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KOVÁCS PETRA TERÉZIA

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Programvezető: Dr. Nyirády Péter, egyetemi tanár, az MTA doktora

Témavezetők: Dr. Riesz Péter, egyetemi docens

Dr. Szarvas Tibor, tudományos munkatárs

Prognostic Factors of Upper Tract Urothelial Carcinoma

PhD thesis

Petra Terézia Kovács

Semmelweis University Doctoral School

Surgical Medicine Division

Supervisors: Péter Riesz, MD, Ph.D.
Tibor Szarvas, D.Sc.

Official reviewers: Tamás Beöthe, MD, Ph.D.
Deján Dobi, MD, Ph.D.

Head of the Complex Examination Committee: György Reusz, MD, D.Sc.

Members of the Complex Examination Committee: László Szabó, MD, Ph.D.
András Horváth, MD, Ph.D.

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List of abbreviations

AC – adjuvant chemotherapy

BC – bladder cancer

COPD – chronic obstructive pulmonary disease

CRP – C-reactive protein

CSS – cancer-specific survival

CTU – computed tomography urography

CTX – systemic chemotherapy

ECM – extracellular matrix

ECOG PS– Eastern Cooperative Oncology Group Performance Status

ELISA – enzyme-linked immunosorbent assay

ICI – immune checkpoint inhibitor

IVR – intravesical recurrence

LN – lymph node

MMP-7 – matrix metalloproteinase-7

NAC – neoadjuvant chemotherapy

OS – overall survival

PD-1 – programmed cell death protein 1

PD-L1 – programmed death-ligand 1

PD-L2 – programmed death-ligand 2

PFS – progression-free survival

RFS – recurrence-free survival

RNU – radical nephroureterectomy

SD – standard deviation

sPD-L1 – soluble programmed death-ligand 1

URS - ureteroscopy

UTUC – upper tract urothelial carcinoma

1. Introduction

Urothelial cancer occurs in the urinary tract, which includes the proximal part of the urethra, bladder, ureters, and the renal pelvis. The majority of urothelial cancer cases develop in the urinary bladder, while only 5-10% of urothelial carcinomas are found in the upper urinary tract. (1) Globocan reported no data on the incidence of upper tract urothelial carcinoma (UTUC), while 573,278 cases of bladder cancer (BC) were detected worldwide in 2020. (2) Approximately 2 new cases of 100,000 people are diagnosed in Western countries annually. (3) According to the data of National Cancer Registry, in Hungary, the disease occurs more often in men than in women, and there is an increasing trend over the years (Figure 1). (4)

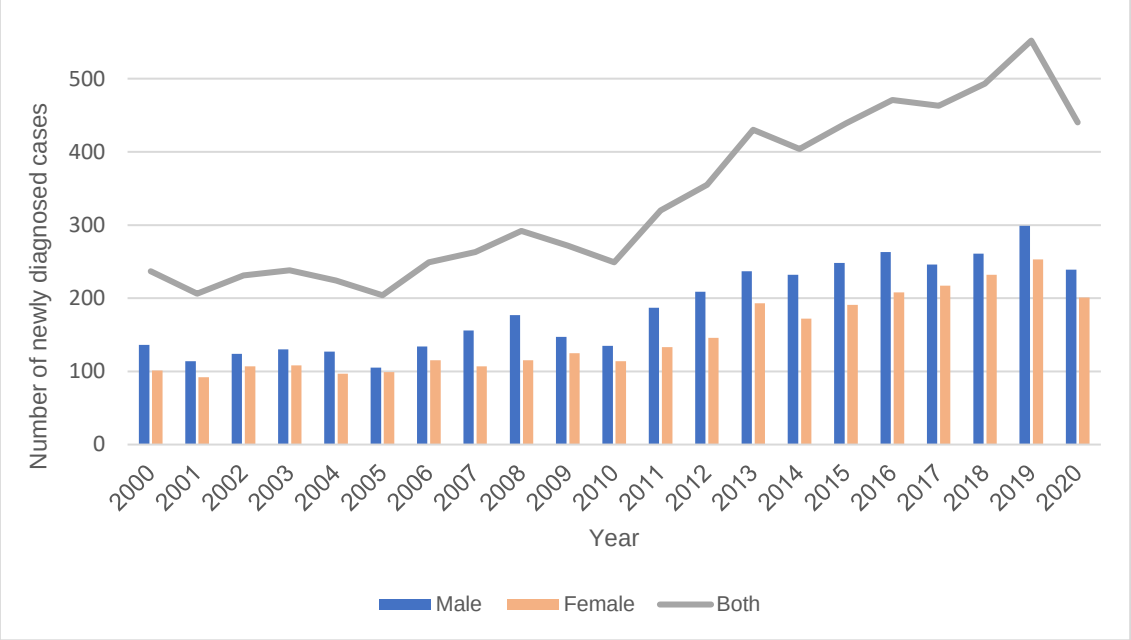

Figure 1. The incidence of UTUC in Hungary between 2000 and 2020 (Source of data: National Cancer Registry (4))

The risk factors for UTUC are similar to those of BC, such as smoking, occupational exposure to certain chemicals and phenacetin usage. However, there are risk factors which are more specific to UTUC, like the Balkan endemic nephropathy, Chinese herb nephropathy, black foot disease, Lynch syndrome. (5) Because of the many similarities between the two diseases, they were considered as identical forms of cancer in different anatomical locations for many years. However, in recent years, an increasing amount of evidence has indicated notable clinical and molecular differences between BC and UTUC. Since 2011, the European Association of Urology has been publishing

different guidelines for BC and UTUC, acknowledging their unique characteristics and the need for separate recommendations. (6)

1.1 Clinical management of UTUC

Urothelial cancer is the most common histological presentation of upper tract tumors. However, in rare cases, non-urothelial cancer can also occur, such as squamous cell carcinoma, adenocarcinoma, or mesodermal tumors. (7)

Localized UTUC is often asymptomatic and may therefore be discovered incidentally through imaging tests. Its most common symptoms include hematuria, flank pain and dysuria. Systemic symptoms such as fever, weight loss and fatigue usually indicate an advanced disease. (1, 8)

Cytology may help to diagnose high-grade UTUC cases, but its sensitivity is even lower for UTUC than for BC. (9) Computed tomography urography (CTU) is the recommended imaging test in cases of suspected UTUC. In a study evaluating the effectiveness of CTU in patients with hematuria, pooled sensitivity was 96%, while pooled specificity was 99% for diagnosing UTUC. (1, 10). CTU is also an important step for tumor staging.

Cystoscopy must be performed to detect possible synchronous BC. If the diagnosis is uncertain, diagnostic ureteroscopy (URS) can help to confirm suspected UTUC and obtain selective cytology from the ureters. (9)

UTUC is classified as low risk when the following conditions are met: the disease is unifocal, the tumor size is less than 2 centimeters, cytology is negative, the result of URS biopsy is low grade and CT scans show no signs of an invasive tumor. All these factors must be true to categorize the tumor as low risk. If any of the following factors are present, the tumor is considered high risk: multifocality, size greater than 2 centimeters, high-grade tumor in cytology, high-grade tumor in URS biopsy, invasiveness on CT scans, hydronephrosis, history of radical cystectomy for high-grade BC and any histological subtype. In low-risk cases, kidney-sparing surgery is possible with various techniques such as endoscopic ablation, ureteral resection, chemoablation. (9) The gold standard procedure for treating UTUC is radical nephroureterectomy (RNU), which can be combined with platinum-based chemotherapy either before (neoadjuvant) or after (adjuvant) surgery. There is a strong recommendation in the EAU guideline to give one postoperative chemoinstillation with mitomycin C or pirarubicin after the RNU between

the postoperative 2-10 days. This treatment can reduce the risk of intravesical recurrence (IVR). (9) For chemotherapy resistant or ineligible patients, immune checkpoint inhibitor (ICI) therapy may be considered as an option. (9)

Performing an URS biopsy can be challenging due to the anatomy of upper urinary tract, and even in a successful procedure, the accuracy of the biopsy remains uncertain. (11) Furthermore, the EAU guidelines mention URS biopsy as a risk-factor for IVR and recommends diagnostic URS preferably without biopsy. (9) Therefore, it is difficult to select the right patients for organ-sparing surgeries and overtreatment is a real clinical problem in the routine management of UTUC. However, without a validated prognostic stratification, high-risk patients are at risk of undertreatment, making it challenging to detect those who would gain benefit from intensive perioperative systemic therapy. (6, 11)

The follow-up strategy depends on various factors, including the risk level of the tumor. If RNU confirms a low-risk tumor and the result of the first control cystoscopy at three months is negative, the next cystoscopy should occur at 12 months, and then annually. In this case, performing a contrast enhanced CT scan to rule out the presence of metastases is optional. However, if the histology confirmed a high-risk tumor after RNU, cystoscopy should be performed every three months until the second year, and every six months until the fourth year. Contrast enhanced CT scan is recommended every six months until the second year, and then annually. (9) Patients with a history of BC should be followed up for ten years due to their increased risk of IVR. (12)

Preoperative predictive factors assist in stratifying patients based on their risk of a worse prognosis. Patient-related factors previously included female gender, higher age, African-American ethnicity as risk-factors. However, these correlations disappeared after adjusting for other predictors. Nonetheless, Lynch syndrome, smoking, high comorbidity and aristolochic acid or phenacetin exposure in medical history are associated with worse prognosis. Tumor-related prognostic factors are higher stage and grade, multifocality, ureteral location, hydronephrosis, larger tumor size, histological subtypes, lymph node (LN) metastasis, lymphovascular invasion, positive surgical margins. These factors are associated with worse prognosis, however their prognostic value is limited. Therefore, tissue, blood, and urine biomarkers were investigated to identify novel prognostic factors. (9, 13) According to Krabbe *et al*., PD-1 expression of tumor infiltrating lymphocytes

was correlated with worse prognosis in UTUC, while PD-L1 expression of tumor cells was independently associated with better recurrence-free survival (RFS) and overall survival (OS). (14) In a meta-analysis, high C-reactive protein (CRP) serum level before RNU was found to be correlated with poor prognosis. (15) Lower preoperative serum pseudocholinesterase level associated with poor prognosis for UTUC in a retrospective study. (16) Pretreatment high alkaline phosphatase and low albumin level were significantly associated with poor OS in another retrospective study. (17) Preoperative albumin-globulin ratio showed a significant correlation with OS as well. (18) However, despite the numerous promising results in the field of biomarker research, no validated prognostic biomarker has been accepted in daily clinical practice to date. (9)

1.2 The investigated biomarkers

The matrix metalloproteinase (MMP) family contains more than 25 zinc-dependent endopeptidases, which are mostly secreted by keratinocytes and fibroblasts. MMPs can be classified into various subgroups such as collagenases, gelatinases, stromelysins, matrilysins, membrane types and others. (19, 20) MMPs play an important role in extracellular matrix (ECM) degradation by cleaving collagens, laminin, fibronectin, and gelatine. (20) They are known for their involvement in physiological processes like tissue remodeling and tissue repair, angiogenesis, and embryogenesis. (21-23) On the other hand, the functions of MMPs can be pathological as well, resulting or playing a role in non-malignant (arthritis, type 2 diabetes mellitus, pulmonary arterial hypertension, idiopathic pulmonary fibrosis, etc.) and malignant diseases (gastric, colorectal, pancreatic, bladder, ovarian, breast, melanoma, lung, etc.) (19, 24-26).

