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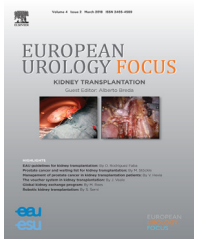

Bladder Cancer

The Value of Preoperative Plasma VEGF Levels in Urothelial Carcinoma of the Bladder Treated with Radical Cystectomy

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Abstract

Background: Elevated preoperative plasma levels of the angiogenesis-related marker VEGF have been associated with worse oncological outcomes in various malignancies.
Objective: To investigate the predictive/prognostic role of VEGF in patients with urothelial carcinoma of the bladder (UCB) treated with radical cystectomy (RC).
Design, setting, and participants: VEGF plasma levels were measured preoperatively in 1036 patients with UCB who underwent RC.
Outcome measurements and statistical analysis: The correlation between plasma VEGF levels and pathological and survival outcomes was assessed using logistic regression and Cox regression analyses. Discrimination was assessed using the concordance index (C index). The clinical net benefit was evaluated using decision curve analysis (DCA).
Results and limitations: Patients with higher pretreatment plasma VEGF levels had poorer recurrence-free survival (RFS), cancer-specific survival (CSS), and overall survival (OS) according to log-rank tests (all $p < 0.001$). Higher VEGF levels were not independently associated with higher risk of lymph node metastasis, ≥pT3 disease, or non–organ-confined disease (all $p > 0.05$). Preoperative plasma VEGF levels were independently associated with RFS, CSS, and OS in preoperative and postoperative multivariable

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models. However, in all cases the C index increased by <0.02 and there was no improvement in net benefit on DCA. A limitation is that none of the patients received current elements of standard of care such as neoadjuvant chemotherapy.
Conclusions: Elevated plasma VEGF levels were associated with features of biologically and clinically aggressive disease such as worse survival outcomes among patients with UCB treated with RC. However, VEGF appears to have relatively limited incremental additive value in clinical use. Further study of VEGF for UCB prognostication is warranted before routine use in clinical algorithms.
Patient summary: Currently available models for predicting outcomes in bladder cancer are less than optimal. A protein called vascular endothelial growth factor (VEGF), which is a marker of the formation of blood vessels (angiogenesis), may have a role in predicting survival outcomes in bladder cancer.

1. Introduction

Radical cystectomy (RC) with lymph node dissection is the standard treatment for very high-risk non–muscle-invasive and muscle-invasive urothelial carcinoma of the bladder (UCB) [1,2]. Owing to high intertumoral heterogeneity, a significant percentage of patients treated with RC for UCB still experience disease progression [3]. Thus, various clinical and pathological factors have been explored to improve risk stratification for patients with UCB and identify those who might benefit from intensified perioperative systemic therapy [4–6]. However, the accuracy of current outcome prediction models remains suboptimal, probably because of their inability to capture the full potential of host-tumor interactions [7]. Furthermore, clinical, radiological, and preoperative pathological factors have significant limitations for outcome prediction, making accurate personalized clinical decision-making difficult [4,8]. Therefore, preoperative biomarkers that capture the biological and clinical potential of each tumor must be identified to improve risk stratification for patients with UCB [9–11].

Angiogenesis, the process for growth of new blood vessels, is necessary for tumor growth and metastasis [12,13]. VEGF plays a central role in regulating tumor angiogenesis [14]. VEGF overexpression can be detected in the majority of cancers, including UCB [15,16]. Moreover, it has been shown that VEGF is associated with oncological outcomes in several cancer types [17–20]. However, associations for circulating levels of VEGF in patients with UCB treated with RC remain poorly investigated, as most previous studies have assessed the prognostic value of tissue VEGF expression [21–23].

We hypothesized that elevated preoperative plasma VEGF levels are associated with features of biologically and clinically aggressive UCB and poor survival. We used data from a large consecutive cohort of patients with nonmetastatic advanced UCB treated with RC and pelvic lymphadenectomy to investigate the relationship between preoperative plasma VEGF levels and established features of UCB invasion, metastasis, and survival outcomes.

