting.

Materials and Methods

Patients

We performed a retrospective analysis of a large multi-institutional series of radical cystectomy patients treated at five different hospitals in Europe and North-America. All 750 patients underwent radical cystectomy and bilateral pelvic lymph-node dissection for cT1-4aN0M0 urothelial carcinoma of the bladder between 1986 and 2015. Neoadjuvant treatment was not administered. Appropriate ethical approval was obtained at each site according to national regulations and the principles of the Declaration of Helsinki. Local ethics committees and/or translational research boards approved the study (Institutional Review Board number at main center: IRBd18126; complete list of numbers provided in supplementary-material1).

Sample Preparation and p53 Immunohistochemistry

The radical cystectomy and pelvic lymph node dissection specimens were locally reviewed by uro-pathologists to determine pT-stage, pN-stage, carcinoma in situ (CIS) and Lympho-Vascular Invasion (LVI). Grade was reported, according to the WHO1973 and WHO2004/2016 classification.

Standard immunohistochemistry was used to assess protein expression of p53 in the five participating laboratories. The freshly cut slides of the radical cystectomy specimens were routinely processed with a monoclonal antibody against p53 (clone DO-7; Dako, CA, USA) on the invasive component of the tumor. TMA technology[8] was used in four of the five participating laboratories. Whole slide staining was applied in Regensburg. Positive and negative controls were included in each batch/run. At each of the five laboratories, the TMAs and slides were read to assess the percentage of p53-positivity by averaging the mean percentage of stained nuclei across the slides and duplicate/triplicate TMA-cores. The reading of the p53 immunohistochemistry was done without knowledge of clinical or molecular data.

p53 Expression Cut-off

Several cut-off values were assessed. First, it was determined whether p53 was “normal” or “aberrant” using the standard 10% cut-off (cut-off1), by averaging the mean percentage of stained nuclei across cores. Secondly, as proposed by Hodgson *et al,*[7] we classified "aberrant" cases as those showing a null-phenotype (complete lack of staining; 0%) and those that stained at or above of 50% (aberrant = 0% or ≥50%) (cut-off2). Cases that showed staining between 1% and 49% were classified as "normal". Finally, an exploratory analysis was done to evaluate the discriminatory ability of a selected cut-off based on our cohort itself. The optimal p53 cut-off value was determined by creating a time-dependent receiver operating characteristic (ROC) curve with DSS as the endpoint to yield the highest Youden-index value (the best trade-off between sensitivity and specificity of a continuous value). Tumors with p53 expression lower than the cut-off value (cut-off3) were the “normal” group, while the remaining were assigned to the “aberrant” p53 group.

FGFR3 Mutation Analysis

In addition to p53 expression analysis, *FGFR3* mutation status of the same tumors at radical cystectomy was determined using the PCR-SNaPshot assay. This method has been previously reported in detail.[9] The reading of this assay was also done without knowledge of clinical or p53-expression data.

Analysis and Statistics

p53 protein expression levels were correlated with histopathological parameters at radical cystectomy (pT-stage, grade pN-stage, CIS, LVI) using the Chi-square or Fisher's exact test, as appropriate for the entire cohort, per site and per method (TMA technique vs. whole slide staining). p53 expression was also correlated with the *FGFR3* mutation status. The Kaplan-Meier method was used to estimate DSS stratified by p53 cut-off and the log-rank method was used to determine significance. Multivariable Cox' regression models were used to assess the Hazard Ratio (HR) with 95% confidence intervals (CI) to test the prognostic effect of p53 on DSS for cut-off1, cut-off2 and cut-off3. The DSS time was calculated from the date of radical cystectomy to the date of death from bladder cancer or the last follow-up visit. All statistical tests were two-sided and significance was set at $P < 0.05$. Analyses were performed using R Version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria, 2020).

Results

Patient and Tumor Characteristics

Median age of the patients at radical cystectomy was 67 years (interquartile range: 58-74 years). Hundred-sixty-two (22%) were female. Sixty-two patients (8%) had non-muscle invasive bladder cancer (NMIBC) (pTa/1/in-situ) at radical cystectomy, while the majority (n = 688; 92%) had muscle-invasive bladder cancer. In our cohort, 432 patients (58%) had no lymph node metastases (pN0), 79% had urothelial carcinoma only, whereas 21% had urothelial carcinoma plus variant histology. The patient and tumor characteristics and the sites are displayed in Table 1.

p53 Immunohistochemistry and Histopathology

Depending on the cut-off value, aberrant p53 expression was found in 489 (65%) tumors (cut-off1) versus 466 (62%) tumors (cut-off2). The association between p53 expression and histopathological variables for cut-off1 and cut-off2 are displayed in Table 2**ab.** This table shows that cut-off1 is significantly associated with more of these variables than cut-off2.

