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Urologic Oncology: Seminars and Original Investigations 37 (2019) 510–518

UROLOGIC ONCOLOGY

Laboratory-Kidney cancer

BioScore (B7-H1, survivin, and Ki-67) does not predict cancer-specific mortality in surgically treated patients with renal cell carcinoma: An external validation study

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Received 11 December 2018; received in revised form 9 March 2019; accepted 8 April 2019

Abstract

Background: To externally validate, BioScore', a biomarker-based scoring system using immunohistochemical tumor expression levels of B7-H1, survivin, and Ki-67, in a single-center cohort of renal cell carcinoma (RCC) patients. Additionally, we investigated the potential benefit of BioScore as compared to the Mayo Clinic stage, size, grade, and necrosis (SSIGN) score.

Materials and Methods: The validation cohort comprised 393 nonmetastatic RCC patients treated with radical nephrectomy or nephron-sparing surgery from 1999 to 2004. Kaplan-Meier estimators, the log-rank test, uni- and multivariable Cox regression models, and measures of discrimination were used to quantify the prognostic performance of BioScore regarding cancer-specific mortality (CSM).

Results: During a median follow-up of 7.8 years, 69/132 (52%) deaths were adjudicated to progressive disease. BioScore was weakly associated with CSM in univariable analysis (hazard ratio per 1 point increase = 1.12, 95% confidence interval = 1.02−1.23, $P = 0.023$). However, this association did not prevail after adjusting for other adverse prognostic factors as represented by the SSIGN score. The discriminative performance of BioScore was very modest (Harrell's C-Index = 0.60) and did not improve the SSIGN score ($P = 0.341$), which already showed an excellent discrimination, as evidenced by Harrell's C-Index of 0.81. In a sensitivity analysis regarding clear cell RCC patients only, B7-H1 positivity did not emerge as a statistically significant predictor of CSM.

Conclusion: Although a higher BioScore was significantly associated with a higher CSM, the magnitude of this association was weak and not independent from other prognosticators. Moreover, BioScore did not improve the prognostic accuracy of the SSIGN score.

Keywords: BioScore; B7-H1; Ki-67; Prognosis; Renal cell carcinoma

1. Introduction

Regarding cancer of the kidney and renal pelvis, the estimated 2018 rates in the US population are 65,340 new cases and 14,970 kidney cancer-related deaths [1]. Currently, the 5-year survival rate is over 50% for locoregional

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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https://doi.org/10.1016/j.urolonc.2019.04.004
1078-1439/

(stage III) disease and ~8% for metastatic renal cell carcinoma (RCC) [2].

Several clinico-pathologic scoring systems to better predict RCC patients prognosis have been published, such as the 2002 American Joint Committee on Cancer (AJCC) tumor, node, metastasis classification system (TNM) stage groupings [3], the UCLA Integrated Scoring System [4], postoperative prognostic nomograms [5,6], the Mayo Clinic stage, size, grade, and necrosis (SSIGN) score [7], the Karakiewicz nomogram [8], and the Leibovich prognosis score [9], to name some prominent ones.

Naturally, various problems arise, regarding the comparability of medical scoring systems in general: (1) They are each generated in different patient cohorts with distinct clinico-pathologic features. (2) They concern different timelines, using various methodological approaches, as well as endpoints. (3) Some major scoring systems were externally validated, while others were not. (4) They often lack biomarker-driven information and data, which would help to better understand underlying molecular pathways and explains, why several groups of researchers (including our own) focused on integrating biomarkers (mostly blood based), as well as clinico-pathologic patient features into combined models [10–18].

In 2008, Parker et al. from the Mayo Clinic were the first to publish an alternative approach in combining tumor expression levels of 3 biomarkers (B7-H1, survivin, and Ki-67), each previously demonstrated as an independent predictor of RCC outcome, into a single scoring panel, termed "BioScore‘," that can be used to refine outcome prediction provided by existing biomarkers with existing clinico-pathologic algorithms as a means of improving outcome prediction in clear cell RCC (ccRCC) [11]. It is important to note that BioScore was developed in a dataset comprising >630 ccRCC patients and demonstrated to exert a statistically significant impact on cancer-specific mortality (CSM) [11]. A detailed analysis of BioScore in ccRCC was provided by McGuire et al. in 2009 [19]. To the best of our knowledge, BioScore has not been externally validated in an independent RCC patient cohort so far, which was the purpose of the recent analysis.

2. Materials and Methods

2.1. Study design and endpoint

The primary validation cohort comprised 393 consecutive patients with clinically localized (N0M0) RCC, who underwent surgery between 1999 and 2004 at a single tertiary academic center. All data were retrieved from a university electronic documentation system. Mortality was adjudicated as either being RCC-related death or death-from-other-causes. Out of 393 RCC patients, 1 was excluded due to missing follow-up information, 7 were excluded due to missing tissue specimens for BioScore assessment, or because at least 1 out of 3 BioScore items could not be pathologically determined ($n = 3$).