MMP-7 - the smallest enzyme in the MMP family - is a member of the subgroup matrilysins. It is also called matrilysin, putative metalloproteinase or punctuated metalloproteinase; PUMP1) (19). While other MMPs are mainly secreted by the stromal component of the tumor, MMP-7 can be released by tumor cells directly. MMP-7 is involved in tumor growth by several different ways. By its ECM degrading effect, it is able to enhance the invasive tumor growth, thus promoting metastatic spreading. MMP-7 has proteolytic products, which have antiangiogenic effect, and on the other hand, it secretes proangiogenic agents as well, providing an angiogenesis promoting microenvironment. MMP-7 can activate other MMPs as well. It can save tumor cells from

apoptosis by processing proapoptotic proteins, such as Fas ligand. MMP-7 is also involved in tumor-induced osteolysis as well as in epithelial-to-mesenchymal transition. (20)

In a previously published study, higher serum MMP-7 level was significantly associated with LN or distant metastatic BC. Moreover, increased serum MMP-7 concentration correlated with shorter cancer-specific and metastasis-free survival. (27) Similarly, in a further study, increased serum MMP-7 levels were found to be correlated with OS and cancer-specific survival (CSS) in BC, thus the prognostic value of serum MMP-7 levels could be confirmed. (28) Svatek *et al.* using a multiplex assay assessed the preoperative plasma levels of MMP-1, -2, -3, -7, -8, -9, and -12 in samples of 135 patients with ≥T1, high grade BC patients. Of the seven MMP types only MMP-7 concentrations were independently associated with poor CSS. (29) In a more progressed BC cohort El Demery *et al*. also confirmed high MMP-7 levels as an independent prognostic factor. (30) According to the results of a systematic review and meta-analysis by Kubik *et al.*, serum preoperative MMP-7 concentrations were significantly higher in patients with urothelial carcinoma than in control groups. In addition, cases with LN metastases had the highest MMP-7 levels. However, about half of the patients with lymphatic metastases had low concentrations of MMP-7, thus serum MMP-7 levels alone are not eligible to predict the need for an extended lymphadenectomy. On the other hand, patients with higher MMP-7 levels and no LN metastases at radical cystectomy had worse prognosis, suggesting the presence of undetected (micro)metastases detected by MMP-7. MMP-7 was an independent predictor of shorter OS and elevated MMP-7 levels meant a 2.5-fold higher mortality risk. (31) Taking together, many independent studies confirmed the prognostic value of MMP-7 in BC, but no data is available regarding the prognostic value of MMP-7 in UTUC.

Programmed cell death protein 1 (PD-1) is a co-inhibitory receptor, which has two ligands: programmed death-ligand 1 (PD-L1) and programmed death-ligand 2 (PD-L2). PD-L1 is expressed on hematopoietic (T cells, B cells, macrophages, dendritic cells) and on non-hematopoietic cells, like vascular endothelial cells, keratinocytes, pancreatic cells, astrocytes, etc. PD-L2 is expressed mostly on macrophages, dendritic cells, and mastocytes, while expression of PD-1 receptor can be observed on activated T cells. The PD-1 and PD-L1 interaction is a checkpoint blockade mechanism that plays a crucial role

in regulating the immune response. When PD-1 on T cells binds to PD-L1 on other cells, it inhibits the immune response, preventing T cells from attacking those cells. This interaction is exploited by some cancer cells as an immune escape mechanism. Checkpoint blockade therapies, such as PD-1 inhibitors, work by disrupting this interaction, allowing the immune system to recognize and attack cancer cells effectively. (32)

Ward *et al.* analyzed the data of 37 UTUC cases and their PD-L1 expression. Overall tissue expression of PD-L1 was 29.7%, but the expression of PD-L1 was 55.6% in more advanced stages (pT3 and pT4), while it was 5.3% in lower stages. Accordingly, tissue PD-L1 level was correlated with higher pathological stage in UTUC. (33) Lu *et al*. performed a systematic review and meta-analysis regarding the prognostic value of PD-L1 in UTUC patients. This study included the data of 1406 UTUC patients. The overall expression of PD-L1 was 21%. PD-L1 expression was significantly associated with higher tumor stages (pT2, pT3 and pT4) and higher tumor grade. Moreover, higher PD-L1 expression was correlated with poor CSS after RNU. (34)

In addition to its tissue expression, PD-L1 can be determined in the blood circulation as its extracellular domain is measurable in soluble form in serum and plasma samples. How the extracellular domain of PD-L1 is cleaved and appears in the circulation is poorly understood. (35) However, Hira-Miyazawa *et al*. identified MMP-7 as a proteolytic enzyme that is able to cleave the extracellular domain of PD-L1. (36) Accordingly, a recent study found a significant correlation between the serum levels of PD-L1 and MMP-7 in BC. (37) The prognostic value of sPD-L1 has not yet been analyzed in UTUC.

2. Objectives

2.1 The aims of the clinical data analysis were:

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

2.2 The aims of the present biomarker studies 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 preoperative serum biomarkers, which can improve risk-stratification and clinical decision-making in UTUC
4) To assess the association between the serum levels of MMP-7 and sPD-L1

3. Methods

3.1 Methods of the clinical data evaluation

The objective of our retrospective study was to identify the clinicopathological risk factors for bladder recurrence in patients who underwent RNU at the Department of Urology, Semmelweis University, between January 1st, 2005, and December 31st, 2016. Data collection was performed using the local hospital healthcare IT system (Medsol).

To categorize symptoms, we followed the classification suggested by Raman *et al*. (38 - Raman), which classified patients into three groups: (1) no symptoms at the time of the diagnosis, (2) local symptoms like flank pain, micro- or macrohematuria or palpable mass and (3) systemic symptoms including general symptoms like weight loss, weakness, fever or decreased health condition.

We classified patients according to the localization of their primary tumor into two groups: patients with exclusive involvement of the renal pelvis and patients with ureteral infiltration. Furthermore, within the ureteral cohort, we categorized patients into three subgroups based on the specific localization of the primary tumor in the upper, middle or lower ureter.

Additionally, we grouped patients depending on the number of comorbidities. First, we created two groups (patients with none or one and patients with two comorbidities). For further analysis, we classified individuals into three groups also based on the number of comorbidities: the first group comprised people without any comorbidities, the second group consisted of patients with one comorbidity, and the last group included patients with two or more comorbidities.

Statistical analyses were performed using the IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA). Data analysis involved the utilization of chi-squared, Mann-Whitney, Kruskal-Wallis tests, univariate and multivariate cox analysis. Survival data have been updated on October 9, 2023.

3.2 Methods of the serum biomarker analysis

3.2.1 Patient cohort of the biomarker studies

We collected serum samples prior various therapies from UTUC patients who underwent different treatments like RNU, platinum-based systemic chemotherapy or

immune checkpoint inhibitor therapy at the Department of Urology, Semmelweis University between August 2014 and July 2020. The UTUC cohorts used for the analysis of MMP-7 and PD-L1 were only slightly different with 57 patients for the MMP-7 study and 61 patients for the PD-L1 study. We collected samples before, during and after various treatments. For the RNU cohort, we collected samples before surgery and on the first or second postoperative day. For the CTX cohort, baseline samples were collected immediately before the first treatment, while on-treatment serum samples were collected at the beginning of the second chemotherapy cycle (on-treatment samples). For all ICI-treated patients, baseline and on-treatment samples (after 3 months of therapy) were available for analysis. We also collected serum samples from three patients who had a pT0 finding at RNU histology. These pT0 cases highlight the challenging differential diagnosis of UTUC with suspicions of cancer before RNU. Although their MMP-7 concentrations were compared with patients with confirmed UTUC, they were subsequently excluded from further analysis.

The studies were carried out according to the principles outlined in the Declaration of Helsinki, and the study protocol received approval from the institutional ethics committee (TUKEB 256/2014). All participants of the studies provided written informed consent prior to their involvement.

Blood samples were collected from patients in 9 ml tubes (Greiner, Nürnberg, CatNr.: 455071) and processed to obtain serum samples, which means they were left at room temperature for 30-90 minutes, then were separated with the help of an Eppendorf 5702R centrifuge at 1500x g for 10 minutes at room temperature, were aliquoted in anonymized tubes and stored at -80°C until further analysis.

The main focus of these studies was to evaluate OS as the primary endpoint and PFS as the secondary endpoint. The study considered the time from the initiation of therapy (RNU, CTX or ICI) to the occurrence of disease progression, death or survival as the relevant endpoint. The date of the last follow-up update was in June 2021.

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

Serum PD-L1 and MMP-7 levels were analyzed using the sandwich ELISA method. For the PD-L1 analysis we utilized the PD-L1/B7-H1 Quantikine ELISA kit (R&D Systems, Wiesbaden, Germany, DB7H10), while for the MMP-7 analysis the Human Total MMP-7 Quantikine ELISA kit (R&D Systems, Wiesbaden, Germany,

Catalog Number: DMP700) was applied. The analysis was done by following the manufacturer's instructions.