2. Patients and methods

2.1. Patient selection

All procedures were undertaken with the approval and oversight of the institutional review board for the protection of human subjects (1011011386 and 069826900). This retrospective study included 1036 patients treated with RC for nonmetastatic UCB between 2003 and 2015. No patient received neoadjuvant chemotherapy (NAC) or radiotherapy. Adjuvant chemotherapy was administered to 167 patients (16.1%) at the clinician's discretion. No patient received adjuvant radiotherapy.

2.2. Measurement of VEGF plasma levels

Preoperative plasma samples for VEGF testing were typically collected on the morning of surgery after an overnight fast; collection and measurement were performed as previously described [20]. In brief, blood was collected into an 8-ml Vacutainer CPT tube containing 1 ml of 0.1 M sodium citrate (Becton Dickinson, Franklin Lakes, NJ, USA) and centrifuged at 1500 × g for 20 min at room temperature. The top layer (plasma) was decanted using a sterile transfer pipette. The plasma was immediately frozen and stored at −80 °C in a polypropylene cryopreservation vial (Nalgene; Nalge Nunc, Rochester, NY, USA). It has previously been found that VEGF levels are higher when measured in serum than when measured in plasma. Since VEGF is present in platelet granules and is released on platelet activation, the higher levels of VEGF in serum are probably as a result, at least in part, of release from damaged platelets, making quantification of nonplatelet-derived VEGF less accurate. Therefore, before assessment, an additional centrifugation step was performed at 10 000 × g for 10 min at room temperature for complete platelet removal. We used quantitative immunoassays to measure VEGF levels (R&D Systems, Minneapolis, MN, USA).

2.3. Pathological evaluation

All surgical specimens were processed according to standard pathological procedures as previously described [24]. The 2002 American Joint Committee on Cancer-Union International Centre le Cancer TNM classification and the 1973 World Health Organization/International Society of Urological Pathology consensus classification were used for pathological staging and grading, respectively.

2.4. Management and follow-up

All patients were followed in accordance with the relevant institutional protocols and local guidelines at the time. In general, routine follow-up included physical examination, radiological imaging, and urine cytology every 3 mo for 2 yr. Between year 2 and year 5, follow-up was performed semiannually, and annually thereafter in most cases. Recurrence was

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Table 1 – Patient demographics

Characteristic	Overall	Stratified by median log VEGF		
	(*n* = 1036)	Low (*n* = 518)	High (*n* = 518)	*p* value[a]
Median age, yr (IQR)	67 (60–73)	66 (59–73)	67 (60–73)	>0.9
Gender, *n* (%)				0.6
Male	814 (79)	404 (78)	410 (79)	
Female	222 (21)	114 (22)	108 (21)	
Blood transfusion, *n* (%)	268 (26)	139 (27)	129 (25)	0.5
Thrombocytosis, *n* (%)	113 (11)	52 (10)	61 (12)	0.4
Clinical tumor grade, *n* (%)				>0.9
Grade 2	6 (0.6)	3 (0.6)	3 (0.6)	
Grade 3	1022 (99)	511 (99)	511 (99)	
Unknown	8	4	4	
Clinical tumor stage, *n* (%)				0.003
cTa	23 (2.2)	7 (1.4)	16 (3.1)	
cTis	105 (10)	67 (13)	38 (7.4)	
cT1	336 (33)	176 (34)	160 (31)	
cT2	498 (48)	241 (47)	257 (50)	
cT3	38 (3.7)	13 (2.5)	25 (4.9)	
cT4	29 (2.8)	11 (2.1)	18 (3.5)	
Unknown	7	3	4	
Pathological tumor grade, *n* (%)				0.6
Grade 1	62 (6.0)	29 (5.6)	33 (6.4)	
Grade 2	11 (1.1)	4 (0.8)	7 (1.4)	
Grade 3	963 (93)	485 (94)	478 (92)	
Pathological tumor stage, *n* (%)				0.3
pT0	62 (6.0)	29 (5.6)	33 (6.4)	
pTa	22 (2.1)	12 (2.3)	10 (1.9)	
pTis	131 (13)	79 (15)	52 (10)	
pT1	162 (16)	81 (16)	81 (16)	
pT2	248 (24)	121 (23)	127 (25)	
pT3	281 (27)	134 (26)	147 (28)	
pT4	130 (13)	62 (12)	68 (13)	
Positive surgical margins, *n* (%)	95 (9.2)	48 (9.3)	47 (9.1)	>0.9
Lymphovascular invasion, *n* (%)	295 (28)	137 (26)	158 (31)	0.15
Concomitant CIS, *n* (%)	572 (55)	301 (58)	271 (52)	0.061
Lymph node involvement, *n* (%)	263 (25)	129 (25)	134 (26)	0.7
Adjuvant chemotherapy, *n* (%)	167 (16)	74 (14)	93 (18)	0.11

