Original Study

Prognostic Role of Serum IL-6 Levels in Bladder Cancer Patients and Hints of its Origin

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Abstract

Interleukin-6 (IL-6) is associated with poor outcomes in bladder cancer (BC) patients, including worse overall survival (OS) and cancer-specific survival (CSS). This study analyzed serum IL-6 levels in 179 BC patients undergoing radical cystectomy and IL-6 concentrations in tumor tissue from 20 additional patients. High serum IL-6 was an independent predictor of OS and CSS, and higher levels were linked to advanced tumor stage, lymph node metastasis, and larger tumor size. Tumor tissue IL-6 was significantly elevated in muscle-invasive BC compared to early-stage tumors. These findings underscore the prognostic potential of IL-6 and its relevance in BC theranostics.

Background: Interleukin-6 (IL-6) is associated with adverse clinical outcome in cancer patients. In bladder cancer (BC) patients, higher IL-6 serum levels have been linked with adverse pathologic features, worse overall survival (OS) and cancer-specific survival (CSS). IL-6 is being investigated as a therapeutic target. However, concentrations in tumor-tissue are not investigated in detail. Objective of this study is to analyze the prognostic value of IL-6 in BC patients and to investigate its concentration in tumor tissue. **Methods:** In this single center prospective observational study, preoperative serum samples of 179 BC patients undergoing radical cystectomy were collected between September 2019 and September 2022. Tumor-tissue of additional 20 patients was collected during transurethral resection or radical cystectomy for investigation of IL-6 in tumor tissue supernatant. IL-6 concentration was measured by ELISA. **Results:** Median serum IL-6 concentration was 5.4 pg/mL. High serum IL-6 was an independent predictor of OS (HR 1.95; 95% CI, 1.07-3.55; $P = .03$) and CSS (HR 2.31; 95% CI, 1.14-4.68; $P = .02$) in multivariate Cox regression analyses. Patients with advanced tumor stage, lymph node metastasis, and larger tumor size had significantly higher preoperative serum IL-6 concentration (all $P < .01$). In tumor tissue supernatant, IL-6 concentration was higher in muscle-invasive BC, with a median of 715.4 pg/mL, as opposed to 20.7 pg/mL in pTa tumor stage ($P < .01$). **Conclusions:** Serum IL-6 is a strong predictor of poor survival rates and adverse pathologic features in BC patients. IL-6 concentrations in tumor tissue supernatant correlate with tumor stage. The role of IL-6 in theranostics of bladder cancer deserves more attention.

Clinical Genitourinary Cancer, Vol. 000, No.xxx, 102378
Keywords: Biomarker, Tumor microenvironment, Urothelial cancer, Immunology, Interleukin

Introduction

Bladder cancer represents a significant global health burden, being the nineth most frequently diagnosed cancer worldwide.[1] Despite advancements in treatment modalities, the prognosis for advanced stage bladder cancer (BC) remains poor, necessitating a deeper understanding of its underlying mechanisms and potential therapeutic targets.[2] The exploration of biomarkers has become a critical area of research to enhance detection, predict outcomes and tailor therapies for bladder cancer patients.

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Submitted: Dec 2, 2024; Revised: May 12, 2025; Accepted: May 17, 2025; Epub: xxx

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https://doi.org/10.1016/j.clgc.2025.102378

Please cite this article as: Simon U. Engelmann et al, Prognostic Role of Serum IL-6 Levels in Bladder Cancer Patients and Hints of its Origin, Clinical Genitourinary Cancer, https://doi.org/10.1016/j.clgc.2025.102378

Prognostic Role of Serum IL-6 Levels in Bladder Cancer Patients

Interleukin-6 (IL-6), a multifunctional cytokine involved in inflammation and immune responses, has emerged as a potential biomarker due to its elevated levels in various cancers, including BC. High serum levels of IL-6 in BC patients have been linked to poor prognosis and adverse pathological features in several retrospective studies.[3-6] These existing studies analyzed patient serum IL-6 levels in correlation with patient data in cohorts ranging from 30 patients to over one thousand patients.[3-6] Other existing studies are solely of experimental nature, and have shown that IL-6 may induce tumorigenesis of BC in cell line experiments, and thus correlates with progression and prognosis of disease.[7,8] Due to these facts, IL-6 and the associated IL-6/STAT3/JAK2 signaling pathway have become a target of interest in general experimental cancer research and, specifically, in bladder cancer research.[9-11]

Studies on the prognostic value of serum IL-6 in BC patients are still limited. There are limited existing retrospective studies including clinical patient data. The largest patient cohort on this topic is a multicenter retrospective study with over one thousand patients.[3] Apart from this, single center studies on serum IL-6 in bladder cancer patients are limited to small patient cohorts with limited clinical information, investigating very specific patient groups.[4-6] Our study aimed to elucidate the role of serum IL-6 as a prognostic biomarker in BC patients by analyzing serum IL-6 in a large single center patient cohort with detailed clinical data. Additionally, we aimed to add new information to the existing knowledge base by exploring IL-6 levels in tumor tissue supernatant, thus investigating its possible origin in tumor tissue.