Statistical analyses included the Wilcoxon rank-sum test for group comparisons, Kaplan-Meier log-rank test, Cox analysis and receiver operating characteristic (ROC) curve analysis for determining the ideal cut-off value with the highest sensitivity and specificity to predict the patients' death. A p-value must be less than 0.05 to be considered statistically significant. IBM SPSS Statistics software was used for all statistical analyses (v. 27.0; IBM Corp., Armonk, NY, USA).

4. Results

4.1 Results of the clinical data evaluation

4.1.1 General characteristics of patients treated with RNU

A total of 159 RNU procedures were performed at the Department of Urology, Semmelweis University, between January 1st, 2005, and December 31st, 2016. Because of insufficient data availability, six patients were excluded. Additionally, in 17 cases histopathological examination revealed other diseases, such as endometriosis, chronic inflammation resulting in granulation tissue, renal cell carcinoma, prostate cancer and non-Hodgkin lymphoma. Consequently, these cases were also excluded from the study. Among the remaining patients, histopathological results confirmed the presence of urothelial cancer in 135 cases, while one case of planocellular cancer was excluded from further analysis.

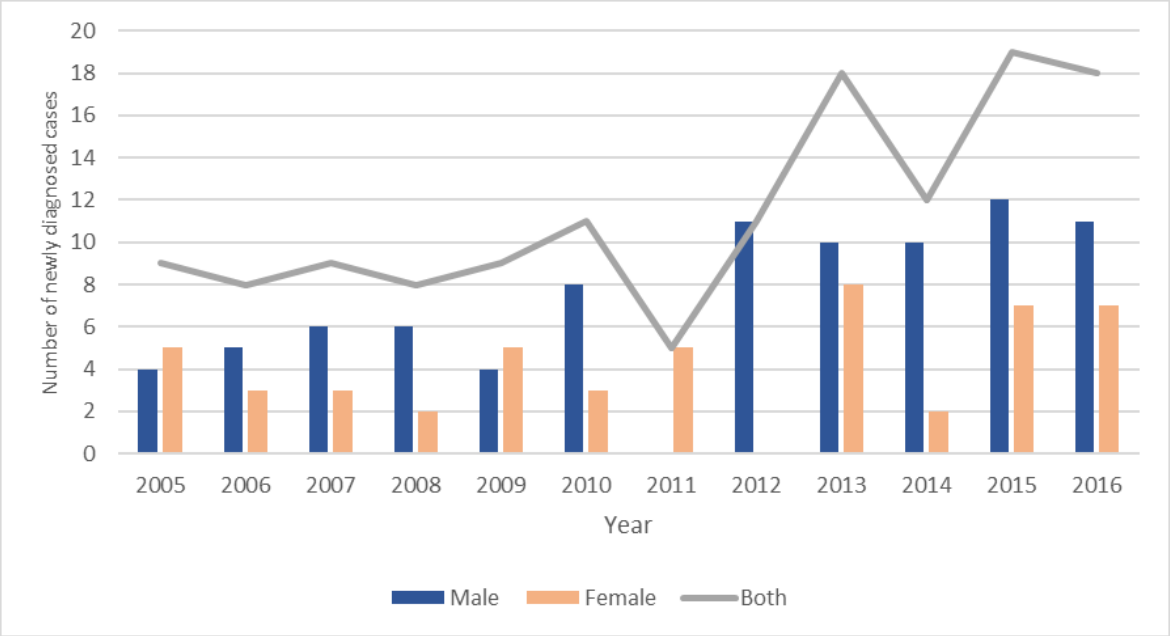

Figure 2. The number of UTUC cases treated with RNU at the Department of Urology, Semmelweis University between January 1st, 2005, and December 31st, 2016.

An average of 12 new UTUC cases were surgically treated annually at our department. Figure 2 shows a rising trend over the time, with a male dominance. The average age at the diagnosis was 68.3 years with a range of 45 and 90 years. As the peak incidence of the disease is in an older age, comorbidities are frequent in the cohort, which

data is summarized in Table 1. It is apparent that the majority (68.9%) of patients had hypertension. The second most common comorbidities are cardiovascular diseases (excluding hypertension), and the third most common are various pulmonary diseases. The category of oncological diseases excludes urological cancers.

Table 1. The comorbidities of the patients (39)

N=135	N	%
Hypertension	93	68.9
Cardiovascular disease	47	34.8
Pulmonary disease	34	25.2
Diabetes mellitus	33	24.4
Oncological disease	26	19.3
Neurological disorder	15	11.1
Deep vein thrombosis	11	8.1
Endocrine disorder	8	5.9

The most common symptom of UTUC was hematuria, which was experienced at 64.5% of the patients, and it was visible in over half of the cases (54.1%). The second most common symptom was flank pain (25.2%) followed by dysuria (7.4%). Many of the patients had mixed symptoms as well. We categorized the symptoms based on the suggestion by Raman *et al*., which is detailed in the Methods section (38). Figure 3 shows that in most cases local symptoms were dominant at the time of the diagnosis.

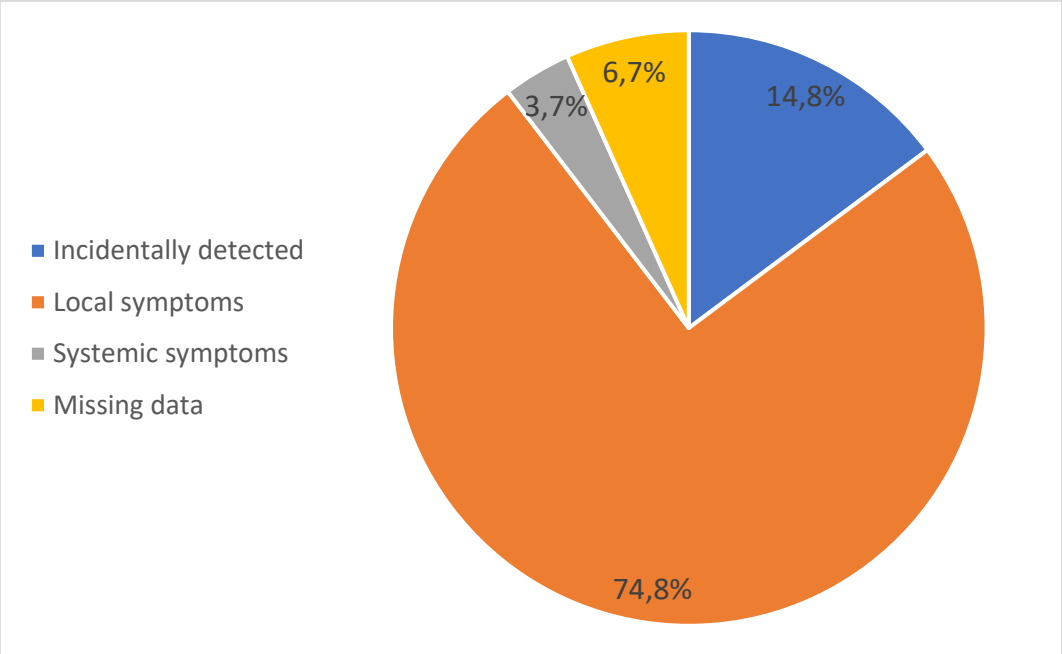

Figure 3. Symptoms at diagnosis as categorized by Raman *et al*. (39)

UTUC was diagnosed more frequently on the left side compared to the right side, and bilateral involvement was observed in one case. In terms of gender, left side UTUC was more prevalent in males, while right-side UTUC was more common in females. However, this difference did not reach statistical significance. The localization of the primary tumor was also analyzed. In 53 cases, the tumor infiltrated the renal pelvis only, while in the remaining cases, the ureter was affected. The most common localization of the primary tumor in the ureter was its lower part.

Figure 4 illustrates the histopathological findings regarding the infiltration of the primary tumor. In 40 cases, the stage of the primary tumor was already at stage pT3 at the time of RNU. We also examined the degree of differentiation with G2 characterizing 57.8% of the lesions, G3 for 28.1%, while G1 was observed in only 5.2% of cases.

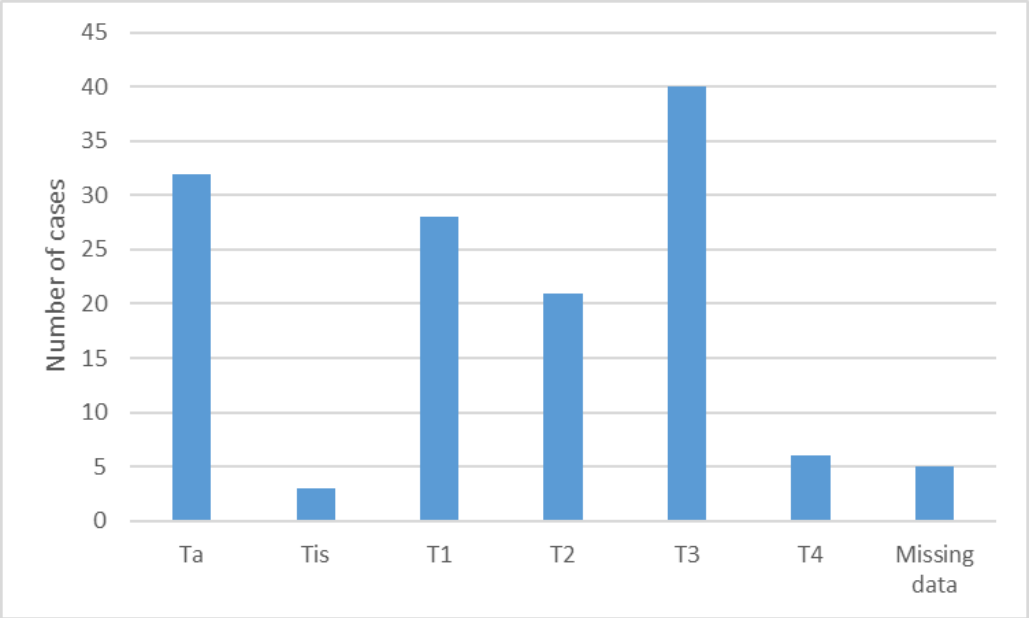

Figure 4. The histopathological staging of the primary tumor (39)

4.1.2 Characteristics of bladder recurrence after RNU

The mean postoperative follow-up time was 32 months (SD: 30.25). Bladder recurrence occurred in 31 patients (23%), with a mean interval of 19.6 months (SD: 29.7, range 2-125 months). Table 2 shows the patients' characteristics of the cohort depending on IVR.