Table 1. Patient and Tumor Characteristics of the 750 Patients (cT1-4aN0M0) who Underwent Radical Cystectomy with Pelvic Lymph-Node Dissection. Median and Interquartile Range is Shown for age; Frequencies and Percentages for the Other Variables.

Variable, n = 750		Frequency	Percentage
Age (Median; IQR)		67 years (median)	58–74 years (IQR)
Sex	Female	162	21%
pT-stage at cystectomy	pTa/is/1	62	8%
	pT2	164	22%
	pT3	368	49%
	pT4	156	21%
Grade (WHO2004/2016)	LG	26	3.5%
	HG	724	96%
Grade (WHO1973)	G1	1	0.1%
	G2	69	9%
	G3	680	91%
FGFR3 mutation status	mutation	70	9%
	wild-type	680	91%
Carcinoma in situ		286	38%
Lymphovascular invasion		363	48%
pN-stage	pN0	432	58%
	pN+	318	42%
City & Laboratory	Regensburg	156	21%
	Toronto	103	14%
	Dallas	132	18%
	Amsterdam	192	26%
	Paris	167	22%

Abbreviations: pT-stage, pathological tumor-stage & pN-stage, pathological nodal-stage (according to the 2002 TNM classification of urothelial carcinoma of the urinary bladder); IQR, interquartile range; *FGFR3,* Fibroblast Growth Factor Receptor 3.

Table 2. Associations Between p53 IHC Expression and the Clinicopathological Variables Stage, Grade (WHO1973), Carcinoma in Situ (CIS), Lympho-Vascular Invasion (LVI) and Nodal status are Shown for the 750 Patients who Underwent Radical Cystectomy.

Table 2a. Estimates including odds ratios, their 95% CIs, and P-values were calculated for aberrant p53 expression versus normal p53 expression according to the "traditional" threshold of >10% indicating aberrant expression.

p53	Aberrant. (%)	Normal expr. (%)	Odds ratio (95% CI)	P-value
Total no. of patients	489 (65)	261 (35)		
pTa/1/is	34 (7)	28 (11)	ref.	**0.047**
pT2	98 (20)	66 (25)	1.2 (0.7-2.2)	
pT3	246 (50)	122 (47)	1.7 (1.0-2.9)	
pT4	111 (23)	45 (17)	2.0 (1.1-3.7)	
Grade 1–2	28 (6)	42 (16)	ref.	
Grade 3	461 (94)	219 (84)	3.2 (1.9-5.2)	**<0.0001**
No CIS	321 (66)	143 (55)	ref.	
CIS	168 (34)	118 (45)	0.63 (0.47-0.86)	**0.004**
No LVI	243 (52)	144 (54)	ref.	
LVI	246 (48)	117 (46)	1.2 (0.92-1.7)	0.15
pN0	267 (55)	165 (63)	ref.	
pN+	222 (45)	96 (37)	1.4 (1.1-1.9)	**0.02**
FGFR3 wild type	452 (92)	228 (87)	ref.	
FGFR3 mutant	37 (8)	33 (13)	0.57 (0.35-0.93)	**0.02**

Abbreviations: CI, confidence interval; CIS, carcinoma in situ; FGFR3, fibroblast growth factor receptor 3; LVI, lympho-vascular invasion.

ROC Curve Analysis and Cut-off Value for p53 Expression

The ROC analysis for p53-staining showed that the area under the curve predicting DSS was 0.51 (95%CI 0.47-0.55) (**Supplementary-Figure 1**). According to the maximum Youden-index value, the optimal cut-off (cut-off 3) was determined at 25% with a sensitivity, specificity and accuracy of 0.56 (95%CI 0.46-0.61), 0.48 (95%CI 0.44-0.59) and 0.52 (95%CI 0.48-0.55), respectively. The associations between p53-expression and histopathological variables for cut-off3 are shown in Table 2**c.**

Site-Specific Analysis for Histopathology

To elucidate potential site-specific differences, we calculated the association between p53 expression and histopathological variables using cut-offs1-3 for each specific hospital (**Supplementary-table 1).** We found that cut-off1 was associated with more histopathological variables than cut-off2 and cut-off3 in two hospitals (Regensburg and Paris); cut-off2 and cut-off3 were each associated with the most histopathological variables in one hospital (Amsterdam and Dallas, respectively).

