Prognostic Factors of Upper Tract Urothelial Carcinoma

PhD thesis

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1. Introduction

Upper tract urothelial carcinoma (UTUC) is a rare disease with a poor prognosis and challenging clinical management. The first diagnosis is difficult, and performing a ureterorenoscopy (URS) biopsy, which is a helpful diagnostic tool, can be challenging and yield uncertain results, while it may also increase intravesical recurrence (IVR). The clinical benefit of lymphadenectomy at radical nephroureterectomy (RNU) is also a matter of debate and patient selection for this procedure is uncertain. While single postoperative chemoinstillation is highly recommended in multiple guidelines, it is rarely performed in daily clinical practice. Moreover, there are no validated preoperative biomarkers for the selection of patients who would benefit from a neoadjuvant systemic chemotherapy, thus posing a risk of undertreatment. On the other hand, there is also a risk of overtreatment in patients, who could be cured with organ-sparing surgery instead of RNU. A validated, prognostic biomarker would be useful to answer most of these questions.

The members of matrix metalloproteinase (MMP) family are able to degrade the extracellular matrix besides many other functions. They are involved in both physiological and pathological processes as well, therefore, they have an important role in the development of non-malignant and malignant diseases, including urothelial bladder cancer, which has many similarities with UTUC. One of our investigated biomarkers, MMP-7, plays an important role in invasive tumor growth and metastatic spreading. The prognostic value of serum MMP-7 level in bladder cancer was confirmed in multiple independent studies, however, there is no data about the prognostic value of MMP-7 concentration in UTUC.

The other investigated biomarker, PD-L1, is an immune checkpoint protein. If it binds to PD-1 receptor, the cell with the PD-L1 on its surface will escape from T cells, thus avoiding the response of the immune system. Various types of cancers use this mechanism to prevent T cells from attacking them. Immune checkpoint inhibitors (ICI) inhibit the interaction between PD-1 and PD-L1, helping the immune system to restore their antitumoral activity. The prognostic value of tissue PD-L1 is already confirmed in UTUC patients. PD-L1 can be found in a soluble form (sPD-L1) in the circulation, however its origin is still unknown. MMP-7 was confirmed as one of the proteolytic

enzymes, which is involved in proteolytic degradation of PD-L1 resulting in the appearance of its extracellular domain in the blood. The prognostic value of sPD-L1 has not been investigated in UTUC patients yet.

2. Objectives

2.1 The aims of the present clinical study were:

1) To identify clinicopathological prognostic factors in our institutional UTUC cohort that are associated with bladder recurrence after RNU
2) To identify high-risk patients who would benefit from a stricter follow-up
3) To identify certain clinicopathological parameters as independent prognostic factors for OS after RNU

2.2 The aims of the present biomarker study were:

1) To analyze the association of pre-treatment serum MMP-7 concentrations with clinicopathological factors in UTUC
2) To identify clinicopathological parameters, which are associated with OS and PFS
3) To find preoperatively available serum biomarkers, which can improve risk-stratification and clinical decision-making in UTUC
4) To see if there is any association between the levels of MMP-7 and sPD-L1

3. Methods

3.1 Methods of the clinical trial evaluation

Our retrospective study aimed to identify clinicopathological risk factors associated with bladder recurrence in patients undergoing RNU at the Department of Urology, Semmelweis University between January 1st, 2005 and December 31st, 2016. We collected data by using the local hospital healthcare IT system (Medsol).

Statistical analyses were conducted using IBM SPSS Statistics version 24.0 for Windows (IBM Corp., Armonk, NY, USA). Analysis methods included chi-squared, Mann-Whitney and Kruskal-Wallis tests, along with univariate and multivariate Cox analysis. Survival data were last updated on October 9, 2023.

3.2 Methods of the serum biomarker analysis

3.2.1 Patient cohort for the biomarker studies

We collected serum samples from UTUC patients who underwent different therapies such as RNU (RNU cohort), systemic chemotherapy (CTX cohort) or immune checkpoint inhibitor therapy (ICI cohort). All patients were treated at the Department of Urology, Semmelweis University between August 2014 and July 2020. The two investigated serum biomarkers were MMP-7 and PD-L1 and cohorts for the analyses of these two markers were almost the same with 57 patients in the MMP-7 study and 61 patients in the sPD-L1 study.

Serum samples were collected not just prior various treatments (pre-treatment), but also during (on-treatment) or after (post-treatment) different therapies. In the RNU cohort, serum samples were available before the radical surgery and on the first or second postoperative day. Pre- and on-treatment serum samples were collected in the CTX cohort, where the on-treatment samples were collected at the beginning of the second chemotherapy cycle. In the ICI cohort, baseline samples and samples after 3 months of therapy (on-treatment samples) were available in the ICI cohort. Due to the difficult differential diagnosis of UTUC, preoperative samples from three patients with pT0 histopathological findings were also available. Besides the preoperative serum samples

comparison with confirmed UTUC patients, they were not the part of further statistical analysis.