Table 2. Association between clinicopathological parameters and intravesical recurrence

		Intravesical recurrence			
		Σ	Yes	No	
General data		**n**	**n**	**n**	**p**
Time until recurrence (months)		19.6 (1.3-125.4)			
All patients		135	31	104	
Number of patients died		85	21	64	
Age (years)	≤65	55	13	42	0.877
	>65	80	18	62	
Sex	Male	86	22	64	0.338
	Female	49	9	40	
Smoking	Smoker	67	18	49	0.659
	Non-smoker	35	8	27	
	Missing	33	5	28	
Comorbidities	None or 1	55	12	43	0.793
	2 or more	80	19	61	
BC before RNU	Yes	29	7	22	0.865
	No	106	24	82	
pT-stage	pTa	32	6	26	
	CIS	3	0	3	
	pT1	28	8	20	
	pT2	21	7	14	
	pT3	40	7	33	
	pT4	6	2	4	
	Missing	5	1	4	
pT-stage	pTa, pT1, CIS	63	14	49	0.823
	pT2-pT4	67	16	51	
Grade	1	7	2	5	
	2	78	17	61	
	3	38	8	30	
	Missing	12	4	8	
Grade	1 or 2	85	19	66	0.872
	3	38	8	30	
Multifocality	Yes	18	5	13	0.569
	No	117	26	91	
Necrosis	Yes	41	10	31	0.795
	No	94	21	73	
Vascular invasion	Yes	23	7	16	0.350
	No	112	24	88	
Margin positivity	Yes	21	5	16	0.920
	No	114	26	88	

As Table 2 illustrates, we found no significant correlation between IVR and the investigated factors. Besides them, the localization of the primary tumor was categorized according to the affected part of the ureter (upper-, middle or lower part). In this cohort, if the upper ureter was infiltrated, recurrence did not occur. In 11 cases (35.5%), bladder recurrence was diagnosed after distal ureteral infiltration. The results did not reach statistical significance.

As mentioned above, hypertension was the most commonly occurring comorbidity in the cohort. Out of 31 cases from the recurrence group, 20 patients had hypertension and, in these cases, the time until bladder recurrence was significantly shorter (p=0.036). We also categorized the patients into three groups depending on the number of comorbidities. Bladder recurrence occurred earlier in patients who had two or more comorbidities compared to those without known comorbidities. However, the observed difference did not reach the significance level (p=0.145).

4.1.3 Association between clinicopathological factors and OS

Shorter OS was observed in elderly patients (HR: 2.492; 95% CI 1.537-4.041; p<0.001), in patients with pulmonary disease (HR: 1.987; 95% CI 1.245-3.171; p=0.004) or in cases with BC in their medical history (HR: 1.853; 95% CI 1.088-3.156; p=0.023). From the histopathological evaluation, high-grade (HR: 2.701; 95% CI 1.682-4.338; p<0.001), surgical margin positivity (HR: 1.898; 95% CI 1.039-3.467; p=0.037), necrosis (HR: 1.647; 95% CI; 1.045-2.595; p=0.031) and vascular invasion (HR: 2.249; 95% CI 1.311-3.856; p=0.003) were correlated with worse OS (Table 3).

Table 3. Association between clinicopathological factors and OS (* - missing data: smoking – 33; pT-stage – 5; grade – 12)

			Overall survival		
General Data		**n**	**HR**	**95% CI**	**p**
Age	≤65	55	ref.		
	>65	80	2.492	1.537-4.041	**<0.001**
Sex	male	86	ref.		
	female	49	1.007	0.643-1.579	0.975
Smoking*	Non-smoker	35	ref.		
	Smoker	67	0.828	0.497-1.379	0.469
Hypertonia	No	42	ref.		
	Yes	93	1.243	0.775-1.995	0.367
Cardiovascular disease	No	88	ref.		
	Yes	47	1.122	0.718-1.753	0.615
Pulmonary disease	No	101	ref.		
	Yes	34	1.987	1.245-3.171	**0.004**
Diabetes mellitus	No	102	ref.		
	Yes	33	0.914	0.553-1.510	0.725
Comorbidities	None or 1	55	ref.		
	2 or more	80	1.537	0.987-2.394	0.057
BC before RNU	No	106	ref.		
	Yes	29	1.853	1.088-3.156	**0.023**
Multifocality	No	117	ref.		
	Yes	18	1.669	0.901-3.091	0.103
pT-stage*	pTa, pT1, CIS	63	ref.		
	pT2-pT4	67	1.554	0.995-2.429	0.053
Grade*	1 or 2	85	ref.		
	3	38	2.701	1.682-4.338	**<0.001**
Margin positivity	No	114	ref.		
	Yes	21	1.898	1.039-3.467	**0.037**
Necrosis	No	94	ref.		
	Yes	41	1.647	1.045-2.595	**0.031**
Vascular invasion	No	112	ref.		
	Yes	23	2.249	1.311-3.856	**0.003**

We also performed OS analysis. As Table 4 illustrates, older age, pulmonary disease, BC in medical history, high-grade tumor and necrosis in histopathological evaluation proved to be independent prognostic factors for OS.

Table 4. 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

4.2.1 Clinical background of MMP-7 study

In the MMP-7 study, we collected 103 serum samples from 57 patients with UTUC (40 males and 17 females). The patients underwent different treatments, including surgical intervention (RNU cohort; n=34), platinum-based systemic chemotherapy (CTX cohort; n=25), or immune checkpoint inhibitor therapy (ICI cohort; n=5) at the Department of Urology, Semmelweis University between August 2014 and July 2020. There were three patients who were part of both the RNU and CTX treatment groups and two patients who were included in both the CTX and ICI treatment groups. Furthermore, one patient was included in all three groups (RNU, CTX and ICI).

Regarding the sample availability, for the RNU cohort, 34 samples were collected before surgery, and an additional 16 serum samples were collected postoperatively (on the first or second postoperative day). For the CTX cohort, 25 baseline serum samples were collected, and 18 on-treatment serum samples were available (collected at the beginning of the second chemotherapy cycle). As for all the ICI-treated patients, baseline serum samples as well as on-treatment samples (collected after 3 months of therapy) were available for analysis. Lastly, the study also included preoperative serum samples from

three patients who had a pT0 finding at RNU. The detailed patients' characteristics are given in Table 5.

Table 5. Patients' characteristics of the MMP-7 cohort (Non-malignant* - histopathological evaluation revealed pT0 stage in three cases, R+ - positive surgical margin, N+ - lymph node metastasis, M+ - distant metastasis) (40)

		RNU			CTX			ICI	
General data	**n**	**median (range)**	**p**	**n**	**median (range)**	**p**	**n**	**median (range)**
Follow-up in months, median (range)	34	24.23 (1.08 - 81.93)	-	25	16.56 (1.05 - 67.70)	-	5	27.97 (6.92 - 30.2)
Age at baseline, median (range)	34	68.50 (45 - 90)	-	25	71 (46 - 84)	-	5	65 (64 - 75)
Number of patients died	11	-	-	13	-	-	1	-
Parameters / MMP-7 cc.	**n**	**MMP-7 cc.**	**p**	**n**	**MMP-7 cc.**	**p**	**n**	**MMP-7 cc.**
All patients, median (range)	34	10.63 (3.30 - 31.40)	0.071	25	8.76 (4.13-26.74)	-	5	9.28 (4.46-13.90)
Non-malignant*	3	5.96 (5.80 - 6.30)						
Age ≤ 65	10	7.37 (3.3 - 27.36)	0.086	5	7.3 (4.13 - 9.17)	0.129	2	9.14 (9.00 - 9.28)
Age > 65	24	14.19 (4.05 - 31.40)		20	9.99 (4.92 - 26.74)		3	9.60 (4.46 - 13.90)
Sex male	21	10.3 (3.3 - 27.36)	0.381	21	7.91 (4.13 - 26.74)	0.081	4	9.14 (4.46 - 13.90)
female	13	13.84 (4.84 - 31.40)		4	12.93 (10.28 - 23.28)		1	9.60
ECOG PS 0	19	13.84 (3.30 - 27.70)	-	11	8.08 (5.86 - 17.18)	-	5	9.28 (4.46 - 13.90)
1	10	9.32 (4.77 - 31.40)	-	10	10.91 (4.13 - 26.74)	-	0	
2	4	9.89 (8.08 - 17.00)	-	4	13.18 (6.68 - 23.28)	-	0	
3	1	15.92		0			0	
ECOG PS 0-1	29	10.44 (3.30 - 31.40)	0.888	21	8.76 (4.13 - 26.74)	0.695	5	9.28 (4.46 - 13.90)
ECOG PS 2-3	5	10.81 (8.08 - 17.00)		4	13.18 (6.68 - 23.28)		0	
Nephrouretherectomy data								
pTa	7	9.80 (3.30 - 26.30)	-	0	-	-	0	-
CIS	1	4.84	-	0	-	-	1	13.90
pT1	9	8.21 (4.05 - 23.73)	-	1	19.13	-	0	-
pT2	2	22.97 (14.53 - 31.40)	-	5	8.76 (6.73 - 24.02)	-	1	9.00
pT3	14	10.63 (4.77 - 27.70)	-	15	7.91 (4.13 - 18.59)	-	3	9.28 (4.46 - 9.60)
pT4	1	17.88	-	2	7.31 (4.92 - 9.70)	-	0	-
n.a.	0			2		-	0	
pTa-pT1-CIS (non-invasive)	17	8.21 (3.30 - 26.30)	0.140	1	19.13	-	1	13.90
pT2-pT4 (invasive)	17	14.53 (4.77 - 31.40)		22	7.99 (4.13 - 24.02)	-	4	9.14 (4.46 - 9.60)
G1	7	8.06 (4.05 - 13.84)	-	0		-	0	
G2	12	13.03 (3.30 - 26.30)	-	5	13.2 (6.73 - 24.02)	-	2	9.14 (9.00 - 9.28)
G3	15	15.60 (4.84 - 31.40)	-	16	7.53 (4.13 - 18.59)	-	2	9.18 (4.46 - 13.90)
n.a.	0			4			1	
G1-G2	19	8.21 (3.30 - 26.30)	0.077	5	13.2 (6.73 - 24.02)	0.075	2	9.14 (9.00 - 9.28)
G3	15	15.60 (4.84 - 31.40)		16	7.53 (4.13 - 18.59)		2	9.18 (4.46 - 13.90)
R0	26	10.37 (3.30 - 31.40)	0.827	14	7.99 (4.13 - 24.02)	0.224	3	9.00 (4.46 - 9.60)
R+	8	12.67 (4.77 - 23.73)		9	8.76 (4.92 - 18.59)		1	9.28
n.a.	0			2			1	
Metastatic status at RNU								
N0/M0	25	8.30 (3.30 - 31.40)	**0.045**	14	7.60 (5.86 - 24.02)	0.781	2	11.45 (9.00 - 13.90)
N+ or M+	9	15.92 (8.96 - 27.70)		9	9.26 (4.13 - 19.13)		3	9.28 (4.46 - 9.60)
n.a.	0			2			0	
Metastatic status at CTX baseline								
M0	-			17	7.77 (4.13 - 17.18)	**0.040**	-	
M+	-			7	18.59 (6.68 - 26.74)		-	
n.a.	-			1			-	