Furthermore, a distinction was made between sites where TMA technology was used to assess p53-expression (Dallas, Paris, Toronto, Amsterdam) versus whole slide staining (Regensburg). If only the centers that used TMA-techniques were considered, we found that cut-off1 was significantly associated with all histopathological variables, whereas cut-off2 was only significantly associated with higher grade and *FGFR3* wild-type tumors and cut-off3 with higher pT-stage, grade, CIS and *FGFR3* wild-type.

p53-Expression and Survival Analyses

The median follow-up of the entire cohort was 5.3 years (IQR 4.0-6.6 years); the median DSS was 3.8 years (95% CI, 3.0-4.6 years). Using cut-off1, the median DSS of patients with normal p53 expression was 5.0 years (95% CI, 3.1-7.0 years) versus aberrant p53 expression 3.6 years (95%CI, 2.9-4.4 years) (P-logrank = 0.566) (Figure 1**a)**. With cut-off2, the difference in DSS between patients with normal versus aberrant p53 expression was also not statistically significantly different; Median DSS normal p53 expression: 5.0 years (95%CI, 3.5-7.9 years) versus aberrant p53 expression: 3.6 years (95%CI, 2.8-4.3 years) (P-logrank = 0.19) (Figure 1**b)**. Finally, cut-off3 was not able to differentiate both groups in terms of DSS; Median DSS normal p53-expression: 4.2 years (95%CI, 3.2-6.5 years) versus aberrant p53 expression: 3.7 years (95%CI, 2.9-4.9 years) (P-logrank = 0.50) (Figure 1**c)**.

Zooming in on patients with locally advanced tumors (≥pT3 and/or pN+; n = 577), the difference in DSS between normal p53-expression and versus aberrant p53-expression according to cut-off1, was not statistically significant (median DSS: 2.9 years (95%CI, 2.2-3.7 years) versus 2.5 years (95%CI, 2.1-3.0; P = 0.92). Also, there was no statistically significant difference in median DSS using cut-off2 (normal p53-expression: 2.4 years (95%CI,