Patients and Methods

Study Design and Patient Selection

After obtaining informed consent, preoperative serum samples were prospectively collected from patients undergoing radical cystectomy (RC) for BC between September 2019 and September 2022 in this single center study. Among 213 patients who underwent RC for BC in this time period, 34 patients were not included in the study cohort due to missing preoperative serum sample or missing follow-up information, resulting in a final cohort of 179 patients (84%). Serum samples were collected preoperative on the day of surgery. After centrifuging at room temperature for 10 min at 1500 x g, the top layer corresponding to patient serum was decanted using sterile pipettes and frozen and stored in aliquots at −80°C.[12] For investigation of IL-6 levels in the tumor tissue supernatant, additional 20 patients with BC were included in the study. Between May 2023 and April 2024 tumor samples of these patients were collected during transurethral resection of bladder tumor (TURBT) or RC and immediately transported (10-15 mins) to the laboratory facility to be processed for a 24-hour culture. Weighed fragments of each sample of tumor tissue were each placed in 1 ml of culture medium (RPMI 1640 Thermo Fischer, 10% Fetal Calf Serum, 2% Glutamine, 1% Penicillin/Streptomycin) in an incubator for 24 hours (21% Oxygen, 37° C). Tumor supernatant was then collected and centrifuged (1500 rpm, 5 min), and immediately stored as aliquots at −80°C for optimal stability.[12] The study adheres to the STROBE checklist for observational cohort studies (Supplemental Information).

Clinical Data Acquisition

Clinicopathologic characteristics were retrospectively collected. Tumor classification and macroscopic tumor size were obtained from pathology reports. Follow-up data were collected from hospital records, patient interviews, local urologists and general practitioners. Overall survival (OS) was defined as the time from radical cystectomy to death, regardless of cause, and cancer-specific survival (CSS) was defined as the time from radical cystectomy to death due to bladder cancer progression or metastasis.

Measurement of IL-6 in Serum and Tumor Tissue Supernatant

Serum IL-6 was measured using an IL-6 ELISA assay (Human IL-6 Quantikine HS ELISA, R&D systems). Every sample was run in duplicate, and the mean was used. In samples where values were out of range ELISA was repeated with a 1:10 dilution and in cases still out of range a further dilution with 1:100 was repeated subsequently. Sample accuracy and reproducibility were ensured by running standards and controls in parallel and using consistent handling protocols. As commonly used in clinical laboratories and research studies, a cut-off of 7 pg/mL was used to distinguish between low and high IL-6 concentrations in serum.[13,14] Measurement of cytokines using ELISA in tumor tissue supernatant is well-established and used by various research groups.[15-18] For measurement of IL-6 in tumor tissue supernatant, 3 small fragments of each sample of tumor tissue were each weighed, and each placed in 1 ml of culture medium and processed as described above. IL-6 levels in supernatant were determined using an ELISA assay (Human IL-6 ELISA, BD OptEIA) run in duplicate, and the mean was used. Concentrations were normalized to 1 mg of tumor tissue, as previously done by other research groups.[19] For each tumor tissue sample, the mean of all 3 fragment measurements was used. The ELISA kits used were validated by the manufacturer for sensitivity and specificity in natural human IL-6. Manufacturer instructions were followed thoroughly when performing ELISA to ensure accuracy and reproducibility of results. Both ELISA kits used are recommended and validated by the manufacturer for the use in human serum and human tumor culture supernatant, respectively.

Statistical Analysis

Frequencies are presented as absolute numbers and percentages. Continuous data are presented as median with interquartile range (IQR). After testing for normal distribution, differences between groups were analyzed using the Mann-Whitney U test for dichotomous parameters and the Kruskal-Wallis test for categorical data. Survival data were analyzed using univariate and multivariate Cox regression. Kaplan-Meier curves were used to illustrate OS and CSS. Statistical analysis was performed using SPSS software (version 29.0; SPSS Inc., Chicago, IL, USA). Graphs were created using Graph Pad Prism software (Prism 10 for macOS, Version 10.2.1).

Results

Descriptive Data

Figure 1A shows the distribution of IL-6 serum levels throughout the entire cohort. Table 1 shows the distribution of clinicopatho-