The institutional ethics committee approved the study protocol (TUKEB 256/2014), and it was conducted according to the principles of Declaration of Helsinki. Written informed consent is available from all patients.

We collected blood samples using 9 ml tubes and processed them to obtain serum samples, allowing them to stand at room temperature for 30-90 minutes, followed by separation using an Eppendorf 5702R centrifuge at 1500x g for 10 minutes. Subsequently, the samples were aliquoted into cryotubes and stored at -80 °C until further analysis.

The primary endpoint of these studies was overall survival (OS) and the secondary endpoint was progression free survival (PFS). The last update on survival was in June 2021.

3.2.2 Serum MMP-7 and PD-L1 analysis and statistical analysis

The sandwhich ELISA method was used to analyze serum MMP-7 and PD-L1 concentrations. The Human Total MMP-7 Quantikine ELISA kit (R&D Systems, Wiesbaden, Germany, Catalog Number: DMP700) was used for the determination of MMP-7 serum levels, and the PD-L1/B7-H1 Quantikine ELISA kit (R&D Systems, Wiesbaden, Germany, DB7H10) was applied to analyze the PD-L1 concentrations.

Wilcoxon rank-sum test, Kaplan-Meier log-rank test, Cox analysis and receiver operating characteristic (ROC) curve were used for the statistical analyses. A statistical difference was considered significant if the p-value was less than 0.05. Statistical analyses were performed using IBM SPSS Statistics software version 27.0 (IBM Corp., Armonk, NY, USA).

4. Results

4.1 Results of the clinical data evaluation

159 RNU were performed at the Department of Urology, Semmelweis University between January 1st, 2005 and December 31st, 2016. Histopathological evaluation confirmed UTUC in 135 cases. The mean annual incidence was 12 cases in our department, with a male dominance. Comorbidities are frequent in this cohort, as the average age was 68 years at the time of diagnosis. The most common comorbidity is hypertension, which can be found in the medical history of 68.9% of cases. Of the patients, 64.5% experienced hematuria, which was the primary symptom preceding flank pain and dysuria. In most of the cases (74.8%), local symptoms were present at the time of the diagnosis. Histopathological results after RNU showed muscle-invasive pT-stage (≥pT2) in half of the cases. The majority of cases were G2 degree of differentiation (57.8%).

The average time of follow-up after RNU was 32 months. IVR was diagnosed in 31 cases (23%), after an average of 19.6 months. We found no significant association between the risk of IVR and age, sex, smoking, comorbidities, BC in medical history, tumor localization, pT-stage, grade, multifocality, necrosis, vascular invasion or surgical margin positivity. We also investigated the time until bladder recurrence. If the patients had hypertension in their medical history, IVR occurred significantly earlier ($p=0.036$).

We also observed the association between various clinicopathological factors and OS. Significantly shorter OS could be observed in patients with advanced age, pulmonary disease, previous bladder cancer before RNU, high grade tumor, surgical margin positivity, necrosis or vascular invasion. From these factors age, pulmonary disease, previous BC in medical history, high grade tumor and necrosis was identified as independent prognostic factors of OS.

Table 1. Multivariate survival analysis

			Overall survival		
General data		**n**	**HR**	**95% CI**	**p**
Age	≤ 65	55	ref.		
	> 65	80	3.193	1.857-5.492	**<0.001**
Pulmonary disease	No	101	ref.		
	Yes	34	1.739	1.012-2.990	**0.045**
BC before RNU	No	106	ref.		
	Yes	29	1.887	1.034-3.444	**0.038**
Grade	1 or 2	85	ref.		
	3	38	3.254	1.891-5.599	**<0.001**
Margin positivity	No	114	ref.		
	Yes	21	1.510	0.742-3.070	0.255
Necrosis	No	94	ref.		
	Yes	41	1.930	1.076-3.462	**0.028**
Vascular invasion	No	112	ref.		
	Yes	23	0.870	0.419-1.809	0.710

4.2 Results of the MMP-7 study

A total of 103 serum samples were collected from 57 UTUC patients in the MMP-7 study. In three cases, pathological examination revealed pT0 findings. Their preoperative MMP-7 levels were also compared with serum MMP-7 levels of confirmed UTUC cases, but they were excluded from further analysis. Serum MMP-7 concentrations of non-malignant cases tended to be lower (p=0.071). Age, sex, ECOG status and surgical margin positivity were also not significantly associated with serum MMP-7 levels. However, as Figure 1 shows, we found a significantly higher preoperative MMP-7 concentration in case of lymphatic or distant metastases (p=0.045). The results showed elevated MMP-7 levels in higher grade and muscle-invasive cases, however, these associations missed the significance level (p=0.077, p=0.140, respectively). In the CTX cohort, distant metastases before systemic chemotherapy were also associated with elevated MMP-7 concentrations (p=0.040).