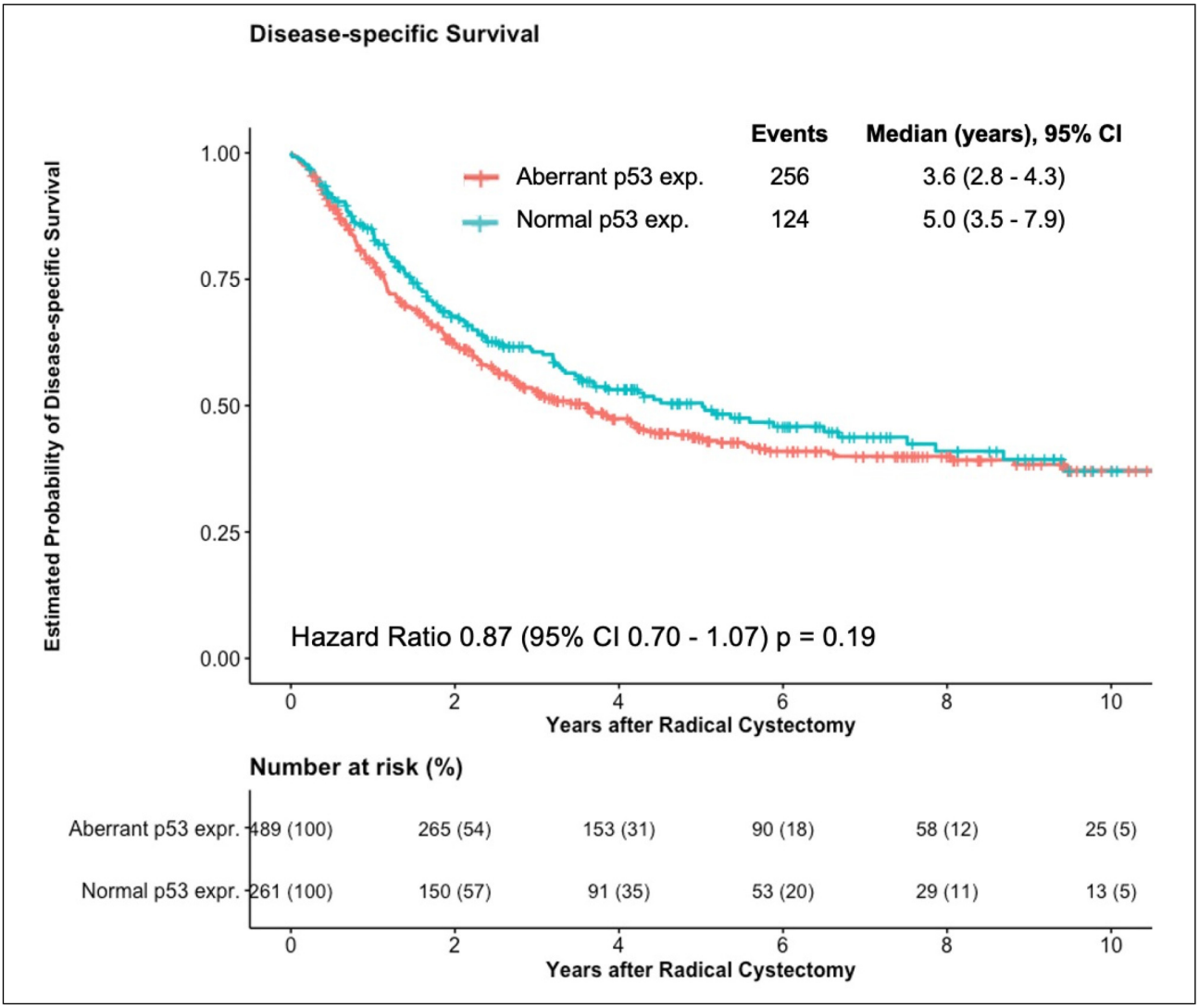

Figure 1a. Kaplan Meier estimates of disease-specific survival (DSS) for patients with aberrant versus normal p53 expression using different cut-offs. **a** Kaplan Meier estimates of disease-specific survival (DSS) for patients with aberrant versus normal p53 expression using cut-off 1.

1.8-3.0) versus aberrant p53-expression: 2.7 years (95%CI, 2.1-3.2), P = 0.62) or cut-off3 (normal p53-expression: 2.3 years (95%CI, 1.8-2.9) versus 2.7 years (95%CI, 2.1-3.3), P = 0.72)

Zooming in on pT2-3aN0 patients (n = 250), none of the cut-offs was able to significantly indicate a difference in DSS (median DSS not reached; cut-off1: P = 0.88; cut-off2: P = 0.47; cut-off3: P = 0.35).

Site-Specific Analysis for Survival

Using either cut-off1, cut-off2 or cut-off3, p53 expression did not indicate a statistically significant difference in DSS in a site-specific analysis (**Supplementary-table 2).** If we distinguished between sites were TMA-techniques were used versus whole-slide staining, we found that cut-off1 revealed a statistically significant difference in DSS between normal versus aberrant p53 expression (P-logrank = 0.04), whereas cut-off 2 and cut-off 3 did not lead to a statistically significant difference, P-logrank = 0.87 and P-logrank = 0.19, respectively.

Multivariable Analysis

In multivariable analysis, pT-stage, pN-stage and LVI were found to be independently associated with DSS, whereas p53 expression was not (not for cut-off1, neither for cut-off2 nor for cut-off3) (**Supplementary-table 3).** Furthermore, if we only selected the TMA-sites, again pT-stage, pN-stage and LVI were found to be independently associated with DSS.