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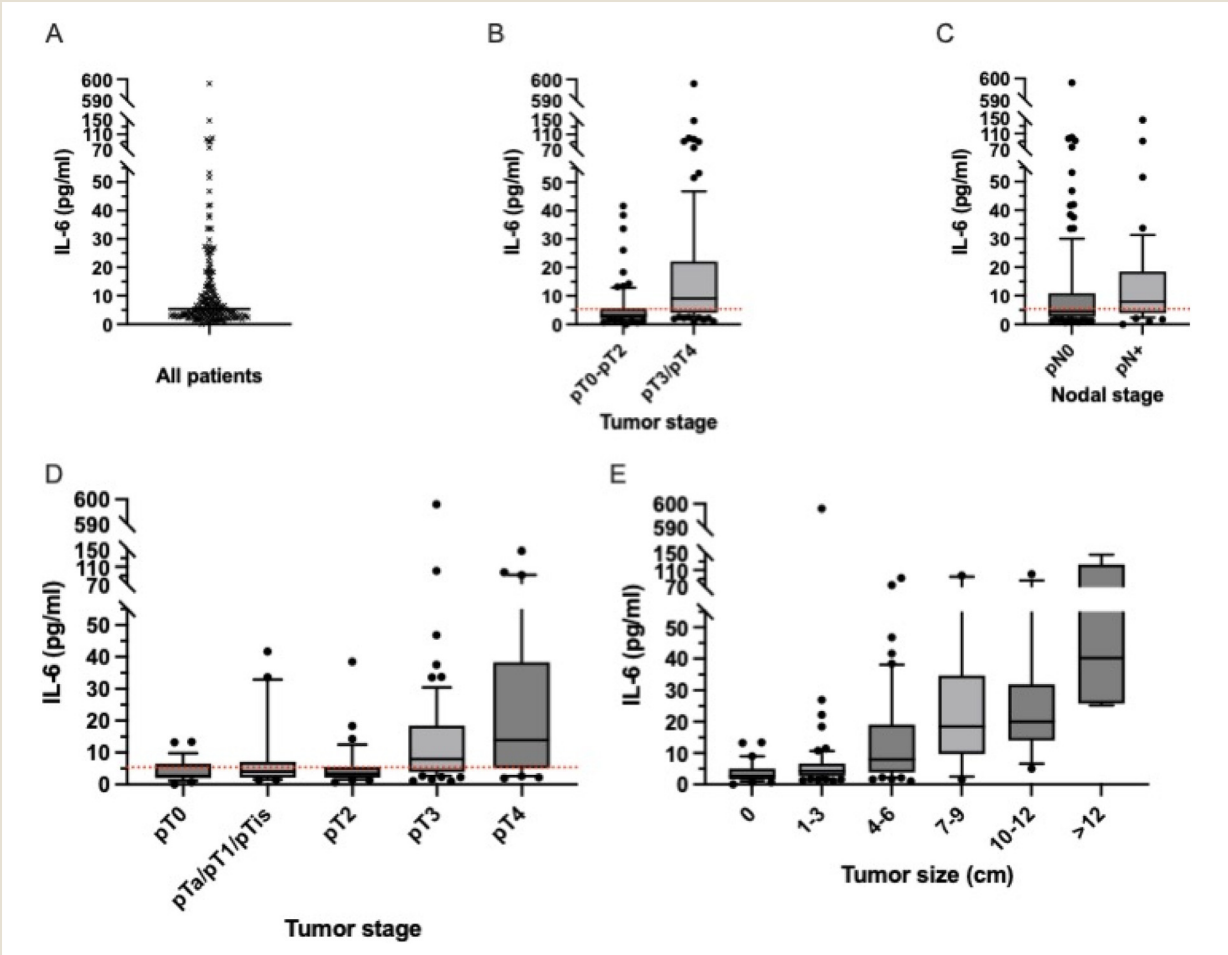
Figure 1 Box plots showing preoperative IL-6 serum levels. A: distribution of IL-6 serum concentration in the entire cohort. B: IL-6 serum levels in organ-confined and nonorgan-confined tumor stages. C: comparison of IL-6 serum levels in patients with pN0 and pN+ nodal stages. D: IL-6 serum levels in patients grouped by tumor stage. E: IL-6 serum levels grouped by tumor size in pathology. Red dotted line indicates median IL-6 concentration.



logic characteristics and comparison of median IL-6 serum levels among groups. Median IL-6 serum level for the study cohort was 5.4 pg/mL (IQR 2.9-13.4). Higher tumor stage, larger tumor size, positive lymph nodes and positive surgical margins were associated with higher IL-6 serum levels (all $P < .05$). In total, 114 (63.7%) patients were alive at the censoring date with a median survival of 21 months (IQR 12-31 months). Death by any cause occurred in 65 (36.3%) patients, of whom 46 (25.7%) died of BC. Posthoc power analysis showed adequate power of our study cohort size for the comparison of serum IL-6 levels in patients with adverse pathologies and overall and cancer specific survival (posthoc power 100%). Same is true for the analysis of IL-6 in tumor tissue supernatant of different tumor stages.[20]

Serum IL-6 and Pathological Parameters

Patients with unfavorable histopathologic features had higher IL-6 serum levels. As shown in Figure 1B, patients with tumor stages pT0-pT2 had a median IL-6 level of 3.19 pg/mL (IQR 2.07-5.65) compared to 9.13 pg/mL (IQR 4.06-22.19) in patients with pT3/pT4 tumor stages, respectively. The difference between these groups was statistically significant ($P < .01$). Figure 1D and Table 1 show IL-6 levels among individual tumor stages. Notably, patients with pT2-stage BC had similarly low IL-6 levels compared to stages pT0, and pTa/pTis/pT1, as opposed to stages pT3/pT4. Lymph node metastasis was associated with higher IL-6 levels ($P = .02$, Table 1 and Figure 1C). Larger tumor sizes in pathology were in line with higher serum IL-6 levels ($P < .01$), as shown in Figure 1E.