B7-H1, survivin, Ki-67, age, body mass index, gender, Eastern Cooperative Oncology Group-performance status (≥1 vs. 0), presence of symptoms at diagnosis, localization and focality of the primary tumor, pathological tumor (pT-) and nodal (N-) stage, adjusted to 2002 AJCC TNM and additionally assessed based on the 2009 AJCC TNM staging system, were assessed as described previously [20]. Furthermore, tumor size, collecting system invasion, histological subtype according to the Heidelberg classification, and the 3-tiered grading according to the Mainz classification were assessed [20]. Cancer-specific survival was defined as the time between the day of surgery and death from RCC, or in case a patient did not die from RCC, the date of death from other causes or loss to follow-up.

2.2. Determination of BioScore

BioScore was assessed according to Parker et al. in paraffin-embedded tumor tissue blocks available for each individual patient with immunohistochemical staining, as described in the original publication [11]. Briefly, 3,5-μ-thick slides from paraffin-embedded blocks with representative tumor tissue for each patient were obtained. For subsequent immunohistochemical staining antibodies were used similar to those applied by Parker et al.: Ki-67 (Dako, stock number: M7240, mouse monoclonal antibody; dilution 1:200), Survivin (Abcam, stock number; #ab469, rabbit polyclonal antibody; dilution 1:250), and B7-H1 (CD274; Sigma, stock number: #SAB2900365, rabbit polyclonal antibody; dilution 1:300). Standardized positive and negative antibody controls were conducted in each immunohistochemical assay.

An experienced pathologist supervised by a board-certified uro-pathologist quantified the membranous staining pattern of B7-H1, which was quantified as percentage of positive tumor cells in 5% to 10% increments. Nuclear staining patterns of survivin and Ki-67 were quantified as number of positive tumor cells in each of 5 representative high-powered microscope fields. As such, survivin and Ki-67 expression were quantified as the number of positive tumor cells per mm^2. The development of BioScore in the external validation cohort followed exactly the previously published methodology by Parker et al. [11], where a patient with a B7-H1-negative, survivin-low, and Ki-67-low tumor defines a baseline patient with a BioScore of 0. In case that a tumor was B7-H1-positive, 2 points were added. Similarly, patients with survivin-high and Ki-67-high tumors would have 3 and 2 points added to their BioScore, respectively (maximum BioScore possible = 7).

2.3. Statistical analyses

All statistical analyses were performed using Stata 14.0 (Stata Corp., Houston, TX). Continuously coded variables

are summarized as medians with interquartile ranges (IQRs), whereas count data are reported as absolute frequencies (percentages). Rank-sum tests, χ^2-tests (expected cell count ≥5) and Fisher's exact tests (expected cell count < 5) were applied for quantifying the association between variables. CSM was analyzed with Kaplan-Meier estimators and compared between 2 or more groups with the log-rank test. Hazards of death-from-RCC were modeled with uni- and multivariable Cox proportional hazards models. Time-dependent associations of BioScore and the SSIGN score were assessed by fitting interactions between these scores and linear follow-up time within univariable Cox models. The discriminative performance of BioScore and the SSIGN score was quantified with Harrell's concordance (C)-index. In a sensitivity analysis, only ccRCC-patients were considered.

3. Results

3.1. Analysis at baseline

Three hundred eighty-two RCC patients were included in the validation analysis. The median age of the study cohort was 64 years (IQR = 57–71), patients were predominantly males (232 [61%]) and had histologically ccRCC (277 [73%]). Descriptive clinico-pathological parameters of the study cohort are shown in Table 1.

3.2. Analysis of CSM

Patients were followed for a median (IQR: 6.3, 9.9 years) interval of 7.8 years (range: 6 days–10 years). During this follow-up, we observed 132 deaths, of which 69 (52%) were adjudicated to RCC progression, whereas 63 (48%) were attributed to other-cause mortality. The 1-, 3-, 5-, and 10-year Kaplan-Meier CSS estimates were 96% (95% CI = 93–98), 89% (95% CI = 85–92), 86% (95% CI = 82–89), and 79% (95% CI = 74–83), respectively (Supplementary. Fig. 1). Patients who died during follow-up were significantly older and had a higher prevalence of adverse tumor characteristics, such as high Fuhrmann nuclear grade, renal/caval vessel invasion, or microvascular invasion. Table 1 shows a comparison of patients who died during follow-up because of their RCC vs. patients who did not die because of RCC. Univariable analysis of clinico-pathological parameters for the prediction of CSM in the study cohort is reported in Table 2.

3.3. Analysis of BioScore and CSM

Among the 3 items of BioScore, 55% were positive for B7-H1, and 26% and 41% had a high nuclear expression of survivin and Ki-67, respectively (Table 1). A high expression of survivin ($P = 0.014$) and Ki-67 ($P = 0.034$), but not B7-H1 positivity ($P = 0.803$) were more prevalent in patients who died from RCC during follow-up. Median BioScore (range: 0–7 points) was 2 points (IQR = 0–4) in the total study cohort, 2 points in patients who did not die from RCC progression (0–4), and 3 points (0–5) in patients who died from RCC, respectively (rank-sum $P = 0.073$).