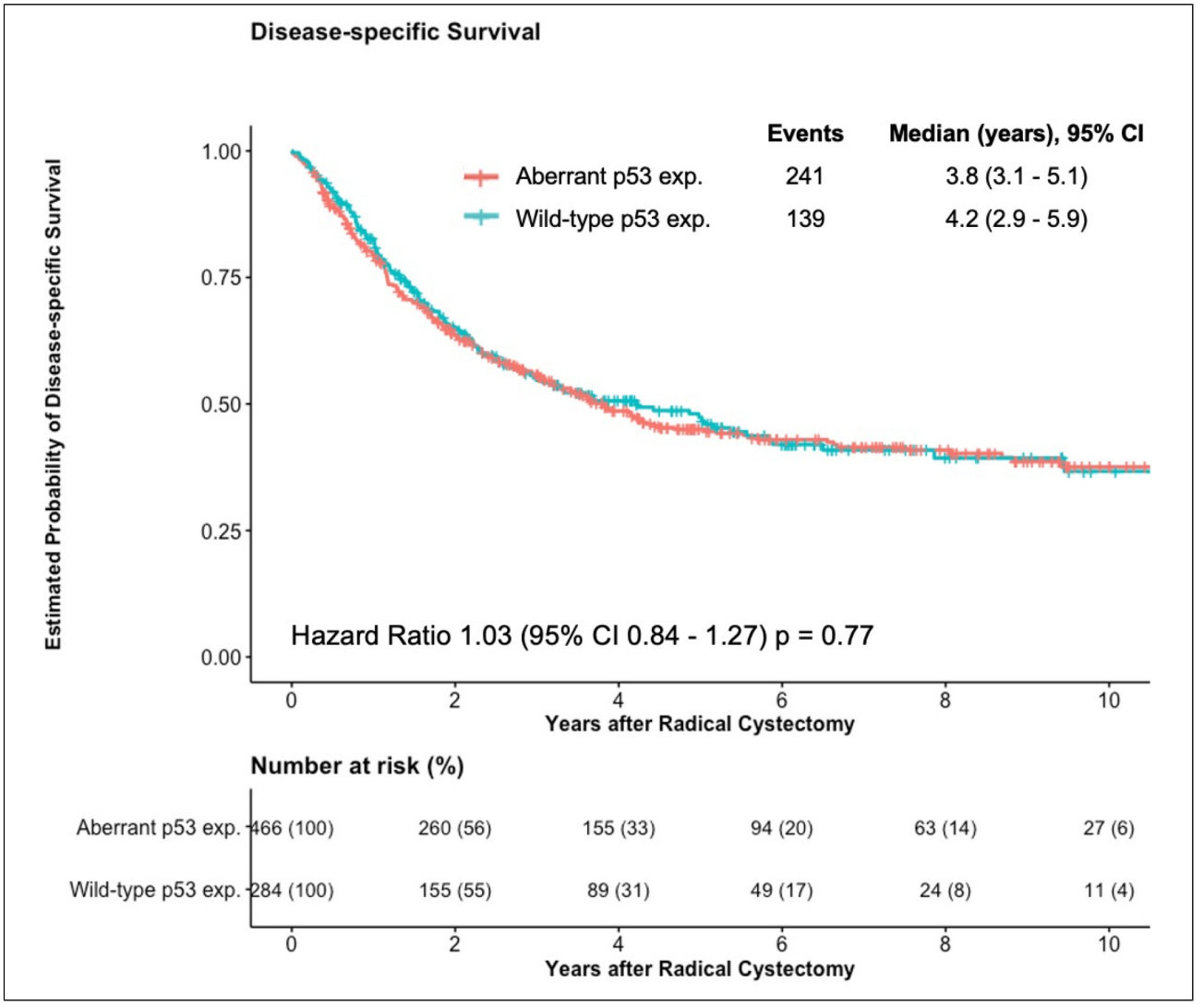

Figure 1b. Kaplan Meier estimates of disease-specific survival (DSS) for patients with aberrant versus normal p53 expression using cut-off 2.

Discussion

Muscle-invasive bladder cancer is one of the deadliest malignancies affecting modern society despite extensive treatments. This highlights the need for biomarkers to predict outcome. In muscle-invasive bladder cancer, p53-expression is one of the most widely used biomarkers generally associated with worse outcome.[4–6] However, biomarkers in general and p53–expression in particular suffers from a lack of multi-laboratory validation as a prognostic marker for invasive bladder cancer.[2,8] Therefore, the present study was done to analyze the clinical value of p53-expression within a radical cystectomy cohort and to search for the optimal cut-off value in a multi-center, multi-laboratory setting. Key findings of this study were that both the traditional and novel p53 expression cut-offs were associated with several adverse histopathologic features. However, neither cut-off1 nor cut-off2 were associated with DSS, neither in the entire cohort, nor in locally advanced or pT2-3aN0 patients. And third, in search for a better cut-off to optimize the prognostic power, we found that the calculated "optimal" cut-off3 of 25%, did also not yield any additional value to predict prognosis. Based on our results, the value of p53-immunohistochemistry alone in invasive bladder cancer should be called into question.