4.2.2 Correlation between serum MMP-7 levels and clinicopathological parameters

In the case of three patients, the histopathological examination after RNU revealed no malignancy (stage pT0 in Table 5). Histopathological evaluation revealed urothelial papilloma in one case and chronic inflammation - presumably caused by stones - in the other two cases. Additionally, selective urine cytology previously conducted was positive in the last two cases. These pT0 patients have a lower preoperative MMP-7 concentration (5.96 ng/ml) compared to those patients with >pT0 histological finding (10.63 ng/ml). However, this difference slightly missed statistical significance (p=0.071). Table 5 and Figure 5 show that preoperative serum MMP-7 levels were higher in patients present with LN or distant metastases at RNU (p=0.045). Figure 5 also illustrates that muscle-invasive and high-grade tumors tended to have higher MMP-7 concentrations (p=0.140 and p=0.077). Age, sex, ECOG performance status and surgical margin positivity were not associated with preoperative serum MMP-7 levels.

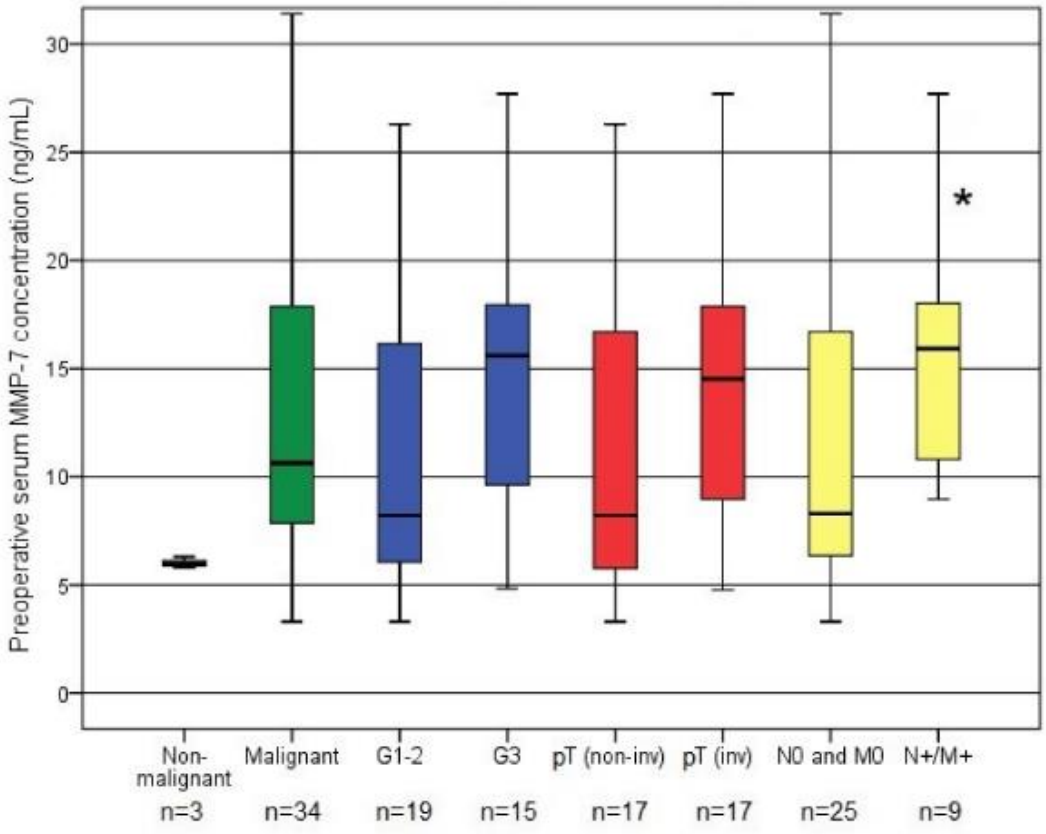

Figure 5. Correlations between preoperative serum MMP-7 levels and clinicopathological parameters in RNU-treated UTUC patients (* significant difference) (40)

In the CTX cohort, the presence of distant metastases at the start of chemotherapy were correlated with higher MMP-7 levels (7.8 ng/ml and 18.6 ng/ml; p=0.040). Age, sex and pathological parameters at RNU were not associated with serum MMP-7 concentrations before systemic chemotherapy.

Table 6. Patients' characteristics in the ICI cohort (N – lymph node metastasis, M – distant metastasis) (40)

	Patient 1.	**Patient 2.**	**Patient 3.**	**Patient 4.**	**Patient 5.**
Age	76	64	64	75	65
Sex	female	male	male	male	male
Clinicopath. parameters at RNU					
Stage (pT)	3	2	3	in situ	3
Grade (G)	-	2	2	3	3
N	yes	no	yes	no	yes
M	no	no	no	no	no
Chemotherapy pre-treatment	Gem/Carb	Gem/Cis	Gem/Carb	n.a.	Gem/Carb
Clinicopath. parameters at ICI baseline					
N	yes	yes	yes	no	yes
M	yes	yes	no	no	yes
MMP-7 cc. at baseline (ng/ml)	9.60	9.00	9.28	13.90	4.46
MMP-7 cc. at 3 months (ng/ml)	10.30	11.42	10.33	14.13	5.12

As the ICI cohort consisted of only five patients the low case numbers did not allow us to perform a solid statistical analysis. Therefore, in Table 6, we provide data on individual patient level.

4.2.3 Changes of serum MMP-7 levels under various therapies

Postoperative MMP-7 levels were significantly lower compared to the pre-operative concentrations (6.0 ng/ml vs. 10.6 ng/ml, p <0.001) in the RNU cohort (Figure 6). We also assessed the prognostic value of MMP-7 level changes using four other classifications: MMP-7 decrease or increase, and whether the reduction exceeded 30, 40 or 50 percent. However, we found no significant association in any of the groups.

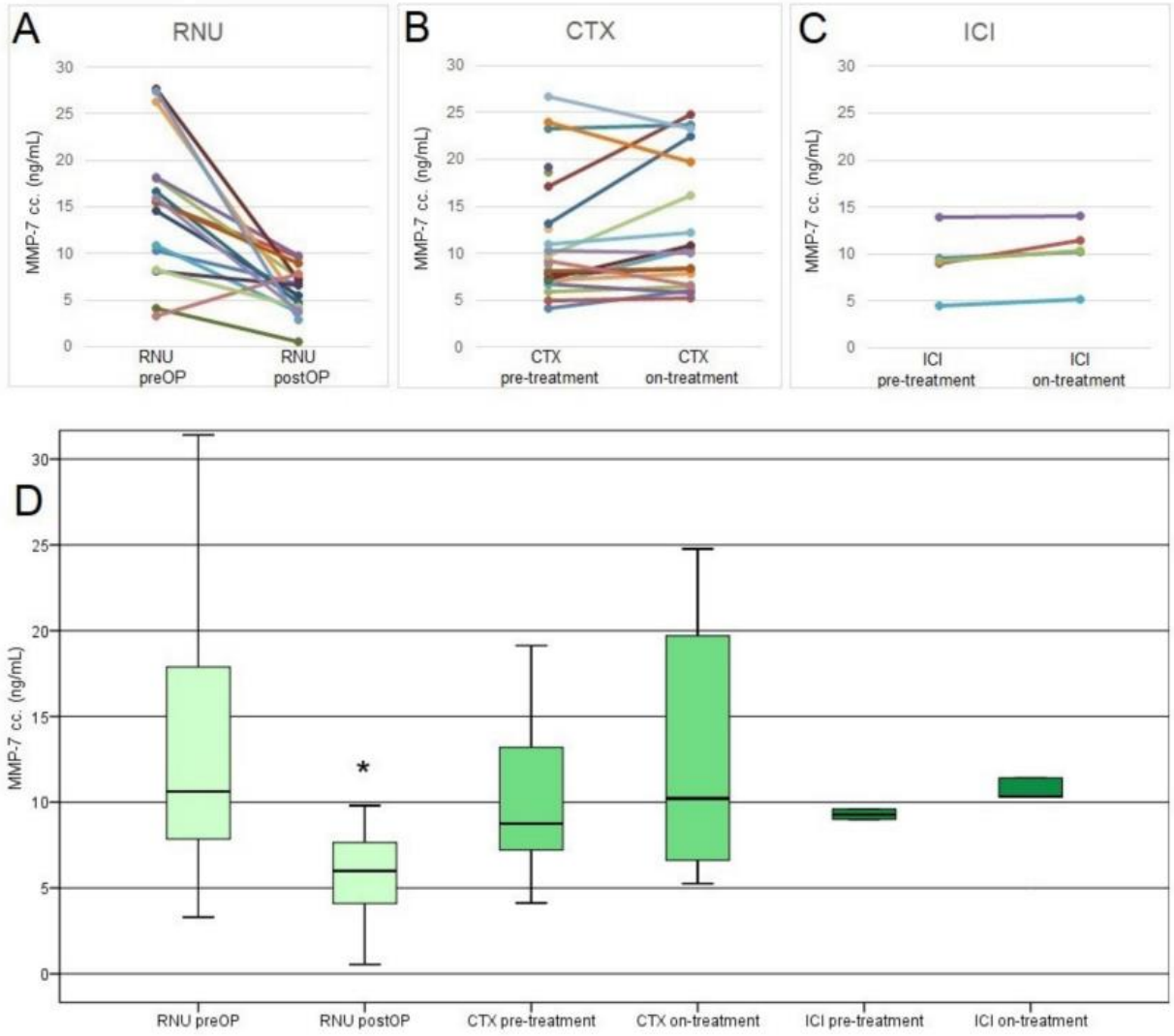

Figure 6. Changes of MMP-7 levels depending on the time of treatment (A) Pre- and postoperative MMP-7 levels in the RNU cohort (B) MMP-7 levels at the start of the chemotherapy and on the first day of second cycle (C) Pre-treatment and on-treatment (at 3 months) values in the ICI cohort (D) Summarized changes in all the cohorts (* - significant difference) (40)

As Figure 6 illustrates, there were no significant differences between baseline and on-treatment MMP-7 concentrations in platinum-treated patients and serum MMP-7 level changes were not correlated with survival.