In univariable Cox regression, patients with higher survivin (hazard ratio [HR] = 1.85, $P = 0.014$) or Ki-67 (HR = 1.70, $P = 0.029$) expression, but not B7-H1-positivity (HR = 1.00, $P = 0.995$) experienced a higher CSM (Table 2). Ten-year Kaplan-Meier CSS estimates were 71%, 74%, and 79% in patients with high expression of survivin, Ki-67, or B7-H1-positivity, and 82%, 82%, and 79% in patients without these immunohistochemical features, respectively (Table 3 and Suppl. Figs. 2–4). A higher BioScore was weakly associated with a higher risk of death from RCC (HR per 1 point increase = 1.12, 95% CI: 1.02–1.23, $P = 0.023$). In detail, 10-year CSS estimates were 82% in the 233 patients with a BioScore of 0 to 2 points, 79% in the 57 patients with a score of 3 to 4 points, and 71% in the 92 patients with a score ≥5 points, respectively (log-rank $P = 0.048$, Fig. 1).

3.4. BioScore prognostic stratification after surgical RCC-resection

The discriminative performance of BioScore, as expressed by its discrimination between patients who did and did not die from RCC during follow-up, was modest (Harrell's C-Index = 0.60, Suppl. Fig. 5) and only slightly better than a toss of a coin (95% CI of the C-Index = 0.53–0.67).

In contrast, the externally validated Mayo Clinic SSIGN score showed a much higher discrimination (Harrell's C-Index = 0.81, 95% CI = 0.75–0.87, s. Fig. 2). Moreover, when considering the score on a common scale (either by z-standardization or log2-transformation), the association of the SSIGN score with CSM was considerably stronger than the corresponding association of the BioScore (HR per doubling of BioScore = 1.20, 95% CI = 0.96–1.51, $P = 0.107$; HR per doubling of the SSIGN score = 3.92, 95% CI = 2.81–5.45, $P < 0.0001$; s. Table 2). In a multivariable Cox regression model regarding the endpoint CSM, including both BioScore and the SSIGN score, only the SSIGN score emerged as a statistically significant determinant of CSM (Table 4). Furthermore, the addition of BioScore to the SSIGN score did not improve its discriminative performance (change in Harrell's C-Index = 0.01, 95% CI = −0.01 to 0.03, $P = 0.341$).

As a sensitivity analysis, we used the B7-H1 expression as a binary variable dichotomized into "high" at the 75th percentile ($n = 85$, cut-off ≥30% pos. cells), and as a binary variable only categorizing those patients as "high" with a B7-H1 expression ≥15% ($n = 19$), consistent with the original publication by Parker et al. [11]. Additionally, the analysis revealed that B7-H1 positivity did not emerge as a

Table 1
Descriptive clinico-pathological parameters of the study cohort comprising of patients with renal cell carcinoma ($n = 382$)

Variable	N (% miss.)	Overall ($n = 382$)	No death-from-RCC during follow-up ($n = 313$)	Death-from-RCC during follow-up ($n = 69$)	P value*
Demographic characteristics					
Age at entry (y)	382 (0%)	64 [57-71]	63 [56-70]	67 [62-72]	**0.004**
Female gender	382 (0%)	150 (39%)	123 (39%)	27 (39%)	0.980
BMI (kg/m^2)	382 (0%)	27.7 [25.4−30.7]	27.9 [25.5−30.9]	26.2 [24.5−29.1]	**0.007**
ECOG PS ≥ 1	382 (0%)	60 (16%)	21 (7%)	39 (57%)	**<0.0001**
Ethnicity: Caucasian	382 (0%)	382 (100%)	313 (100%)	69 (100%)	**1.000**
Tumor characteristics					
RCC-related symptoms at Dx	382 (0%)	115 (30%)	86 (27%)	29 (42%)	**0.017**
Tumor location (side)	382 (0%)	/	/	/	0.960
Right	/	171 (45%)	141 (45%)	30 (43%)	/
Left	/	196 (51%)	160 (51%)	36 (52%)	/
Bilateral	/	15 (4%)	12 (4%)	3 (4%)	/
Tumor location (kidney)	382 (0%)	/	/	/	0.519
Upper renal third	/	147 (38%)	117 (37%)	30 (43%)	/
Middle renal third	/	121 (32%)	99 (32%)	22 (32%)	/
Lower renal third	/	114 (30%)	97 (31%)	17 (25%)	/
Tumor size (mm)	382 (0%)	45 [32−65]	40 [30−60]	80 [53−108]	**<0.0001**
TNM pT3b-pT4	382 (0%)	14 (4%)	3 (1%)	11 (16%)	**<0.0001**
TNM pN	382 (0%)	/	/	/	**<0.0001**
pN0	/	89 (23%)	66 (21%)	23 (33%)	/
pN1	/	17 (4%)	4 (1%)	13 (19%)	/
pNX	/	276 (72%)	243 (78%)	33 (48%)	/
TNM cM	382 (0%)	28 (7%)	6 (2%)	22 (32%)	**<0.0001**
Fuhrmann Grade	382 (0%)	/	/	/	**<0.0001**
G1	/	84 (22%)	78 (25%)	6 (9%)	/
G2	/	256 (67%)	217 (69%)	39 (57%)	/
G3	/	42 (11%)	18 (6%)	24 (35%)	/
Tumor histology	382 (0%)	/	/	/	0.805
Clear cell RCC	/	277 (73%)	225 (72%)	52 (75%)	/
Papillary RCC	/	87 (23%)	72 (23%)	15 (22%)	/
Chromophobe RCC	/	18 (5%)	16 (5%)	2 (3%)	/
Adrenal involvement	382 (0%)	5 (1%)	0 (0%)	5 (7%)	**<0.0001**
Microvasular invasion	382 (0%)	60 (16%)	34 (11%)	26 (38%)	**<0.0001**
Microlymphovascular invasion	382 (0%)	15 (4%)	6 (2%)	9 (13%)	**<0.0001**
Tumor necrosis > 10%	352 (8%)	110 (8%)	78 (27%)	32 (50%)	**<0.0001**
Multifocality	382 (0%)	18 (5%)	12 (4%)	6 (9%)	0.084
Surgical characteristics					
Nephron-sparing/partial nephrectomy	382 (0%)	46 (12%)	44 (14%)	2 (3%)	**0.010**
Adrenalectomy	382 (0%)	122 (32%)	84 (27%)	38 (55%)	**<0.0001**
Renal/caval vessel invasion	382 (0%)	40 (10%)	18 (6%)	22 (32%)	**<0.0001**
Fat invasion	382 (0%)	71 (19%)	38 (12%)	33 (48%)	**<0.0001**
Collecting duct invasion	382 (0%)	45 (12%)	26 (8%)	19 (28%)	**<0.0001**
R1 resection margin	382 (0%)	8 (2%)	2 (1%)	6 (9%)	**0.001**
BioScore variables					
B7-H1 “Positive”	382 (0%)	210 (55%)	173 (55%)	37 (54%)	0.803
Nuclear survivin “High”	382 (0%)	99 (26%)	73 (23%)	26 (38%)	**0.014**
Nuclear Ki-67 “High”	382 (0%)	156 (41%)	120 (38%)	36 (52%)	**0.034**
Risk prediction					
BioScore (points)	382 (0%)	2 [0−4]	2 [0−4]	3 [0−5]	0.073
SSIGN score (points)*	382 (0%)	0 [2−4]	2 [0−3]	7 [4−8]	**<0.0001**