Our findings contrast the results of Hodgson *et al,*[7,10] who proposed an updated model for p53-immunohistochemistry assessment (cut-off2). This model, almost exclusively investigated in muscle-invasive bladder cancer, was shown to be more congruent with oncological outcome[10] and *TP53* mutations status[7] as it takes into account missense mutations leading to protein overexpression or nonsense mutations leading to complete lack of protein expression. Although we did not assess the

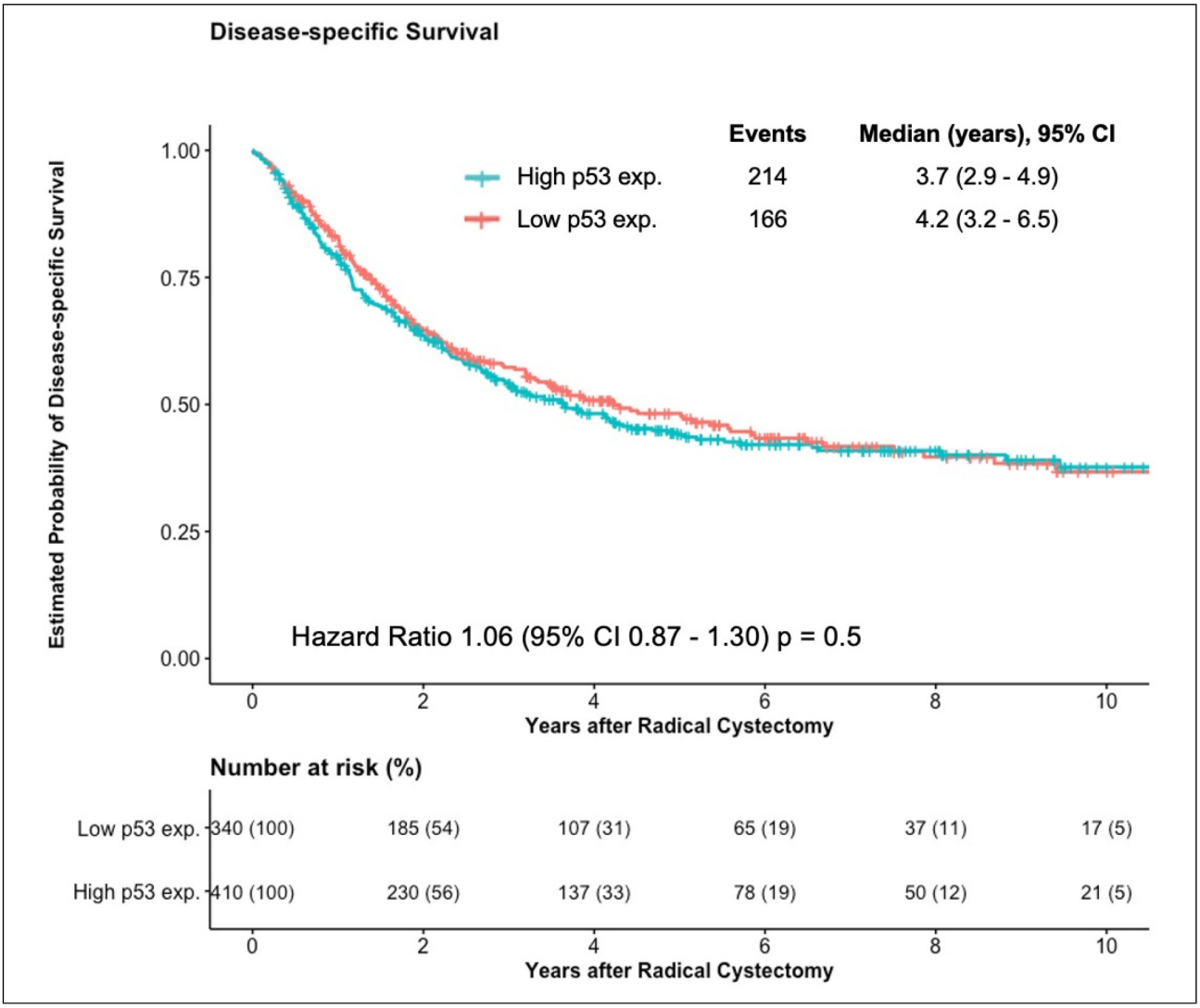

Figure 1c. Kaplan Meier estimates of disease-specific survival (DSS) for patients with aberrant versus normal p53 expression using cut-off 3.

association between p53 expression and *TP53* mutations in this study, we found that the promising prognostic results of Hodgson *et al*[7] could not be validated in a multi-laboratory setting.