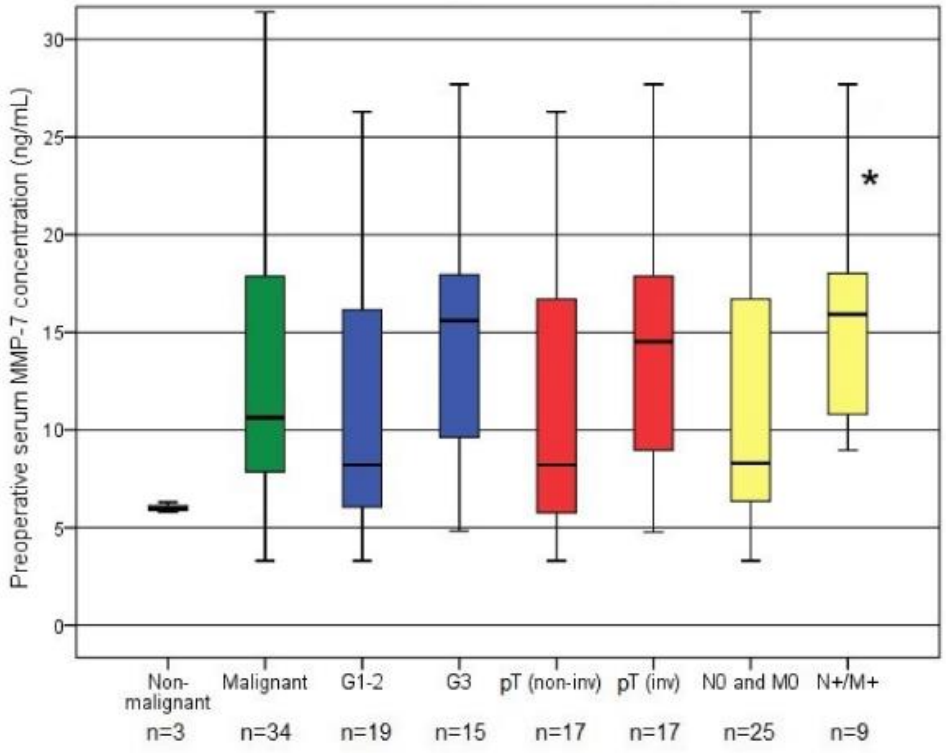

Figure 1. Correlations between preoperative serum MMP-7 levels and clinicopathological parameters in RNU-treated UTUC patients (* significant difference)

Post-treatment MMP-7 concentrations significantly dropped after RNU ($p<0.001$), while no significant correlations were observed in the changes of MMP-7 levels in the CTX or ICI cohort.

In the RNU cohort, worse OS and PFS were found in case of high pT-stage or high-grade tumors, or in the presence of lymphatic or distant metastases. We found significantly shorter OS and PFS in the CTX cohort also in case of lymphatic or distant metastases. Moreover, higher MMP-7 levels were associated in both cohorts with significantly worse OS.

4.3 Results of the sPD-L1 study

We collected serum samples from 61 patients who were treated with different therapies (RNU, CTX, ICI) because of UTUC at the Department of Urology, Semmelweis University between August 2014 and July 2020.

In the RNU cohort, significantly worse OS was observed in higher pT-stage cases ($p=0.013$) and in patients with lymphatic or distant metastases ($p=0.014$). In accordance with the previous results, significantly shorter PFS was found in muscle-invasive cases ($p<0.001$), and in the presence of LN or distant metastases ($p=0.001$). Elevated sPD-L1 levels were associated with significantly worse OS when using the median ($p=0.041$) and ROC cut-off ($p < 0.001$) as well.

In the CTX cohort, lymphatic or distant metastases associated with worse OS ($p=0.012$) and higher sPD-L1 concentrations were associated with significantly shorter OS as well ($p=0.015$).

We also found a significant correlation between MMP-7 and sPD-L1 levels in the RNU cohort ($p=0.008$), while no significant results were identified in the other two cohorts.

5. Conclusions

1. Intravesical recurrence after RNU occurs earlier in patients with hypertension.
2. We found a significantly shorter OS in patients with advanced age, pulmonary disease, bladder cancer in medical history, high-grade tumor, positive surgical margin, tumor necrosis and vascular invasion.
3. We identified age, pulmonary disease, bladder cancer in medical history, high-grade tumor and necrosis as independent prognostic factors for OS after RNU.
4. Preoperative serum MMP-7 levels were significantly higher in case of lymphatic or distant metastases.
5. Pretreatment MMP-7 levels showed a significant association with the presence of distant metastases at the start of the systemic chemotherapy.
6. Serum MMP-7 concentrations significantly decreased after RNU.
7. Higher serum MMP-7 and sPD-L1 levels were significantly associated with worse OS in RNU and CTX cohorts as well.
8. A significant association between MMP-7 and sPD-L1 levels was also revealed in the RNU cohort.

6. Bibliography of the candidate's publications

Related to the dissertation

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treatment soluble PD-L1 as a predictor of overall survival for immune checkpoint inhibitor therapy: a systematic review and meta-analysis. Cancer Immunol Immunother. 2023 May;72(5):1061-1073.

IF: 5.8 (expected IF value)