Baseline characteristics are presented as summary measures. In detail, continuous variables are reported as medians (IQR) and count data are reported as absolute frequencies (%). Data are displayed in the overall cohort ($n = 382$), as well as in patients who did not ($n = 313$) or did ($n = 69$) die from RCC during the observational period. The P-value is from Wilcoxon’s rank-sum tests or chi-squared tests, comparing the distribution of the respective value between patients who did not or did die from RCC.

BMI = body mass index; Dx = diagnosis; ECOG PS = Eastern Cooperative Oncology Group performance status; RCC = renal cell carcinoma; R1 = pos. surgical margin; SSIGN score = stage, size, grade, and necrosis score; TNM = tumor, node, metastasis classification system.

Bold numbers in Table 1 shows statistically significant p-values.

Table 2
Univariable analysis of clinico-pathological parameters for the prediction of cancer-specific mortality (CSM) in patients with renal cell carcinoma ($n = 382$)

Variable	Hazard ratio (HR)	95% CI	*P* value
Demographic characteristics			
Age at entry (per 5 y increase)	1.27	1.12−1.45	**<0.0001**
Female gender	1.10	0.68−1.79	0.691
BMI (per 5 kg/m^2 increase)	0.66	0.48−0.90	**0.008**
ECOG PS ≥ 1	13.95	8.54−22.78	**<0.0001**
Tumor characteristics			
RCC-related symptoms at Dx	1.88	1.16−3.04	**0.010**
Tumor location (side)	/	/	/
Right	Ref.	Ref.	Ref.
Left	1.05	0.64−1.70	0.856
Bilateral	1.38	0.42−4.51	0.599
Tumor location (kidney)	/	/	/
Upper renal third	Ref.	Ref.	Ref.
Middle renal third	0.82	0.47−1.44	0.493
Lower renal third	0.69	0.38−1.26	0.230
Tumor size (per 10 mm increase)	1.17	1.13−1.22	**<0.0001**
TNM pT3-pT4	10.49	5.46−20.15	**<0.0001**
TNM pN	/	/	/
pN0	Ref.	Ref.	Ref.
pN1	6.23	3.12−12.43	**<0.0001**
pNX	0.44	0.26−0.75	**0.002**
TNM cM	10.44	6.25−17.42	**<0.0001**
Fuhrmann grade	/	/	/
G1	Ref.	Ref.	Ref.
G2	2.39	1.01−5.67	**0.047**
G3	12.96	5.28−31.77	**<0.0001**
Tumor histology	/	/	/
Clear cell RCC	Ref.	Ref.	Ref.
Papillary RCC	0.99	0.56−1.77	0.981
Chromophobe RCC	0.62	0.15−2.55	0.507
Adrenal involvement	27.68	10.43−73.49	**<0.0001**
Microvasular invasion	4.38	2.68−7.15	**<0.0001**
Microlymphovascular invasion	6.19	3.06−12.51	**<0.0001**
Tumor necrosis > 10%	2.70	1.65−4.43	**<0.0001**
Multifocality	2.30	0.99−5.33	0.052
Surgical characteristics			
Nephron-sparing/partial nephrectomy	0.21	0.05−0.86	**0.030**
Adrenalectomy	2.89	1.79−4.66	**<0.0001**
Renal/caval vessel invasion	5.60	3.36−9.32	**<0.0001**
Fat invasion	5.31	3.29−8.55	**<0.0001**
Collecting duct invasion	3.48	2.05−5.91	**<0.0001**
Positive surgical margins	12.93	5.53−30.26	**<0.0001**
BioScore variables			
B7-H1 “positive”	1.00	0.62−1.61	0.995
Nuclear survivin “High”	1.85	1.14−3.02	**0.014**
Nuclear Ki-67 “High”	1.70	1.05−2.73	**0.029**
Risk prediction			
BioScore (per 1 point increase)	1.12	1.02−1.23	**0.023**
BioScore (per 1 SD increase)	1.31	1.04−1.65	**0.023**
BioScore (per doubling)	1.20	0.96−1.51	0.107
SSIGN score (per 1 point increase)*	1.51	1.41−1.61	**<0.0001**
SSIGN score (per 1 SD increase)	3.29	2.71−3.99	**<0.0001**
SSIGN score (per doubling)	3.92	2.81−5.45	**<0.0001**