CIS = carcinoma in situ; IQR = interquartile range.
[a] Wilcoxon rank-sum test, Pearson's χ^2 test, or Fisher's exact test, as appropriate.

defined as any local recurrence (in the retroperitoneum or renal fossa) or distant metastasis. Recurrences in the bladder or contralateral upper urinary tract were considered as second primary tumors.

2.5. Statistical analysis

Results for continuous variables are reported as the median and interquartile range (IQR). Owing to non-normal distribution of preoperative VEGF levels, log transformation was performed to reduce skewness and allow valid inference on multivariable analysis. Patient characteristics and median preoperative VEGF plasma levels were treated as categorical variables; thus, group comparisons were performed using a Mann-Whitney U test or Kruskal-Wallis test with subsequent significance testing as appropriate.

Binominal logistic regression analysis was performed to assess the association between preoperative VEGF plasma levels and lymph node metastasis (LNM), ≥pT3 disease, or any non–organ-confined disease (NOCD; defined as ≥pT3 disease and/or LNM).

Recurrence-free survival (RFS), cancer-specific survival (CSS), and overall survival (OS) were graphically visualized using the Kaplan-Meier method. The difference between groups was assessed using a log-rank test. Univariable and multivariable Cox regression models were used to investigate the associations of VEGF with RFS, CSS, and OS. Clinical and pathological tumor grade were excluded as variables for all predictive models, as almost all patients had high-grade UCB. Separate Cox regression models that featured either preoperative clinical variables or postoperative histopathological variables were developed. The discriminatory ability of these models and the additional information provided by plasma VEGF levels was tested using Harrel's concordance index (C index). The additional clinical net benefit of VEGF was evaluated using decision curve analysis (DCA) to investigate whether preoperative plasma VEGF levels improved the accuracy of separate predictive and prognostic models and whether these models had a relevant net benefit in the preoperative or postoperative setting. All p values were two-sided and significance was defined as $p < 0.05$. Statistical analyses were performed using R v3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) and Stata/MP 14.2 statistical software (Stata Corp., College Station, TX, USA).

3. Results

3.1. Patient demographics and VEGF association

Patient characteristics are presented in Table 1. The median age of the cohort was 67 yr (IQR 60–73). There was no association between median VEGF levels and adverse pathological features such as LNM and advanced pathological tumor stage ($p > 0.05$).