Serum IL-6 and Survival Prediction

In univariate Cox regression analysis tumor size, high IL-6, pT3/pT4 tumor stage, positive nodal stage and positive surgical margin(s) were risk factors for OS and CSS (all $P < .01$, Supplemental Table 1). Additionally, age and ASA-score 4 were risk factors for OS only ($P < .05$). In multivariate Cox regression, high serum IL-6 (OS: HR 1.95; 95% CI, 1.07-3.55; $P = .03$, CSS: HR

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Table 1 Patient Characteristics and Group Comparison of Median Serum IL-6 Levels in the Entire Cohort of 179 Patients

Characteristic	n (%)	Median IL-6 pg/mL (IQR)	*P*-Value[a]
Age (Median, IQR)	70 (62-78)		
Gender			.54
Male	131 (73.2)	5.38 (2.92-11.22)	
Female	48 (26.8)	5.89 (2.66-22.96)	
ASA-score			**.04**
1	3 (1.7)	1.59 (1.45-n/a)	
2	70 (39.1)	3.87 (2.15-8.24)	
3	97 (54.2)	5.52 (3.33-17.59)	
4	9 (5.0)	23.48 (10.02-72.4)	
Smoking			.33
Yes	97 (54.2)	4.45 (2.72-13.27)	
no	82 (45.8)	6.04 (3.01-14.44)	
Neoadjuvant chemotherapy			.59
Yes	52 (29.1)	6.23 (2.77-9.85)	
No	127 (70.9)	4.74 (2.89-15.14)	
Preoperative cM-Stage			.08
cM0	176 (98.3)	5.15 (2.79-13.36)	
cM+	3 (1.7)	9.72 (9.01-n/a)	
Tumor stage at cystectomy			**< .01**
pT0	27 (15.1)	2.71 (1.95-6.53)	
pTa, pT1, pTis	20 (11.2)	4.06 (2.15-7.14)	
pT2	33 (18.4)	3.40 (2.11-5.60)	
pT3	67 (37.4)	7.93 (3.89-18.46)	
pT4	32 (17.9)	13.94 (5.06-38.29)	
Macroscopic tumor size (cm)			**< .01**
0[b]	36 (20.1)	2.74 (1.64-5.03)	
1-3	61 (34.1)	4.27 (2.71-6.68)	
4-6	53 (29.6)	7.93 (3.86-19.08)	
7-9	13 (7.3)	18.46 (9.65-34.70)	
10-12	12 (6.7)	19.98 (14.00-31.90)	
> 12	4 (2.2)	40.27 (25.75-122.80)	
Nodal stage			**.02**
pN0	134 (74.9)	4.50 (2.64-10.90)	
pN+	45 (25.1)	7.93 (3.94-18.51)	
Surgical margins			**< .01**
R0	133 (74.3)	4.42 (2.67-9.79)	
R+	43 (24.0)	13.14 (4.69-26.94)	
Rx	3 (1.7)	1.35 (n/a)	
Serum IL-6 pg/mL (Median, IQR)	5.4 (2.9-13.4)		
High IL-6 (Cut-off: 7pg/mL)			
Yes	74 (41.3)		
No	105 (58.7)		

Abbreviations: ASA-score = American society of anesthesiologists—score; n/a = not available.
[a] Differences in groups were determined by Mann-Whitney U or Kruskal-Wallis test. Bold Values represent statistically significant values with p<0.05.
[b] Tumor size 0 cm means no macroscopic tumor in pathology report of cystectomy. This was either due to pT0 (27 patients) tumor stage or no macroscopic tumor detectable (9 patients).

2.31; 95% CI, 1.14-4.68; $P = .02$), nonorgan confined disease (OS: HR 3.35; 95% CI, 1.44-7.77; $P < .01$, CSS: HR 6.82; 95% CI, 1.92-24.18; $P < .01$) and positive nodal status (OS: HR 2.24; 95% CI, 1.28-3.90; $P < .01$, CSS: HR 2.98; 95% CI, 1.56-5.71; $P < .01$) were all independent predictors of OS and CSS, respectively (Table 2).

Patients with high preoperative serum IL-6 had significantly worse OS and CSS (log-rank ≤ 0.01, Figure 2). As stated above, positive nodal stage and nonorgan confined disease are also risk factors for poor OS and CSS. In subgroup analysis within these groups, patients with high IL-6 had significantly worse OS and CSS (log-rank ≤ 0.01, Figure 2)

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Figure 2 Kaplan-Meier curves showing overall and cancer specific survival comparing low- and high serum IL-6 levels in (A) the entire cohort and subgroups (B: only patients with pT3 or pT4 tumor stages; C: only patients.