95% CIBMI = body mass index; Dx = diagnosis; ECOG PS = Eastern Cooperative Oncology Group performance status; RCC = renal cell carcinoma; Ref. = Reference; TNM = tumor, node, metastasis classification system; SD = standard deviation; SSIGN score = stage, size, grade, and necrosis score;95% CI = 95% confidence intervals.

Bold numbers in Table 2 shows statistically significant *p*-values.

Table 3
The 1-, 3-, 5-, and 10-year Kaplan-Meier cancer-specific survival (CSS) estimates in renal cell carcinoma patients with high, low or negative expression levels of B7-H1, survivin, and Ki-67

	1-y CSS (95% CI)	3-y CSS (95% CI)	5-y CSS (95% CI)	10-y CSS (95% CI)	log-rank *P*
B7-H1 expression					
B7-H1 "positive"	96% (92–98)	90% (85–93)	85% (80–90)	79% (72–85)	0.995
B7-H1 "negative"	96% (92–98)	88% (82–92)	86% (80–91)	79% (70–85)	
Survivin expression					
Survivin "high"	92% (84–96)	80% (71–87)	78% (68–85)	71% (60–80)	0.012
Survivin "low"	97% (95–99)	92% (88–95)	89% (84–92)	82% (76–86)	
Ki-67 expression					
Ki-67 "high"	95% (90–97)	85% (78–90)	80% (72–85)	74% (66–81)	0.028
Ki-67 "low"	97% (94–98)	92% (88–95)	90% (85–93)	82% (75–87)	
BioScore					
0–2 points	97% (94–99)	92% (87–95)	90% (85–93)	82% (75–87)	0.048
3–4 points	100% (100–100)	91% (79–96)	81% (68–89)	79% (66–88)	
5–7 points	91% (83–95)	81% (71–88)	78% (68–86)	71% (60–80)	
SSIGN score					
0–2 points	100% (97–100)	97% (94–99)	97% (93–98)	93% (89–96)	<0.0001
3–4 points	97% (90–99)	91% (81–95)	89% (79–94)	80% (66–89)	
5–6 points	96% (77–99)	93% (74–98)	82% (62–92)	66% (42–82)	
7–9 points	87% (68–95)	55% (36–71)	34% (17–51)	0% (0–0)	
≥10 points	46% (19–70)	8% (0–29)	8% (0–29)	0 (0–0)	

SSIGN score = Mayo Clinic stage, size, grade, and necrosis score; 95% CI = 95% confidence interval.

significant predictor of CSM (HR for cut-off ≥30% pos. cells = 0.85, $P = 0.594$; HR for cut-off ≥15% = 0.28, $P = 0.207$). Finally, albeit not in a statistically significant fashion, the prognostic potential of BioScore appeared to decrease over time (multiplicative change in the univariable HR of the BioScore for every year after baseline = 0.97, $P = 0.130$), whereas the corresponding potential of the SSIGN score did not appear to weaken with time (multiplicative change = 0.99, $P = 0.360$).

3.5. Sensitivity analysis in ccRCC

Among the 277/382 (72.5%) patients with ccRCC, 51% were positive for B7-H1, and 27% and 45% had a high