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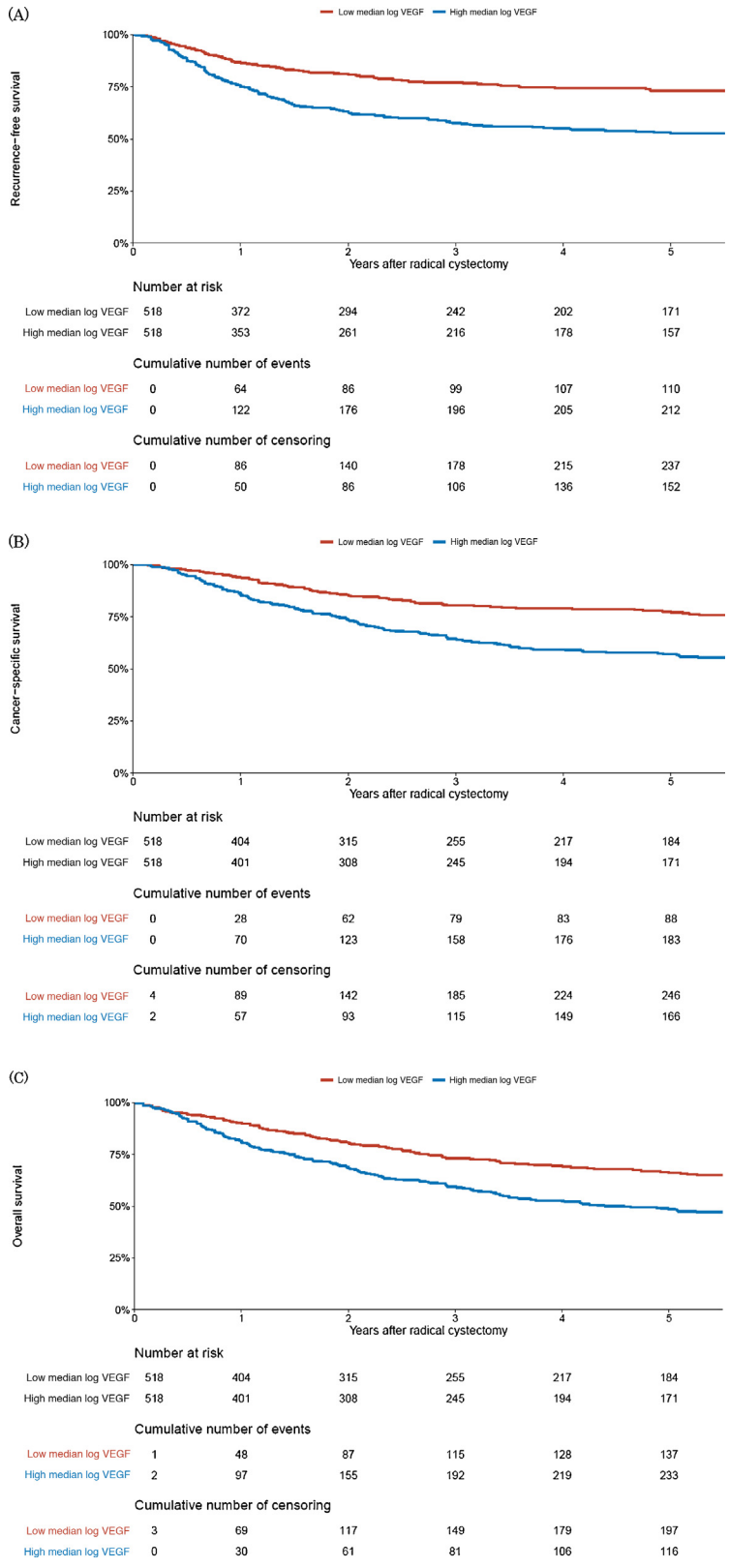

Fig. 1 – Kaplan Meier estimates of oncological outcomes stratified by VEGF level among 1036 patients with urothelial carcinoma of the bladder treated with radical cystectomy. (A) Recurrence-free survival, (B) cancer-specific survival, and (C) overall survival.