MMP-7 concentrations slightly elevated in the ICI cohort after 3 months of treatment in all cases, but as mentioned above, valid statistical analysis was not possible because of the low number of cases.

4.2.4 Correlations of pre-treatment clinicopathological parameters as well as MMP-7 levels with OS and PFS

In the RNU cohort, shorter OS was found in high-stage (≥pT2) (HR: 7-115; 95% CI 1.504-33.659; p=0.013) and high-grade (G3) cases (HR: 5.060; 95% CI 1.325-19.323; p=0.018). Similarly, patients present with LN or distant metastases had worse OS (HR: 4.891; 95% CI 1.379-17.345; p=0.014). In accordance, significantly shorter PFS was observed in patients with high-stage (HR: 23.899; 95% CI 3.087-185.035); p=0.002), high-grade tumor (HR: 7.670; 95% CI 2.114-27.833; p=0.002) and in presence of LN or distant metastases (HR: 8.250; 95% CI 2.742-24.820; p < 0.001).

Table 7. Association between pre-treatment MMP-7 levels and clinicopathological parameters with OS and PFS (* - median cut-off for RNU is 10.6 ng/ml, for CTX is 8.8 ng/ml; ** - ROC cut-off for RNU is 14.2 ng/ml, for CTX is 17.9 ng/ml) (40)

		RNU							CTX						
			OS			PFS				OS			PFS		
General data		**n**	**HR**	**95%CI**	**p**	**HR**	**95%CI**	**p**	**n**	**HR**	**95%CI**	**p**	**HR**	**95%CI**	**p**
Age	≤ 65	10	ref.			ref.			5	ref.			ref.		
	> 65	24	2.142	0.459 - 9.994	0.332	1.775	0.494 - 6.378	0.379	20	1.675	0.370 - 7.584	0.503	0.552	0.171 - 1.783	0.321
Sex	male	21	ref.			ref.			21	ref.			ref.		
	female	13	0.281	0.060 - 1.301	0.104	0.330	0.091-1.191	0.090	4	0.374	0.049 - 2.884	0.345	0.337	0.044- 2.579	0.295
ECOG PS	0-1	29	ref.			ref.			21	ref.			ref.		
	2-3	5	3.451	0.707 - 16.832	0.126	2.926	0.807 - 10.608	0.102	4	2.124	0.571 - 7.899	0.261	0.954	0.212 - 4.291	0.951
Nephrouretherectomy data															
Stage	pTa-pT1-CIS	17	ref.			ref.			1	ref.			ref.		
	pT2-pT4	17	7.115	1.504 - 33.659	**0.013**	23.899	3.087 - 185.04	**0.002**	22	1.000	0.0 - 14926.7	1.000	21.482	0.000 - 7.515E+19	0.888
Metastases	N0/M0	25	ref.			ref.			14	ref.			ref.		
	N+ or M+	9	4.891	1.379 - 17.345	**0.014**	8.250	2.742 - 24.820	**<0.001**	9	3.065	0.923 - 10.181	0.068	3.651	1.237 - 10.773	**0.019**
Grade	1-2	19	ref.			ref.			5	ref.			ref.		
	3	15	5.060	1.325 - 19.323	**0.018**	7.670	2.114 - 27.833	**0.002**	16	1.379	0.297 - 6.412	0.682	0.945	0.258 - 3.453	0.931
Chemotherapy baseline data															
Metastases	N0/M0	-	-			-			10	ref.			ref.		
	N+ or M+	-	-			-			14	6.722	1.410 - 32.049	**0.017**	8.985	2.309 - 34.961	**0.002**
CTX regimen	Gem/Cis	-	-			-			14	ref.			ref.		
	Gem/Carb	-	-			-			11	0.907	0.296 - 2.777	0.864	1.893	0.658 - 5.448	0.237
Pretreatment serum MMP-7 level															
serum MMP-7	median cut-off*	17	ref.			ref.			13	ref.			ref.		
serum MMP-7	median cut-off*	17	3.324	0.874 - 12.644	0.078	1.694	0.585 - 4.900	0.331	12	1.270	0.425 - 3.797	0.669	0.799	0.267 - 2.394	0.689
serum MMP-7	ROC cut-off**	19	ref.			ref.			20	ref.			ref.		
serum MMP-7	ROC cut-off**	15	4.413	1.159 - 16.798	**0.029**	1.676	0.585 - 4.799	0.336	5	12.063	2.800- 51.968	**0.001**	2.166	0.452 - 10.380	0.334

Two groups were created using patient's pre-treatment MMP-7 levels. For dichotomization, we used the median value as a cut-off (10.6 ng/ml). In addition, to find the cut-off with the highest sensitivity and specificity for predicting death during follow-up, we also used receiver operating characteristics (ROC) analysis. This method identified 14.2 ng/ml as a cut-off with the highest sensitivity (72.7%) and specificity (69.6%) (Figure 7A and 7B). Table 7 shows that higher preoperative MMP-7

concentrations were significantly associated with shorter OS when using the ROC cut-off (p=0.029).

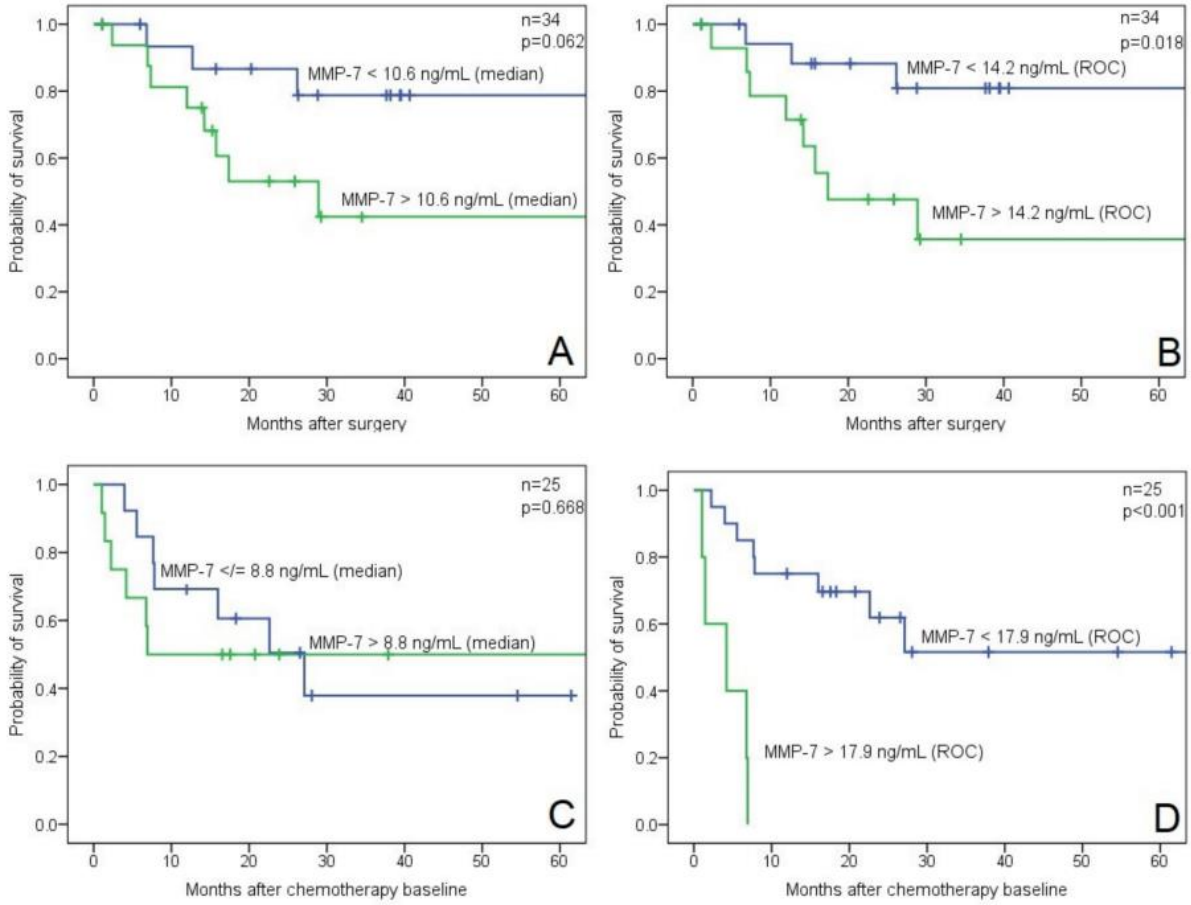

Figure 7. Kaplan-Meier presentation of OS with log-rank test in the RNU and CTX cohorts using different cut-off values (A) RNU cohort and median cut-off (B) RNU cohort and ROC cut-off (C) CTX cohort and median cut-off (D) CTX cohort and ROC cut-off (40)

Table 7 shows that LN or distant metastases at chemotherapy were associated with shorter OS (p=0.017) and PFS (p=0.002) in the CTX cohort. Using the ROC-defined cut-off value (17.9 ng/ml), higher pre-treatment MMP-7 concentrations correlated with poor OS ($p < 0.001$) (Figure7D). However, as this cut-off value was classified only five patients into the high MMP-7 group, this correlation needs to be handled by caution. Multivariate survival analyses were not possible to perform because of the low number of deaths in the cohorts (RNU: 11, CTX: 13, ICI: 1).

4.3 Results of the sPD-L1 study

4.3.1 Clinical background of sPD-L1 study

The patient cohort in the PD-L1 study is largely similar to the previous study with some minor differences that require further clarification and detailed explanation.