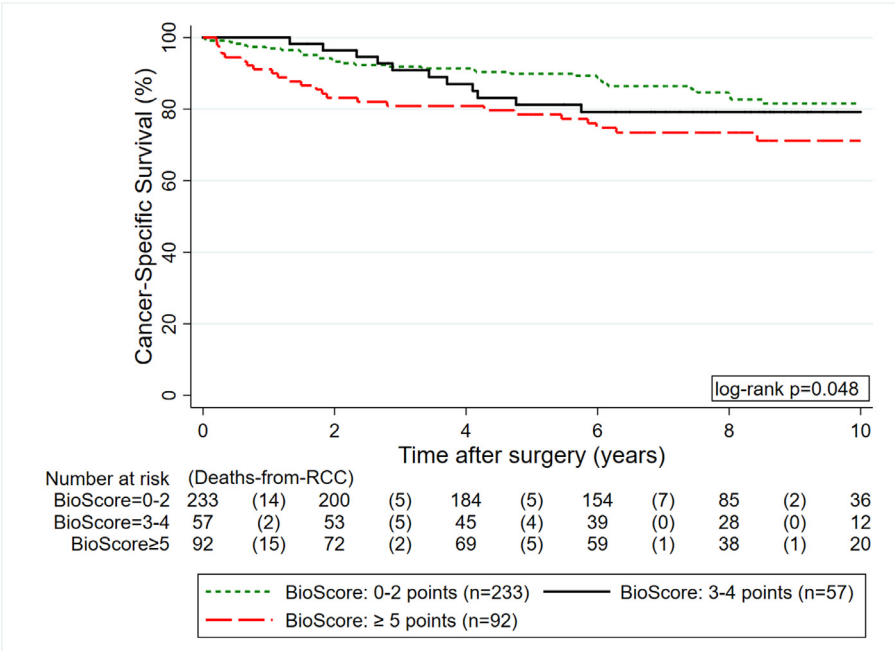

Fig. 1. The 1-, 3-, 5-, and 10-year Kaplan-Meier cancer-specific survival (CSS) estimates of the study cohort according to BioScore.

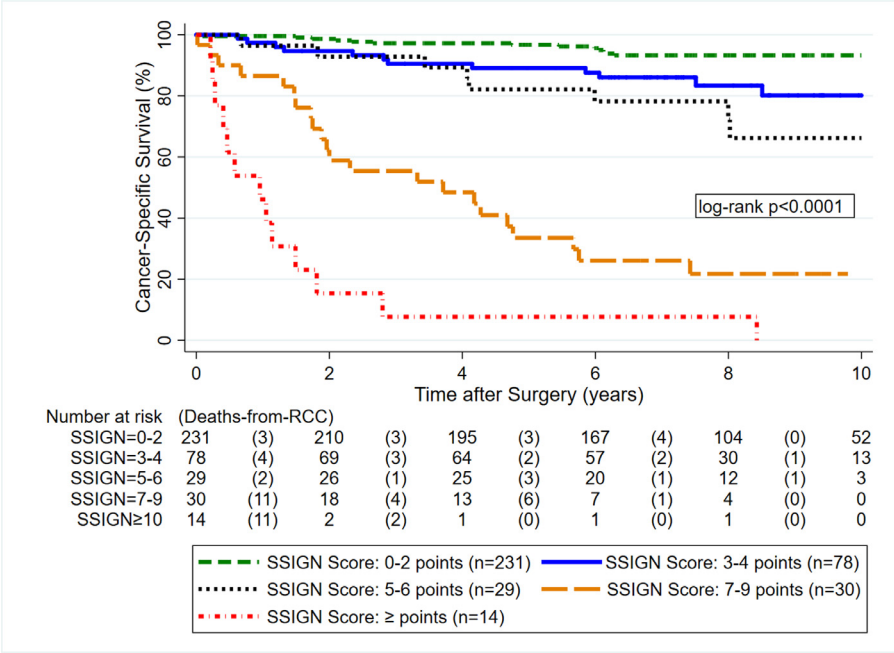

Fig. 2. The 1-, 3-, 5-, and 10-year Kaplan-Meier cancer-specific survival (CSS) estimates of the study cohort according to the Mayo Clinic SSIGN (stage, size, grade, and necrosis) score.

nuclear expression of survivin and Ki-67, respectively. Twenty-one (40%) of the 52 patients who died from RCC had a high nuclear expression of survivin, as compared to only 55 (24%) of the 225 patients who did not die from RCC during the observational period, respectively ($P = 0.020$). Twenty-nine (56%) of the 52 patients who died from RCC had a high nuclear expression of Ki-67, as compared to 95 (42%) of the 225 patients who did not die from RCC during the observational period, respectively ($P = 0.007$). No association was found between B7-H1 positivity and death from RCC ($P = 0.447$). Median BioScore (range: 0–7 points) was 2 points (IQR = 0–54) in all 277 ccRCC patients, 2 points in patients who did not die from RCC progression (0–4), and 3 points (0–7) in patients who died from RCC, respectively (rank-sum $P = 0.170$).

4. Discussion

Current RCC staging systems have shown improved prognostic performance characteristics compared to anatomical staging alone, such as for example the UCLA Integrated Scoring System [4], several postoperative nomograms [5,6,8,21], the Mayo Clinic SSIGN score [7], as well as Parker et al.'s BioScore [11], just to name a few.

An accurate prediction of clinical outcomes in localized RCC after curative surgery is paramount, since it might help to better identify patients with an increased risk for recurrence, as well as metastatic spread of the disease [13]. In general, internally validated nomograms have the advantage of a high predictive accuracy regarding the tested endpoints, as well as good calibration characteristics in the nomogram development dataset [5,6,8,21]. However, as recently pointed out by Bandini et al. in a detailed report analyzing 9 different RCC-nomograms that were published over the last years, most of them lack true external validation and even fewer have investigated the clinical utility, as measured by a model's ability to improve clinical decision-making [22]. A major problem arises, since both, external validation and assessment of the clinical utility of any prognostic model are mandatory before they can be incorporated into daily routine clinical practice [22].

Regarding potentially useful RCC-biomarkers, such as preoperatively assessable blood-based parameters [14–16,23], as well as more complex molecular markers

Table 4
Multivariable Cox regression model regarding cancer-specific survival (CSS) in renal cell carcinoma patients, including both BioScore and the Mayo Clinic stage, size, grade, and necrosis (SSIGN) score

Variable	Hazard ratio (HR)	95% CI	*P* value
BioScore (per 1 point increase)	1.01	0.91–1.11	0.896
SSIGN score (per 1 point increase)	1.50	1.40–1.62	<0.0001

SSIGN score = Mayo Clinic stage, size, grade, and necrosis score; 95% CI = 95% confidence interval.