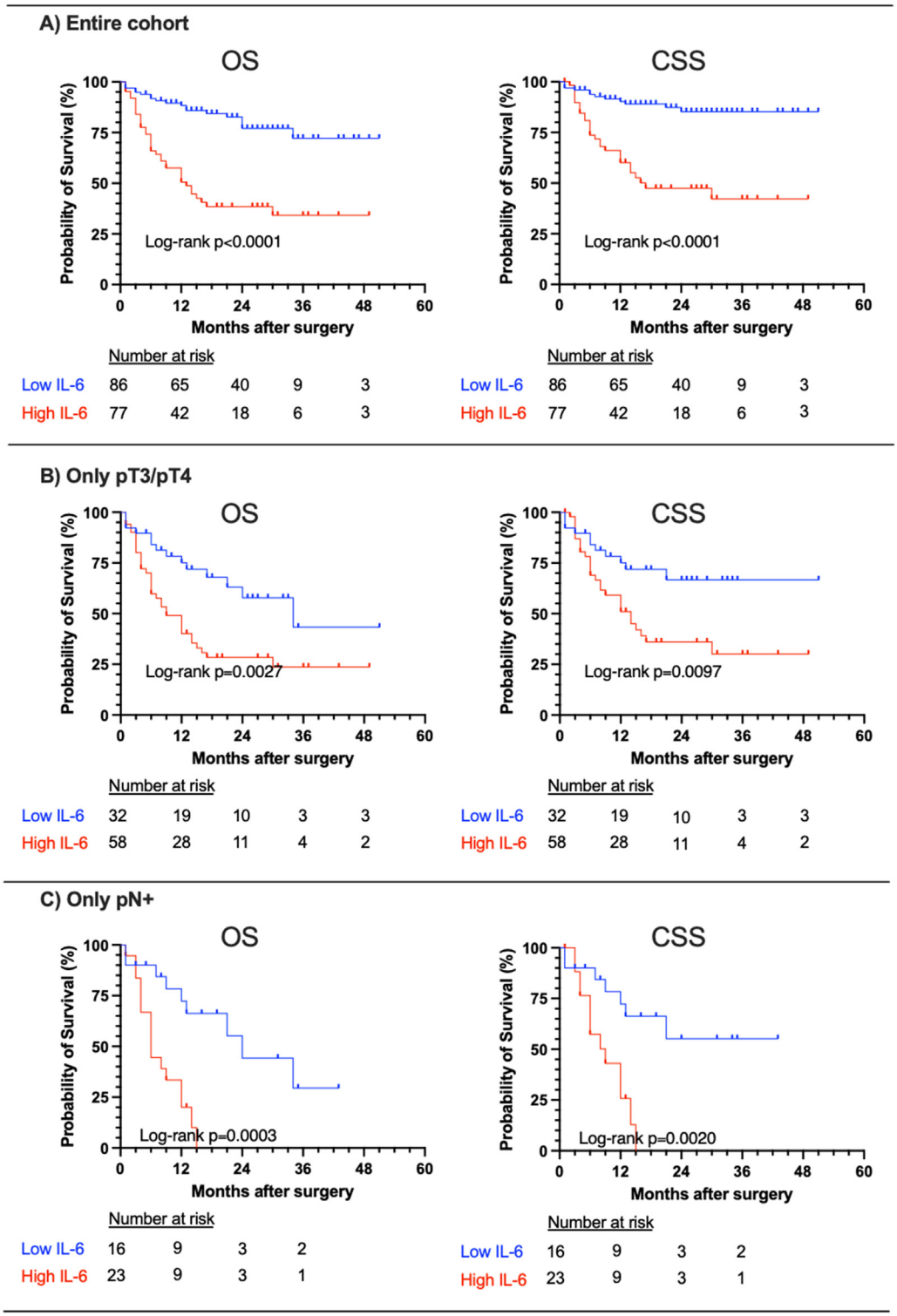

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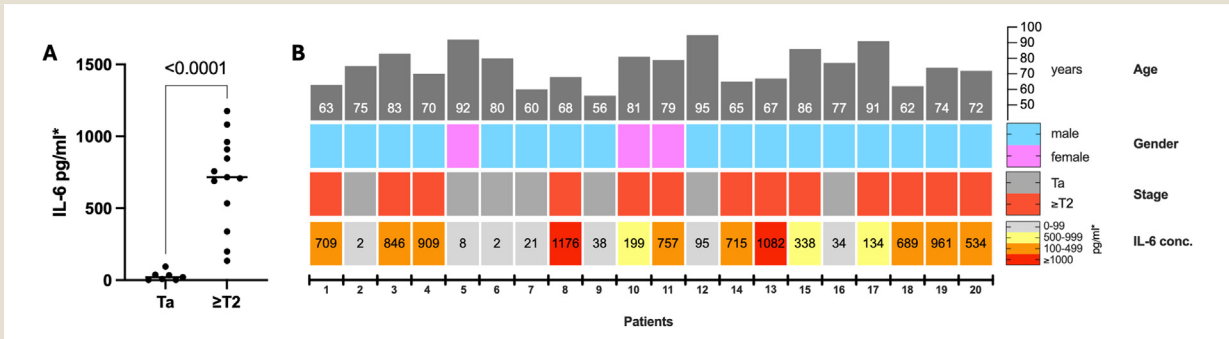
Table 2 Multivariable Cox Regression Models for the Prediction of Overall Survival and Cancer Specific Survival (Age, Tumor Stage, Nodal Stage, Tumor Size and Serum IL-6)