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Table 2 – Cox regression analyses for preoperative parameters

Parameter	Recurrence-free survival		Cancer-specific survival		Overall survival	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Log VEGF	1.42 (1.19–1.70)	<0.001	1.41 (1.17–1.70)	<0.001	1.17 (1.01–1.35)	0.04
Age	1.02 (1.00–1.03)	0.007	1.02 (1.01–1.03)	0.001	1.05 (1.04–1.06)	<0.001
Gender						
Male	Reference		Reference		Reference	
Female	1.51 (1.18–1.93)	0.002	1.64 (1.26–2.10)	<0.001	1.33 (1.09–1.62)	0.004
Clinical tumor stage						
cTa/cTis/cT1	Reference		Reference		Reference	
cT2	1.75 (1.38–2.21)	<0.001	1.89 (1.48–2.43)	<0.001	1.64 (1.37–1.96)	<0.001
cT3/cT4	1.99 (1.30–2.95)	<0.001	2.23 (1.43–3.34)	<0.001	1.88 (1.35–2.56)	<0.001
C index with VEGF	0.618		0.641		0.638	
C index without VEGF	0.602		0.628		0.634	

HR = hazard ratio; CI = confidence interval.

3.2. VEGF association with pathological features

Multivariable logistic regression modeling revealed that elevated preoperative VEGF was not significantly associated with higher risk of LNM (odds ratio [OR] 1.12, 95% confidence interval [CI] 0.86–1.45; p = 0.40), ≥pT3 disease (OR 1.02, 95% CI 0.80–1.30; p = 0.87), or NOCD (OR 1.09, 95% CI 0.86–1.38; p = 0.49; Supplementary Table 1).

3.3. VEGF association with survival outcomes in the preoperative model

The median follow-up for alive patients was 37 mo. The 5-yr estimates for RFS, CSS, and OS were 62.5%, 66%, and 57%, respectively. Patients with higher median pretreatment VEGF had poorer RFS, CSS, and OS in the respective log-rank tests (all $p < 0.001$; Fig. 1).

In a multivariable Cox regression analysis that included available preoperative variables (age, sex, and clinical tumor stage), higher pretreatment VEGF levels were independently associated with poorer RFS (hazard ratio [HR] 1.42, 95% CI 1.19–1.70; $p < 0.001$), CSS (HR 1.41, 95% CI 1.17–1.70; $p < 0.001$), and OS (HR 1.17, 95% CI 1.01–1.35; p = 0.04; Table 2). Addition of preoperative plasma VCAM-1 levels slightly improved the C index of the same reference models for prognosticating RFS (+1.6%), CSS (+1.3%), and OS (+0.4%; Table 2). On DCA, there was no relevant gain in net benefit for prognosis of RFS, CSS, or OS (Supplementary Fig. 1) across any threshold probabilities after addition of plasma VEGF levels in comparison to a reference model consisting of established clinicopathological characteristics.

3.4. VEGF association with survival outcomes in the postoperative model

In a multivariable Cox regression model that included established postoperative variables, higher pretreatment VEGF levels remained independently associated with poorer RFS (HR 1.53, 95% CI 1.29–1.82; $p < 0.001$), CSS (HR 1.44, 95% CI 1.20–1.73; $p < 0.001$), and OS (HR 1.21, 95% CI 1.04–1.39; p = 0.01; Table 3). VEGF addition to the reference models (Table 3) did not improve the C index for prognosticating RFS, CSS, and OS. On DCA, there was no relevant gain in net benefit for prognosis of RFS, CSS, or OS