Serum samples were collected prior to treatment from a total of 61 UTUC patients (44 males and 17 females) who underwent different treatment approaches (RNU, n=37; CTX, n=25; ICI, n=6) at the Department of Urology, Semmelweis University between August 2014 and July 2020. A total of six patients were included in multiple cohorts within the study. Specifically, three patients were part of both the RNU and CTX cohorts, two patients were included in the CTX and ICI cohorts, and one patient was included in all three treatment groups. The median age at baseline was 69, 72 and 65 years in the RNU, CTX and ICI cohorts, respectively. The patients were followed for 24 months in the RNU, for 18 months in the CTX and for 20 months in the ICI cohort. The number of patients who died during the follow-up was 11, 13 and 2, respectively.

4.3.2 Correlations between pre-treatment sPD-L1 levels and survival

In the RNU cohort, we observed shorter OS in muscle-invasive compared to non-muscle-invasive disease (HR: 7.115; 95% CI 1.504-33.659; p=0.013) as well as in LN or distant metastatic cases (HR: 4.891; 95% CI 1.379-17.345; p=0.014). Similar results were observed for PFS; ≥pT2 stage (HR: 10.836; 95% CI 2.865–40.978; $p < 0.001$); LN or distant metastases (HR: 6.185; 95% CI 2.199–17.397; $p = 0.001$). Higher sPD-L1 concentrations were correlated with worse OS when using the median (84.0 pg/ml; p=0.041) or the ROC-defined cut-off value (118.5 pg/ml; $p < 0.001$) as well (Figure 8A and 8B).

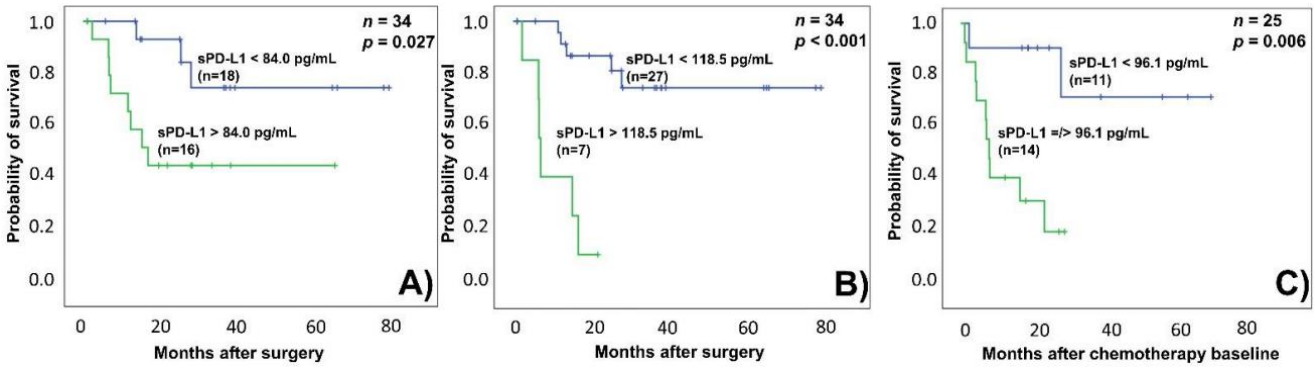

Figure 8. Kaplan-Meier presentation of OS with log-rank tests in the RNU and CTX cohorts using different cut-off values (A) RNU cohort and median cut-off (B) RNU cohort and ROC cut-off (C) CTX cohort and median cut-off (41)

The presence of LN or distant metastases was associated with shorter OS in the CTX cohort (HR: 14.737; 95% CI 1.810–119.987; p = 0.012). In this cohort, the median and the ROC-based cut-off values were close (96.1 pg/ml *vs.* 93.9 pg/ml) and classified the same patients into the high and low sPD-L1 level groups. Figure 8C and Table 8 show that higher sPD-L1 concentrations were associated with worse OS (HR: 6.956; 95% CI 1.461-33.110; p=0.015).

Table 8. Association between pre-treatment sPD-L1 levels and prognosis (* - median cut-off for RNU is 84.0 pg/mL, median cut-off for CTX is 96.1 pg/mL; ** - ROC cut-off for RNU is 118.5 pg/mL, ROC cut-off for CTX is 93.9 pg/mL) (41)

	RNU							CTX						
		OS			PFS				OS			PFS		
	n	HR	95% CI	p	HR	95% CI	p	n	HR	95% CI	p	HR	95% CI	p
Pretreatment sPD-L1 cc.														
median cut-off*	17	ref.			ref.			11	ref.			ref.		
median cut-off*	17	4.023	1.06-15.27	**0.041**	2.793	1.01-7.72	0.048	14	6.956	1.46-33.11	**0.015**	1.584	0.56-4.48	0.386
ROC cut-off**	27	ref.			ref.			11	ref.			ref.		
ROC cut-off**	7	12.114	2.99-49.08	**<0.001**	6.667	2.14-20.76	0.001	14	6.956	1.46-33.11	**0.015**	1.584	0.56-4.48	0.386

4.3.3 Correlation between sPD-L1 and MMP-7 levels

We used the Spearmen's rank correlation analysis to examine the correlation between sPD-L1 and MMP-7 levels in the RNU, CTX, and ICI cohorts. In the 34 patients of the RNU cohort with available both sPD-L1 and MMP-7 levels, we observed a significant association between the two serum proteins (n=34, rs = 0.445, p = 0.008). However, in the ICI (n = 5, rs = 0.900, p = 0.307) and CTX cohorts (n = 25, rs = 0.217, p = 0.297), no significant correlation could be detected.

5. Discussion

In this present study, we aimed to assess clinical parameters and two serum biomarkers for their prognostic values in UTUC patients. Therefore, we conducted a retrospective data analysis of patients who underwent RNU. Our aim was to characterize the patient cohort and examine the commonly available clinical risk factors associated with IVR. Furthermore, we determined the pre-treatment serum levels of two potential biomarkers in UTUC patients who underwent various therapies. For this, we examined the pre-, on- and post-treatment MMP-7 levels as well as pretreatment concentrations of sPD-L1 in UTUC patients who underwent surgical, chemo or immune checkpoint inhibitor therapy. According to our results, patients with lymphatic or distant metastases had significantly higher MMP-7. Moreover, high levels of MMP-7 and sPD-L1 serum levels were correlated with shorter OS in the surgically treated (RNU) and chemotherapy treated (CTX) cohorts.

The rarity of UTUC leads to a lack of high-level evidence in its management, resulting in numerous challenges treating this disease. As the use of URS for the examination of the bladder and the ureters may increase the risk of IVR, the diagnostic procedure of UTUC is complicated. Furthermore, because of the anatomical features of the upper urinary tract, the performance of URS biopsy is difficult, and its accuracy is limited. (42) Smith *et al*. examined 56 patients with UTUC and performed diagnostic URS biopsy followed by further biopsies or resection. According to their results, 43% of the patients' histological results changed from low-grade to high-grade or from non-invasive to invasive disease, representing a large rate of understaging. (43)

Another matter of debate is the clinical benefit of LN dissection at RNU. Roscigno *et al*. analyzed data from 1130 patients to investigate the effect of lymphadenectomy at the time of RNU. They found that pathologically confirmed LN metastasis was an independent predictor of CSS in invasive cases and uncertain LN status was correlated with poor prognosis as well. These suggest that LN dissection may provide benefit for well selected patients. However, because of the low sensitivity of current imaging methods for the detection of LN metastasis, information on LN positivity is only available at the histopathological evaluation of the removed tissue at RNU. Therefore, a preoperatively available marker, which may help to identify the patients who would benefit from a lymphadenectomy, is of great clinical importance (42, 44).

Intravesical chemoinstillation in the postoperative interval after RNU can prevent or decrease the risk of IVR. Tari *et al*. published the first study in 1987 about chemoinstillation as a therapeutic tool to prevent bladder recurrence. Intravesical chemoinstillation was performed in 16 UTUC patients, two IVR were diagnosed in the first two years after RNU (12.5%). In the control group of 26 patients with UTUC, who did not receive chemoinstillation, 11 bladder recurrence was identified after RNU (42%). According to their results, the risk of IVR significantly decreased after intravesical chemoinstillation. (45) According to the results of the ODMIT-C Trial - which was a prospective, randomized, multicenter study - one-time intravesical mitomycin C chemoinstillation significantly reduced the IVR after RNU. (46) The ODMIT-C Trial provided level 1 evidence for the use of chemoinstillation, therefore, in 2013, the EAU UTUC guideline recommended the performance of this therapeutic procedure. (47) Based on the increasing body of evidence on the successful use of single postoperative chemoinstillation, the guideline of the Japanese Urologic Association (JUA) also strongly recommended this therapy from the year of 2014, while the American Urological Association indicated this treatment from 2023. Despite all these recommendations of different UTUC guidelines, the use of postoperative chemoinstillation after RNU to prevent IVR is not well established (9, 47). According to the results of Lu *et al*., only 51% of urologic oncologists perform intravesical chemoinstillation routinely in their practice in the United States. (48) Dobé *et al*. asked the participants of the EAU Section of Oncological Urology (ESOU) via email about the use of postoperative chemoinstillation in 2017, and they found - with a low (12%) response rate - that 47% of the respondents performed chemoinstillation after RNU. (49) Further trials and studies investigated the limitation of use of this therapeutic tool, and the main causes pointed out by the clinicians were lack of evidence, concern about possible complications and limitations of the clinical infrastructure. Overall, the use of single postoperative chemoinstillation after RNU is supported by randomized clinical trials and various guidelines as well, however, the daily utilization remains relatively low. (47)

A further problem in the UTUC management is the identification of patients who would benefit from neoadjuvant systemic chemotherapy. Since 1) there are no validated preoperative markers for the risk-stratification of patients and 2) preoperative staging is challenging, there is a significant risk of both over- and undertreatment. (6)

Furthermore, the uncertainty of preoperative staging may also lead to overtreatment in cases where the disease is noninvasive and an organ-sparing surgery would be enough (6,42).