[10,24–27], that are sometimes integrated into existing prognostic models, the above-mentioned problem does not become smaller. For example, Kim et al. were among the first to demonstrate an enhanced-prognostic ability with molecular marker-based staging compared to clinical variables alone, analyzing 150 metastatic ccRCC-patients who underwent nephrectomy prior to immunotherapy [10]. Nevertheless, the problem of unproven clinical utility, as well as the lack of a comparability with other staging models that were generated in different patient cohorts, persists.

In their study, Parker et al. showed that ccRCC patients with high (4, 5, or 7) BioScores were 5 times more likely to die from RCC compared with patients with low (0, 2, or 3) BioScores (HR = 5.03, 95% CI = 3.82–6.61, $P < 0.001$) [11]. Additionally, BioScore statistically significantly enhanced the prognostic ability of each of the individual prognostic features studied. In contrast, in our validation study, BioScore was only associated with a weak rise in CSM (HR per 1 point increase = 1.12, 95% CI = 1.02–1.23, $P = 0.023$).

In our RCC external validation cohort ($n = 382$), even if evaluating a mixed population of histological RCC subtypes, we were not able to reproduce these results using the same methodology as the authors: Among the 3 items of BioScore, 55% were positive for B7-H1 vs. 15% in Parker et al.'s patient cohort, 26% vs. 31% had a high nuclear expression of survivin, and 41% vs. 39% had a high nuclear expression of Ki-67, respectively [11]. A high expression of survivin and Ki-67, but not B7-H1 positivity, were more prevalent in patients who died from RCC during follow-up in our cohort. Moreover, we found a higher BioScore to be only weakly associated with a higher risk of death from RCC. Additionally, the discriminative performance of BioScore in our cohort was modest, evidenced by Harrell's C-Index of 0.60. Finally, in a multivariable Cox regression model regarding CSS, including both BioScore and the SSIGN score, only the SSIGN score emerged as a statistically significant determinant of CSS. Furthermore, the addition of BioScore to the SSIGN score did not improve its discriminative performance, as evidenced by a change in Harrell's C-Index of only 0.01.

The observed differences in terms of the results regarding Parker et al.'s study [11] and our recent analysis remain somewhat elusive. In particular, patients' baseline characteristics of the original Parker et al. study cohort and our study cohort were very similar, with a greater proportion of patients with nuclear grade G3–G4 tumors (47% vs. 11%), a higher proportion of patients with histologic tumor necrosis (30% vs. 8%), as well as the restriction to clear cell histology tumors in the Parker et al. cohort, representing the only main differences in patients' baseline characteristics. Acknowledging important limitations, the sample size of our study cohort was significantly smaller, resulting in the inability to perform meaningful BioScore subgroup analyses. Additionally, a shorter median follow-up, the retrospective nature of data assessment, as well as possible differences in the immunohistochemical staining process of the paraffin-embedded tissue blocks have to be stated. Moreover, despite our recent validation study showed incomparable results with the original publication by Parker et al., a prospective external validation of this important tool is strongly warranted in independent datasets.

5. Conclusion

Although a higher BioScore was significantly associated with a higher CSM, the magnitude of this association was weak and not independent from other prognosticators. Moreover, BioScore did not improve the prognostic accuracy of the SSIGN score.

Acknowledgments

None of the contributing authors have any conflicts of interest, including specific financial interests, relationships and affiliations relevant to the subject matter or materials discussed in the manuscript.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2019.04.004.

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA: Cancer J Clin 2018;68:7–30.
[2] Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA: Cancer J Clin 2017;67:7–30.
[3] Ficarra V, Galfano A, Mancini M, Martignoni G, Artibani W. TNM staging system for renal-cell carcinoma: current status and future perspectives. Lancet Oncol 2007;8:554–8.
[4] Zisman A, Pantuck AJ, Dorey F, Said JW, Shvarts O, Quintana D, et al. Improved prognostication of renal cell carcinoma using an integrated staging system. J Clin Oncol 2001;19:1649–57.
[5] Kattan MW, Reuter V, Motzer RJ, Katz J, Russo P. A postoperative prognostic nomogram for renal cell carcinoma. J Urol 2001;166:63–7.
[6] Sorbellini M, Kattan MW, Snyder ME, Reuter V, Motzer R, Goetzl M, et al. A postoperative prognostic nomogram predicting recurrence for patients with conventional clear cell renal cell carcinoma. J Urol 2005;173:48–51.
[7] Frank I, Blute ML, Cheville JC, Lohse CM, Weaver AL, Zincke H. An outcome prediction model for patients with clear cell renal cell carcinoma treated with radical nephrectomy based on tumor stage, size, grade and necrosis: the SSIGN score. J Urol 2002;168:2395–400.
[8] Karakiewicz PI, Briganti A, Chun FK, Trinh QD, Perrotte P, Ficarra V, et al. Multi-institutional validation of a new renal cancer-specific survival nomogram. J Clin Oncol 2007;25:1316–22.
[9] Leibovich BC, Blute ML, Cheville JC, Lohse CM, Frank I, Kwon ED, et al. Prediction of progression after radical nephrectomy for patients with clear cell renal cell carcinoma: a stratification tool for prospective clinical trials. Cancer 2003;97:1663–71.