Our findings regarding the poor prognostic value of p53-immunohistochemistry in the multi-laboratory setting contrast our previous findings on the prognostic value and reproducibility of the *FGFR3* mutation in invasive bladder cancer.[9,11,12] While p53-expression was not, the *FGFR3* mutation status seemed promising to guide decision-making on adjuvant anti-FGFR3 therapy as *FGFR3* appeared homogeneous in a multi-center, multi-laboratory setting.[9,11] Based on the current and previous studies, it can be concluded that PCR-based *FGFR3* mutation analysis appears reproducible and contributes as a prognostic marker whereas the prognostic value of p53-immunohistochemistry remained highly questionable.

Immunohistochemistry tests, including p53, have historically suffered from reproducibility issues and caution must be used in comparing results across studies from different laboratories.[13] This was illustrated by our observation that the association of both cut-offs with pathologic variables highly varied in a per-laboratory analysis and that all three cutoffs were not associated with survival. Similar to ours, a recent international study on immunohistochemistry of several biomarkers, including p53, in advanced bladder cancer[13] showed that the agreement of immunohistochemistry among laboratories was poor, even if TMA was used in the most experienced hands. In our study, we found that TMA yielded somewhat better results in terms of histopathologic features and prognosis compared to whole-slide staining, albeit only for the traditional cut-off1. Taken together, p53-immunohistochemistry alone does

Table 2b. Estimates including odds ratios, their 95% CIs, and P-values were calculated for aberrant p53 expression versus normal p53 expression according to the novel threshold aberrant = 0% or ≥ 50%.

p53	Aberrant. (%)	Normal expr. (%)	Odds ratio (95% CI)	P-value
Total no. of patients	466 (62)	284 (38)		
pTa/1/is	26 (6)	36 (13)	ref.	**0.006**
pT2	101 (22)	63 (22)		
pT3	241 (52)	127 (45)		
pT4	98 (21)	58 (20)		
Grade 1–2	24 (5)	46 (16)	ref.	
Grade 3	442 (95)	238 (84)	3.6 (2.1-6.0)	**<0.0001**
No CIS	297 (64)	167 (59)	ref.	
CIS	169 (36)	117 (41)	0.81 (0.60-1.1)	0.18
No LVI	244 (52)	143 (54)	ref.	
LVI	222 (48)	156 (46)	0.92 (0.69-1.2)	0.59
pN0	271 (58)	161 (57)	ref.	
pN+	195 (42)	123 (43)	0.94 (0.70-1.3)	0.96
FGFR3 wild type	435 (93)	245 (86)	ref.	
FGFR3 mutant	31 (7)	39 (14)	0.45 (0.27-0.74)	**0.001**

Abbreviations: CI, confidence interval; CIS, carcinoma in situ; FGFR3, fibroblast growth factor receptor 3; LVI, lympho-vascular invasion.

Table 2c. Estimates including odds ratios, their 95% CIs, and P-values were calculated for aberrant p53 expression versus normal p53 expression according to cut-off 3 (the Youden model threshold) = 25%).

p53	High p53 expr.(%)	Low p53 expr.(%)	Odds ratio (95% CI)	P-value
Total no. of patients	410 (55)	340 (45)		
pTa/1/is	24 (6)	38 (11)	ref. (1.0)	**0.002**
pT2	77 (19)	87 (26)	1.4 (0.8-2.6)	
pT3	215 (52)	153 (45)	2.2 (1.3-3.9)	
pT4	94 (23)	62 (18)	2.4 (1.3-4.4)	
Grade 1–2	19 (5)	51 (15)	ref.	
Grade 3	391 (95)	289 (85)	3.6 (2.1-6.4)	**<0.0001**
No CIS	272 (66)	192 (57)	ref.	
CIS	138 (34)	148 (44)	0.7 (0.5-0.9)	**0.007**
No LVI	206 (50)	181 (53)	ref.	
LVI	204 (50)	159 (47)	1.1 (0.9-1.5)	0.46
pN0	227 (55)	205 (60)	ref.	
pN+	183 (45)	135 (40)	1.2 (0.9-1.6)	0.19
FGFR3 wild type	385 (94)	295 (87)	ref.	
FGFR3 mutant	25 (6)	45 (13)	0.4 (0.3-0.7)	**0.001**