Variable	OS			CSS		
	HR	(95%CI)	*P*-Value	HR	(95%CI)	*P*-Value
Age (years, continuous)	1.01	0.99-1.04	.40	1.00	0.97-1.03	.79
pT3/T4 (Ref. pT0-T2)	3.35	1.44-7.77	**< .01**	6.82	1.92-24.18	**< .01**
pN+ (Ref. pN0)	2.24	1.28-3.90	**< .01**	2.98	1.56-5.71	**< .01**
Tumor size (cm, continuous)	1.08	1.00-1.16	.05	1.05	0.96-1.14	.29
High IL-6 (Ref. low, <7pg/mL)	1.95	1.07-3.55	**.03**	2.31	1.14-4.68	**.02**

Abbreviations: Ref = reference; OS = overall survival; CSS = cancer specific survival. Bold values represent statistically significant values with p<0.05.

Figure 3 A: IL-6 levels in tumor tissue supernatant of 20 patients according to tumor stage. B: Visualization of patient characteristics, tumor stage and IL-6 concentration in tumor tissue supernatant.*Concentration normalized to 1 mg of tumor tissue.



IL-6 in Tumor Tissue Samples

In the data presented above, it is eminent that patients with unfavorable histopathologic features (advanced tumor stages, positive lymph nodes, positive surgical margins) and greater tumor sizes have higher preoperative serum IL-6 levels (Figure 1). To further investigate the possible origin of IL-6 in tumor tissue, IL-6 was measured in culture supernatant after 24 hours of incubation in 20 patients. Four (20%) were samples of BC at RC and 16 (80%) were BC at TURBT. Seven patients had stage pTa urothelial cancer in TURBT, the remaining 13 patients had stage pT2 or higher. Median IL-6 in tumor tissue supernatant was 436 pg/mL (IQR 37-779). Median IL-6 levels were significantly lower in pTa tumors (20.7 pg/mL, IQR 2.2-37.5) when compared with ≥ pT2 (715.4 pg/mL IQR 436.1-935.2), $P < .01$ (Figure 3).

Discussion

The predictive role of serum IL-6 and its role as a therapeutic target are being assessed in several cancer entities. Ubiquitously high IL-6 concentration seems to be a prognostic factor in cancer.[9,21]

Although the predictive role of serum IL-6 in BC patients was previously described in literature, published data are limited. Existing studies claim IL-6 as a predictive biomarker for survival, progression, and recurrence. In a study assessing the role of serum cytokines in bladder cancer, Kumari et al. found significantly higher IL-6 concentrations in patients who had recurrence during follow up ($P < .01$).[22] In line with these findings, we found high IL-6 levels to be a strong independent predictor for poor OS and CSS (OS: HR 1.95, 95% CI, 1.07-3.55, $P = .03$; CSS: HR 2.31, 95% CI, 1.14-4.68, $P = .02$). Our data (see Figure 2) are comparable with studies conducted by Andrews et al. and Schuettfort et al.[3,4] Andrews found significantly shorter CSS in patients with high serum IL-6 (log-rank $P = .015$) and high IL-6 was an independent risk factor for shorter CSS (HR 2.17, 95% CI, 1.29-3.66, $P = .05$).[4] Similar to our study, Schuettfort et al. found high IL-6 associated with both shorter OS (log-rank $P < .01$) and shorter CSS (log-rank $P < .01$). They also found serum IL-6 levels to be an independent predictive marker for OS and CSS (OS: HR 1.2, 95% CI, 1.13-1.27, $P < .01$; CSS: HR 1.33, 95% CI, 1.24-1.42, $P < .01$).[3] Comparing our study to those of Andrews and Schuettfort, there are differences in median serum IL-6 concentration. In our study cohort we found a median of 5.4 pg/mL compared to 4.8 pg/mL and 2.8 pg/mL in Andrews and Schuettforts cohort, respectively. However, the study cohorts also differ by tumor stage, with 55% of our patients having ≥ T3 and only 40% in the multicenter study conducted by Schuettfort. Additionally, published studies use different cut-off values to differ between low and high IL-6 values. The studies by Andrews and Schuettfort used the median of their study cohort as a cut-off value, whereas we used the cut-off of 7 pg/mL, which is well established in prior research and in laboratory diagnostics.[13,14] Despite these differences, it is eminent that serum IL-6 remains a strong predictor of survival outcome in patients with bladder cancer.