Table 3 – Cox regression analyses for postoperative parameters

Parameter	Recurrence-free survival		Cancer-specific survival		Overall survival	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Log VEGF	1.53 (1.29–1.82)	<0.001	1.44 (1.20–1.73)	<0.001	1.21 (1.04–1.39)	0.01
Age	1.01 (1.00–1.02)	0.13	1.02 (1.00–1.03)	0.02	1.04 (1.03–1.05)	<0.001
Gender						
Male	Reference		Reference		Reference	
Female	1.60 (1.24–2.04)	<0.001	1.66 (1.28–2.14)	<0.001	1.39 (1.14–1.69)	<0.001
Pathological tumor stage						
pT0/pTa/pTis/pT1	Reference		Reference		Reference	
pT2	1.52 (1.05–2.21)	0.03	1.51 (1.02–2.25)	0.04	1.44 (1.12–1.83)	0.004
pT3/pT4	3.27 (2.32–4.64)	<0.001	3.18 (2.21–4.62)	<0.001	2.55 (2.00–3.25)	<0.001
Positive surgical margins	1.40 (1.02–1.90)	0.03	1.46 (1.05–2.00)	0.02	1.10 (0.82–1.45)	0.50
Lymphovascular invasion	1.46 (1.14–1.87)	0.003	1.62 (1.25–2.10)	<0.001	1.24 (1.02–1.51)	0.03
Concomitant CIS	1.11 (0.88–1.39)	0.38	1.00 (0.79–1.26)	0.98	1.03 (0.87–1.24)	0.71
Lymph node involvement	2.53 (1.96–3.26)	<0.001	2.56 (1.97–3.34)	<0.001	2.09 (1.69–2.57)	<0.001
Adjuvant chemotherapy	0.90 (0.68–1.17)	0.43	0.97 (0.72–1.28)	0.81	0.85 (0.67–1.07)	0.18
C index with VEGF	0.758		0.781		0.736	
C index without VEGF	0.753		0.776		0.735	

HR = hazard ratio; CI = confidence interval; CIS = carcinoma in situ.

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(Supplementary Fig. 2) across any threshold probabilities after addition of plasma VEGF levels in comparison to a reference model consisting of established clinicopathological characteristics.

4. Discussion

In this study, we confirmed the independent association of elevated preoperative plasma VEGF levels with worse survival outcomes in multivariable Cox regression models adjusted for the effects of established preoperative and postoperative variables. These findings suggest that blood levels of VEGF, as a part of a panel, could help in clinical decision-making regarding perioperative systemic therapy and aid in patient counseling.

The mechanisms underlying the association of VEGF with survival may be explained by its relationship with tumor angiogenesis [12]. Angiogenesis—the formation of new capillaries from pre-existing blood vessels—is vital for tumor growth and metastasis, and is a multifactorial, complex process that draws on signaling pathways involved in regulating several aspects of cell biology. VEGF, which is overexpressed in many human cancers, is a predominant regulator of angiogenesis processes [14,25,26]. The VEGF family comprises several members (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PIGF) [15]. These VEGF members bind to three receptor types (VEGFR-1, VEGFR-2, and VEGFR-3) [13]. VEGF-A interacts mainly with VEGFR-2 expressed on endothelial cells (ECs) and bone marrow–derived endothelial progenitor cells (EPCs) [13]. Receptor binding activates cellular signaling pathways, resulting in increased permeability of blood vessels, EC proliferation and migration, recruitment of EPCs, and maintenance of newly formed vasculature [13]. Of note, VEGF-B interacts with VEGFR-3 primarily to maintain the newly formed blood vessels, and VEGF-C and VEGF-D bind to VEGFR-3 and are primarily expressed in lymphatic vessels [15]. Thus, VEGFR-3 and its ligands play a central role in promoting lymph angiogenesis and cancer cell spread to lymph nodes. Finally, PIGF is a cytokine that plays vital roles in angiogenesis, such as promoting tumor growth via activation of stromal cells, myeloid cells, and bone marrow–derived endothelial progenitors [15]. Thus, overall it is assumed that elevated VEGF levels are associated with greater tumor angiogenesis and poor oncological outcomes.

However, to fully establish VEGF as a biomarker requires more than simply demonstrating that elevated VEGF is an independent predictor of UCB survival outcomes in conventional multivariate models [27,28]. To comprehensively evaluate the clinical benefit of VEGF measurement, we assessed its discriminatory ability given that any potential biomarker is expected to meaningfully improve the performance of a predictive/prognostic reference model [27,28]. Therefore, we investigated whether preoperative plasma VEGF levels improved the accuracy of separate predictive and prognostic models and whether these models had a relevant net benefit in preoperative or postoperative settings on DCA. However, despite the large number of patients included, we found that VEGF addition yielded only a marginal improvement in the preoperative setting for RFS and CSS and no relevant improvement in the C index in the postoperative setting. On DCA, our data showed that preoperative plasma VEGF does not offer a clinically meaningful net benefit in addition to established clinical and histopathological factors.