Abbreviations: pT-stage, pathological tumor stage; NMIBC, non-muscle invasive bladder cancer; WHO, World Health Organization; pN-stage, pathological nodal stage; SMs, surgical margins; PSMs, positive surgical margins; LNs, lymph nodes; LVI, Lympho-vascular Invasion; CIS, carcinoma-in-situ. Using cut-off 1, aberrant p53 expression was significantly associated with higher pT-stage (P = 0.047), higher grade (OR 3.2 (95% CI, 1.9-5.2), P < 0.0001), absence of CIS (OR 0.63 (95% CI, 0.47-0.86), P = 0.004), nodal metastases (OR 1.4 (95% CI, 1.1-1.9), P = 0.02) and with *FGFR3* wild type status (OR 0.57 (95% CI, 0.35-0.93), P = 0.02), on univariable analysis. Using cut-off 2, abnormal expression of p53 was associated with higher pT-stage (P = 0.006), higher grade (OR 3.6 (95% CI, 2.1-6.0), P < 0.0001) and *FGFR3* wild type status (OR 0.45 (95% CI, 0.27-0.74), P = 0.001), but not with CIS or nodal metastases. OR = Odds ratio.

not seem suitable for clinical practice, most likely because of its questionable reproducibility between laboratories.

Limitations of the present study include those inherent to its retrospective design, in which patients underwent radical cystectomy during a relatively long period of time. Moreover, this is a selected series of patients with advanced bladder cancer and persistent tumor at radical cystectomy. Furthermore, when we

calculated the "optimal" cut-off3, we could not take into account the p53 null phenotype. However, this was mainly done to illustrate that the current limitations of p53-immunohistochemistry are not so much related to its precise cut-off. Finally, we did not obtain *TP53* status of the tumors in this study and, as a consequence, we could not evaluate the association between p53 immunohistochemistry and *TP53* here. Different methods to interpret p53 expression may better capture *TP53* status[7,14] and may have implications for its reproducibility.

In conclusion, this multi-center, multi-laboratory study showed that p53-immunohistochemistry of radical cystectomy specimens does not enable a reliable selection of patients at higher risk for poor clinical outcome after radical cystectomy regardless of the cut-off value chosen. As a consequence, p53-immunohistochemistry alone is probably not suitable to guide clinical decision making after radical cystectomy.

Acknowledgments

The authors would like to acknowledge the International Bladder Cancer Network (IBCN) and the Bladder Cancer Advocacy Network (BCAN) for providing the platform to collaborate on this project. We thank the Core Facility for Molecular Pathology & Biobanking of the Netherlands Cancer Institute – Antoni van Leeuwenhoek and the Tissue bank Unit – PRB Mondor for their assistance.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the University of Toronto, a grant from the Dutch Cancer Society – KWF Kankerbestrijding and the French Urological Association. Specific grant numbers are not applicable. FC and YN were supported by the European Urological Scholarship Program (EUSP).

Ethical Approval

Site specific approval and protocol numbers Amsterdam: The Institutional Review Board of the Netherlands Cancer Institute – Antoni van Leeuwenhoek hospital (CFMPB-160 & IRBd18126). Regensburg: Medical ethical board of the University of Regensburg (16-101-0218). Paris: The regional ethics board of Ile-de-France IX – Comité de protection des personnes – Ile-de-France IX - Créteil (11-052). Toronto: The regional ethics board of the University Health Network, Toronto (02-0515-C, 08-0263-T, 09-0826-CE and 09-0556-TE). Dallas: The Institutional Review Board for the Protection of Human Subjects at University of Texas Southwestern Medical School: STU 102014-008.

Informed Consent

Not applicable, because this article does not contain any studies with human or animal subjects.

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Trial Registration

No RCT registration, because no RCTSite specific approval and protocol numbers.

Supplemental Material

Supplemental material for this article is available online.

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