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Our study also elucidates the predictive value of serum IL-6 on adverse pathologic features and confirms the findings of previous studies. Warli et al.[5] found significantly higher serum IL-6 levels in 15 patients with lymph node metastases opposed to 17 patients without lymph node metastasis ($P = .003$). Accordingly, in the current study patients with lymph node metastases showed a median serum IL-6 of 7.93 pg/mL, which was significantly higher than those without lymph node metastasis (4.50 pg/mL, $P = .02$). A study conducted by Andrews et al. showed high serum IL-6 being predictive for invasive tumor stages (pT2-T4) and lymph node metastases in 51 patients with BC. In these patients with adverse pathologic features, preoperative serum IL-6 was significantly higher ($P = .04$ and $P = .03$, respectively).[4] In our study, patients with extravesical tumor disease (pT3/ pT4) had significantly higher preoperative serum IL-6 levels ($P < .01$, Table 1, Figure 1). Patients with stage pT2 BC had relatively low IL-6 serum levels in our study cohort (median 3.4 pg/mL, Table 1). Andrews et al. do not present IL-6 values per individual tumor stage group; results were grouped by NMIBC and MIBC. Schuettfort et al. also confirmed the predictive role of serum IL-6 on adverse pathologic features in a large multicenter study.[3] In this study IL-6 was found to be an independent predictive factor for lymph node metastasis (OR 1.3, 95% CI, 1.19-1.43, $P < .01$) and ≥ pT3 (OR 1.25, 95% CI, 1.15-1.37, $P < .01$). In addition to the findings of the studies mentioned above, we found a positive correlation between IL-6 serum levels and tumor size (Figure 1E).

IL-6 is an interesting interleukin not only with prognostic value, but also as a therapeutic target. In a phase Ib/II study, among others, tocilizumab, an IL-6 receptor antibody, is being tested in metastatic urothelial carcinoma.[23] This knowledge and our findings above led us to further investigate IL-6 concentrations in tumor tissue. Although plenty of research has been conducted on IL-6 and the linked pathways of STAT3/JAK2 and possible therapeutic targets, the literature is limited regarding data describing IL-6 concentrations in human bladder cancer tissue.[10,24,25] To highlight the efforts of other research, investigating IL-6 and the linked pathways as a therapeutic target in bladder cancer, it is important to understand and evaluate IL-6 concentrations in serum and tumor tissue of bladder cancer patients. Our study analyzes and describes these findings. In the 20 patients included in the analysis of tumor supernatant in our study, we found a median IL-6 concentration of 436 pg/mL (IQR 37-779) per mg of tumor tissue. To our best knowledge, no similar data has been published describing IL-6 concentrations in tumor tissue of bladder cancer patients. Previous studies investigating IL-6 in bladder cancer patients have solely concentrated on serum IL-6 levels, indicating its systemic significance. Our novel approach to investigate IL-6 in tumor tissue supernatant gives new insights of localized IL-6 concentrations in tumor tissue. As most of the patients included in this analysis underwent TURBT, we could not differentiate between tumor stages ≥ pT2. Comparing the groups, median IL-6 levels differ significantly between pTa and ≥ pT2 tumors (20.7 pg/mL, IQR 2.2-37.5 vs. 715.4 pg/mL, IQR 436.1-935.2, $P < .01$), (Figure 3). These findings support further research conducted on IL-6 as a therapeutic target in BC patients.[10,11,25,26] In summary, this study uniquely contributes to the existing research by providing detailed comparative analysis of IL-6 levels in both serum and tumor tissues, offering new insights into its prognostic significance across different stages of bladder cancer.

There are several limitations to our study. First our study is limited by cohort size, with 179 patients included in serum IL-6 analysis in our single center study. Therefore, we were also constrained in multivariate statistical analyses. A further limitation is the retrospective analysis with limited information on confounding factors possibly altering IL-6 levels, such as drug interactions or inflammatory diseases. Regarding the subgroup of 20 patients for analysis of IL-6 in tumor tissue, our data is limited by samples originating from TURBT, which makes it impossible to differentiate between ≥ pT2 stage tumors. In this subgroup, we did not collect preoperative serum, restricting the conclusions that can be made linking serum and tumor tissue IL-6 concentrations.

Conclusion

In our study, we confirmed that IL-6 is a strong independent predictive biomarker for OS and CSS after RC in bladder cancer. High IL-6 concentrations in serum correlate with adverse pathologic features. In addition, bladder cancer tumor tissue contains high concentrations of IL-6, especially aggressive and invasive tumor stages. Further investigations of IL-6 in tumor tissue are being continued by our research group to attain a more in-depth understanding of its importance.

Ethics Approval

The study complies with the ethical standards described in the latest declaration of Helsinki; it was approved by the institutional ethics review board of the University of Regensburg (approval number: 08/108 and 24-3636-101).

Disclosure

The authors have no relevant financial or nonfinancial interests to disclose.