Despite these rather negative findings, owing to ease of procurement, low cost, high sample homogeneity, and considerable potential to improve prognosis, VEGF warrants further evaluation in future studies. Of note, a combination of preoperative plasma VEGF levels and other blood-based biomarkers is more likely to have a higher predictive value than any single biomarker. In addition, when combined with tissue levels of VEGF expression in transurethral resection (TUR) specimens, it might help in preoperative patient counseling. While next-generation sequencing and immunohistochemical staining have suggested several other candidate tissue biomarkers, such results are often limited in reproducibility because of to their cost, intratumoral heterogeneity, the absence of a standardized approach for their handling, and the overall complexity of the evaluation process [29,30]; thus, their implementation in clinical practice remains hampered. Unlike classical clinicopathological parameters that can be assessed only postoperatively, VEGF could be used for preoperative risk stratification and could result in more efficient delivery of neoadjuvant systemic therapy. Future studies of VEGF could contribute to better patient selection for bladder-sparing strategies and/or new systemic treatments such as immunotherapy.

While the strengths of this cohort lie in the purity of the treatment allocation and the international, multicenter nature, the study is not without limitations. First, its retrospective and multicenter design may have resulted in variations in laboratory, pathological, and surgical workup, thus confounding the results. Second, unknown pretreatment factors (undetected inflammation or immune diseases) may have affected VEGF levels and led to systematic bias. Furthermore, VEGF levels were assessed at a single time point preoperatively; VEGF was not evaluated in terms of its variability over time, response to therapy, or relationship with UCB oncological prognosis. Third, owing to the recruitment timeframe for the study, no information was available on the predictive value of VEGF with respect to immunotherapies or NAC. While the present analysis led to some negative results, VEGF needs to be re-evaluated in patients receiving the current standard of care. Fourth, the study did not involve a normal control population in evaluating VEGF. Therefore, no data were available regarding normal values, false-positive rates, or stability after years of storage of serum. Fifth, we did not categorize RFS by local (probably affected by staging and surgical quality) and systemic recurrences (more likely to be discriminated by the marker). Interestingly, VEGF is an independent predictor of RFS and CSS and at the same time is not correlated with pathological features, while it is known that advanced pathological features considerably affect RFS and CSS. While the reason for the discrepancy between survival outcomes and pathological features remain unclear, the findings may imply that local tumor growth requires more vasculature. Indeed, our study results show that VEGF is more strongly

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linked to systemic disease progression than to local growth of tumor tissue, suggesting that it might have been useful to assess recurrences categorized as local or systemic. Sixth, the only preoperative variables we included were age, gender, and cT stage. We did not include tumor size, TUR data (such as CIS, LVI, variants), or hydronephrosis, which are all predictors of NOC disease at RC. Finally, VEGF was the only focus of this study, despite growing evidence that a combination of preoperative markers may help in predicting oncological outcomes for UCB patients. Therefore, well-designed prospective studies with long-term follow-up are warranted to validate whether VEGF could be used as part of a panel of biomarkers to enhance current tools for risk stratification in UCB, including patients treated with current standard treatments such as NAC and immunotherapy.

5. Conclusions

This is the first study to investigate the predictive and prognostic value of plasma VEGF levels in patients with UCB treated with RC. We confirmed that elevated preoperative plasma VEGF levels are independently associated with worse survival outcomes for patients with UCB using multivariable Cox regression models adjusted for the effects of established preoperative and postoperative variables. However, VEGF showed little value in improving the discriminatory ability of models relying on either preoperative or postoperative clinicopathological variables. Future studies should include a combination of VEGF and other biomarkers, especially in the era of new systemic therapies.

Author contributions: Keiichiro Mori had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Mori, Schuettfort, Shariat.
Acquisition of data: Mori, Schuettfort, Lotan, Scherr, Shariat.
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Appendix A. Supplementary data

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