[10] Kim HL, Seligson D, Liu X, Janzen N, Bui MH, Yu H, et al. Using tumor markers to predict the survival of patients with metastatic renal cell carcinoma. J Urol 2005;173:1496–501.
[11] Parker AS, Leibovich BC, Lohse CM, Sheinin Y, Kuntz SM, Eckel-Passow JE, et al. Development and evaluation of BioScore: a biomarker panel to enhance prognostic algorithms for clear cell renal cell carcinoma. Urol Oncol 2018;36:94.e15–21.
[12] Morshaeuser L, May M, Burger M, Otto W, Hutterer GC, Pichler M, et al. p53-expression in patients with renal cell carcinoma correlates with a higher probability of disease progression and increased cancer-specific mortality after surgery but does not enhance the predictive accuracy of robust outcome models. Urol Oncol 2017.
[13] Seles M, Posch F, Pichler GP, Gary T, Pummer K, Zigeuner R, et al. Blood platelet volume represents a novel prognostic factor in patients with nonmetastatic renal cell carcinoma and improves the predictive ability of established prognostic scores. J Urol 2017;198:1247–52.
[14] Dalpiaz O, Luef T, Seles M, Stotz M, Stojakovic T, Pummer K, et al. Critical evaluation of the potential prognostic value of the pretreatment-derived neutrophil-lymphocyte ratio under consideration of C-reactive protein levels in clear cell renal cell carcinoma. Br J Cancer 2017;116:85–90.
[15] Bezan A, Mrsic E, Krieger D, Stojakovic T, Pummer K, Zigeuner R, et al. The preoperative AST/ALT (De Ritis) ratio represents a poor prognostic factor in a cohort of patients with nonmetastatic renal cell carcinoma. J Urol 2015;194:30–5.
[16] Hutterer GC, Stoeckigt C, Stojakovic T, Jesche J, Eberhard K, Pummer K, et al. Low preoperative lymphocyte-monocyte ratio (LMR) represents a potentially poor prognostic factor in nonmetastatic clear cell renal cell carcinoma. Urol Oncol 2014;32:1041–8.
[17] Thiel DD, Davidiuk AJ, Meschia C, Serie D, Custer K, Petrou SP, et al. Mayo adhesive probability score is associated with localized renal cell carcinoma progression-free survival. Urology 2016;89:54–60.
[18] Pichler M, Hutterer GC, Stoeckigt C, Chromecki TF, Stojakovic T, Golbeck S, et al. Validation of the pre-treatment neutrophil-lymphocyte ratio as a prognostic factor in a large European cohort of renal cell carcinoma patients. Br J Cancer 2013;108:901–7.
[19] McGuire BB, Fitzpatrick JM. Biomarkers in renal cell carcinoma. Curr Opin Urol 2009;19:441–6.
[20] Morshaeuser L, May M, Burger M, Otto W, Hutterer GC, Pichler M, et al. p53-expression in patients with renal cell carcinoma correlates with a higher probability of disease progression and increased cancer-specific mortality after surgery but does not enhance the predictive accuracy of robust outcome models. Urol Oncol 2018;36:94 e15-94 e21.
[21] Hutterer GC, Patard JJ, Perrotte P, Ionescu C, de La Taille A, Salomon L, et al. Patients with renal cell carcinoma nodal metastases can be accurately identified: external validation of a new nomogram. Int J Cancer 2007;121:2556–61.
[22] Bandini M, Fossati N, Briganti A. Nomograms in urologic oncology, advantages and disadvantages. Curr Opin Urol 2019;29:42–51.
[23] Dalpiaz O, Pichler M, Mrsic E, Reitz D, Krieger D, Venturino L, et al. Preoperative serum-gamma-glutamyltransferase (GGT) does not represent an independent prognostic factor in a European cohort of patients with non-metastatic renal cell carcinoma. J Clin Pathol 2015;68:547–51.
[24] Cao YW, Liu Y, Dong Z, Guo L, Kang EH, Wang YH, et al. Monocarboxylate transporters MCT1 and MCT4 are independent prognostic biomarkers for the survival of patients with clear cell renal cell carcinoma and those receiving therapy targeting angiogenesis. Urol Oncol 2018;36:311.:e15-e25.
[25] Wang K, Ruan H, Song Z, Cao Q, Bao L, Liu D, et al. PLIN3 is up-regulated and correlates with poor prognosis in clear cell renal cell carcinoma. Urol Oncol 2018;36:343.:e9-e19.
[26] Zhang S, He J, Jia Z, Yan Z, Yang J. Acetyl-CoA synthetase 2 enhances tumorigenesis and is indicative of a poor prognosis for patients with renal cell carcinoma. Urol Oncol 2018;36:243.:e9-e20.
[27] Ha M, Son YR, Kim J, Park SM, Hong CM, Choi D, et al. TEK is a novel prognostic marker for clear cell renal cell carcinoma. Eur Rev Med Pharmacol Sci 2019;23:1451–8.