CRediT authorship contribution statement

Simon U. Engelmann: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Christoph Pickl:** Writing – review & editing, Data curation. **Maximilian Haas:** Writing – review & editing, Data curation. **Felix Kasparbauer:** Writing – original draft, Data curation. **Emily Rinderknecht:** Writing – review & editing. **Sebastian Kälble:** Writing – review & editing, Data curation. **Bas W.G. van Rhijn:** Writing – review & editing, Formal analysis. **Peter J. Siska:** Writing – review & editing, Conceptualization. **Sonja-Maria Decking:** Writing – review & editing, Formal analysis. **Kathrin Renner:** Writing – review & editing, Formal analysis, Conceptualization. **Renate Pichler:** Writing – review & editing. **Maximilian Burger:** Writing – review & editing, Supervision. **Miodrag Gužvić:** Formal analysis. **Roman Mayr:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Conceptualization.

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Please cite this article as: Simon U. Engelmann et al, Prognostic Role of Serum IL-6 Levels in Bladder Cancer Patients and Hints of its Origin, Clinical Genitourinary Cancer, https://doi.org/10.1016/j.clgc.2025.102378

Supplemental Information—methodology

Serum sampling

1. Draw blood from patient (2x Sarstedt S-Monovette Serum 9 mL)
2. Let samples stand for 20-30 mins at RT for coagulation
3. Centrifuge at 1500 x g for 10 mins
4. Take sterile pipette and pipette into aliquots of 200 µl
5. Freeze aliquots at −80°C immediately

Tumor tissue sampling

1. Collect Tumor tissue during surgery (TURBT or RC)
2. Immediately place tumor tissue in transport Medium (RPMI 1640, 1% Penicillin/Streptomycin) at RT
3. Transport tissue to laboratory facility immediately (< 15 mins)
4. Under sterile working conditions on ice, use a scalpel to sperate the tissue sample into 3 pieces of 10-20 mg each
5. Weigh each tumor tissue piece using a fine scale
6. Now place each tumor tissue piece into a 24-well plate well
7. Pipette 1 ml of culture medium (RPMI 1640 Thermo Fischer, 10% Fetal Calf Serum, 2% Glutamine, 1% Penicillin/Streptomycin) into each well
8. Additionally, to the 3 wells with tumor tissue, pipette 1 ml Medium into a spare well as control
9. Incubate for 24 hrs (21% Oxygen, 37° C)
10. After 24 hrs, collect supernatants and the control sample with a sterile pipette
11. Transfer supernatant and control sample into a microtube each
12. Centrifuge at 1500 x g for 5 mins
13. Transfer supernatants and control into aliquots of 200 µl and freeze at −80°C

IL-6 measurement in serum samples and tumor tissue supernatant using Human IL-6 Quantikine HS ELISA, R&D systems or Human IL-6 ELISA, BD OptEIA, respectively.

1. Thaw aliquot of serum sample at room temperature
2. Adhere to manufacturer guidelines for IL-6 ELISA
3. Reconstitute the Human IL-6 HS Standard according to manufacturer instructions
4. Carry out ELISA kit with undiluted samples
5. Run a duplicate with a second aliquot for each sample
6. If values are out of range, repeat the assay with a new aliquot (to avoid freeze-thaw cycles) and dilute sample accordingly

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Supplemental Table 1 Univariate Cox Regression Analysis for OS and CSS

Variable	OS			CSS		
	HR	(95%CI)	*P*-Value	HR	(95%CI)	*P*-Value
Age (years, continuous)	1.04	1.01-1.06	**.01**	1.02	0.99-1.05	.23
Gender (Ref. Male)	1.18	0.69-2.02	.54	0.96	0.47-1.96	.91
Tumor size (cm, continuous)	1.21	1.15-1.28	**< .001**	1.20	1.12-1.29	**< .001**
IL-6 (pg/mL, continuous)	1.003	1.001-1.006	**.003**	1.002	1.00-1.006	.38
High IL-6 (Ref. low IL-6, by median)	3.99	2.28-6.96	**< .001**	3.57	1.78-7.18	**< .001**
Tumor Stage at Cx (Ref. T0)						
pTa, Tis, T1	0.45	0.05-4.30	.49	-	-	-
pT2	1.30	0.31-5.46	.72	1.61	0.15-17.722	.70
pT3	6.33	1.95-20.58	**.002**	13.59	1.84-100.13	**.01**
pT4	8.26	2.44-28.01	**< .001**	12.89	1.65-100.98	**.015**
pN+ (Ref. pN0)	3.79	2.31-6.21	**< .001**	5.98	3.15-11.36	**< .001**
R+ (Ref. R0)	1.95	1.30-2.92	**.001**	2.17	1.33-3.56	**.002**
ASA-score (Ref. 1)						
2	0.74	0.10-5.57	.77	0.44	0.06-3.45	.44
3	1.52	0.21-11.04	.68	0.89	0.12-6.58	.91
4	8.96	1.12-71.9	**.04**	4.33	0.50-37.69	.18

Bold values represent statistically significant values with $p<0.05$.
Abbreviations: OS = overall survival; CSS = Cancer specific survival.

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