Based on these, a preoperative prediction of tumor stage, LN status and platinum sensitivity represent a major difficulty along the management of UTUC. Therefore, various pre- and postoperative nomograms were created – to provide a more accurate prediction of oncological outcomes. These nomograms were designed to predict OS, CSS or RFS of UTUC and may help therapeutic decision-making. However, a recent meta-analysis that analyzed 26 postoperative predictive models found that only four nomograms underwent external validation, while the others still need to be confirmed. The analysis identified heterogeneity and the lack of external validation as obstacles to the daily application of these models. (50)

In our institutional cohort, the average age was 68 years at the time of diagnosis, which is identical to the findings of Shariat *et al.,* who found a median patient age of 68 years in a multicentre study conducted in Asia, Canada and Europe. Male dominance is a known phenomenon in UTUC and it was confirmed in our patient cohort as well. (51) Raman *et al.* classified the symptoms of UTUC patients into three groups: incidental, local or systemic. Thirty-three per cent of UTUC cases were asymptomatic and so incidentally identified, 61% of patients had local, while 6% had systemic symptoms. In our cohort, local symptoms were more dominant (75%) with less incidental finding (15%). However, according to Raman *et al.,* there is no significant difference between the oncological outcomes of incidental or local groups, but poor prognosis can be observed in the systemic group (38). After the RNU, pT3 stage was confirmed in 29% of patients and 50% of the tumors were muscle-invasive at the time of diagnosis. Margulis *et al.* found pT3 tumors in 32.5% and muscle-invasive stage in 56% of cases and the EAU guideline mentions that two-third of the cases are muscle-invasive. Our results - with 49.6% muscle-invasive tumor - reflect characteristics that are slightly more favorable. Overall, it is apparent that - in contrast to BC, where 75-85% of tumors are superficial at the time of the diagnosis - the majority of UTUC cases are muscle-invasive at diagnosis. (9, 52)

IVR was observed at 31 (23%) patients. The average time between RNU and recurrence was 19.6 months. The literature reports that 15-50% of patients may

experience bladder recurrence, mostly within two years after UTUC treatment. Our findings are in accordance with these rates. (53) Previously diagnosed BC, ureteral localization of the primary tumor, margin positivity, necrosis of the tumor and vascular invasion is often associated with IVR, however, neither of these factors were significantly correlated to bladder recurrence in our patient cohort. (53-55)

We analyzed the association of various factors with time to disease recurrence and found significantly shorter RFS in patients who were diagnosed with hypertension. Stocks *et al.* analyzed the data of 577,799 individuals who were followed for 12 years. According to their results, higher blood pressure was significantly correlated with higher occurrence of cancer in men, and with higher cancer-related mortality in both genders. While UTUC was not examined in this study, they found a positive correlation in men between higher blood pressure and the incidence of bladder cancer. (56) One study analyzed the risk of urothelial cancer-related death depending on the type of the prescribed antihypertensive drugs. They found a lower risk of cancer-related death in BC patients who were angiotensin-receptor or calcium-channel blocker users. However, no such a risk decrease was observed in their UTUC cohort. (57) In our study, bladder recurrence occurred sooner when one or two comorbidities were present, but this correlation missed the significance level. Metabolic syndrome includes large waist circumference, high blood pressure, higher fasting glucose level and dyslipidemia. According to a study, metabolic syndrome was a prognostic factor for shorter CSS in UTUC patients. (58) In another study, poorly controlled diabetes mellitus was found to be associated with shorter RFS in UTUC patients treated with RNU and it was identified as an independent prognostic factor for IVR besides male gender, ureteral tumor localization and end-stage renal insufficiency. (59) IVR was not correlated with advanced age in a systematic review and meta-analysis, but according to our results, IVR occurred earlier in the elderly patients. (55) However, Ferro *et al.* found that patients aged 70 or above had worse prognosis after RNU than the younger individuals, and age proved to be an independent prognostic factor for OS and CSS. (60) In line with these results, in our cohort advanced age was an independent prognostic factor for OS. Moreover, pulmonary disease, BC in medical history, high-grade tumor and tumor necrosis were also significantly and independently associated with poor OS in our cohort. BC in medical history, high-grade tumor and tumor necrosis were already discussed as possible prognostic factors for IVR, but not pulmonary disease. (9)

Huang *et al.* analyzing 80 BC patients found worse OS and PFS in individuals with COPD. In addition, COPD proved to be an independent prognostic factor for OS. (61)

According to our findings, UTUC patients with elderly age and comorbidities need closer follow-up and regular cystoscopy control examination because they are at higher risk of IVR and shorter OS.

As previously mentioned, there is no validated prognostic biomarker for UTUC in the daily clinical practice currently. (9) The value of serum MMP-7 and sPD-L1 as a biomarker in urothelial bladder cancer has been confirmed by various independent studies, but it has not been evaluated in UTUC yet. (27-31, 35-37) In this study, we determined the preoperative serum levels of MMP-7 and observed significantly increased levels in metastatic UTUC cases. The MMP-7 concentration of 15.9 ng/ml in metastatic UTUC cases was similar to those of 13.9 ng/ml that was formerly found in metastatic BC. (27) In our study, high preoperative serum MMP-7 concentration was significantly associated with shorter OS in UTUC. After RNU, histological evaluation resulted in pT0 finding in three patients, all these patients had low preoperative serum MMP-7 levels (mean concentration: 5.9 ng/ml). These results show that serum MMP-7 concentration may be implemented into preoperative prognostic models in order to identify patients who would benefit from an organ-sparing treatment strategy.

The source of sPD-L1 in the blood circulation is not well established, but it has been shown, that some members of the MMP family – including MMP-7 – is able to cleave membrane-bound PD-L1 thus releasing soluble PD-L1 into the circulation. (36) However, current studies found no significant association between the tissue PD-L1 expression and sPD-L1 level (37, 62). Due to the absence of link between tissue PD-L1 expression and sPD-L1 concentrations, along with the correlation between serum MMP-7 and sPD-L1, our hypothesis suggests that higher sPD-L1 levels might result from a proteolytically active microenvironment. Similar to MMP-7 results, elevated sPD-L1 was associated with poor survival in the RNU and CTX cohorts.

Similar to former findings in BC, we found a significant decrease in MMP-7 concentrations after RNU (preoperative: 10.6 *vs.* postoperative: 6.0 ng/ml) suggesting the tumor tissue as the main origin of serum MMP-7. (27)

We found a significant association between higher pre-treatment MMP-7 levels and worse OS in our platinum-treated CTX cohort, which is similar to former findings

made in BC. (63) Ansell *et al.* identified MMP-7 and MMP-13 as predictive biomarkers for platinum-resistance in head and neck cancer cell lines. (64) Another study suggested that high tissue MMP-7 expression is correlated with poor response to cisplatin-based chemotherapy and poor oncological outcome in non-small cell lung cancer. (65) According to these results, MMP-7 might have a role in chemotherapy resistance also in urothelial cancer.

Overall, literature data and our present results suggest MMP-7 as a potential therapeutic target. MMP-7 has a broad substrate specificity, and it was shown to participate both in physiological and pathological processes. MMP-7 can cleave Fas receptor and its ligand, Fas ligand as well, resulting decreased Fas expression and function. Decreased Fas activation helps tumor cells to avoid Fas-induced apoptosis, therefore supporting tumor growth. (66) MMP-7 by cleaving the extracellular part of E-cadherin was shown to be involved in the epithelial-to-mesenchymal transition, which represents an important step in tumor progression of epithelial tumors. (67) Moreover, degradation of various molecules can release agents with biologically active role in tumor growth, like the anti-angiogenetic endostatin, which is the degradation product of collagen XVIII. (68) MMP-7 can also activate many other MMPs resulting in an enhanced proteolytic cascade. (19) We already discussed the relationship between PD-L1 and MMP-7, which may represent another mechanism of tumor progression. (36) Bolenz *et al.* investigated the impact of broad-spectrum MMP and selective MMP-7 inhibitors in BC cells. Their results showed that selective inhibition of MMP-7 significantly correlated with lower invasive capability, suggesting MMP-7 as potential therapeutic target. (69) Moreover, Lynch *et al.* observed a decreased level of RANKL and osteolysis in MMP-7 deficient rodent with prostate tumor. (70) In another study, reduced MMP-7 expression in UTUC cells showed a significant association with decreased invasive capability. (71) According to these results, MMP-7 may play a major role in tumor growth and metastatic progression in various types of cancer including UTUC. Despite the promising results of *in vitro* studies with broad-spectrum MMP inhibitors (batimastat and marimastat), these agents failed in prospective clinical studies. (72, 73) The use of MMP inhibitors, specific for single MMPs, may provide more specific effect and possibly also better antitumor effects. As MMP-7 is a validated target in various cancer types, a selective MMP-7 inhibitor may have better therapeutic effect compared to broad-spectrum MMP inhibitors.

However, developing of specific MMP-7 inhibitors is still a challenge and not solved yet. (73) Besides the problem of selective inhibition, proper pharmacokinetics of the developed drugs showed the next challenge, however, a recently published study by Oka *et al*. reported about a highly selective inhibitor of MMP-7 with optimal clearance in *in vitro* and *in vivo* experiment, but further trials are needed until clinical usage. (74)

According to our findings, both investigated biomarkers seem to have promising prognostic value, which results need to be tested in larger prospective studies.

6. Conclusions

In the present work, we aimed to identify factors, which can help the prognostication of UTUC patients.

According to our results, IVR occurs earlier in patients with hypertension. We identified patient age, pulmonary disease, BC in medical history, high-grade tumor and tumor necrosis as independent prognostic factors for shorter OS after RNU. Therefore, elderly patients with comorbidities need a stricter follow-up with regular cystoscopy examinations.

Pretreatment MMP-7 concentrations were significantly associated with certain clinicopathological parameters (LN or distant metastases). Higher MMP-7 and sPD-L1 levels were associated with significantly shorter OS in RNU and CTX cohorts as well.

Serum MMP-7 levels significantly dropped after RNU. We also revealed a significant correlation between MMP-7 and sPD-L1 levels in the RNU cohort.

According to our results, higher MMP-7 or sPD-L1 levels predict an advanced pathological stage and shorter OS in UTUC patients. These findings could help the daily therapeutic decision-making and reduce the risk of both under- and overtreatment.

7. Summary

The management of UTUC patients can be challenging due to the obstacles of diagnosis and preoperative staging, which can lead to the risk of both under- and overtreatment. There are no validated preoperative serum biomarkers available to aid in daily clinical decision-making.

In the present work, our aim was to determine the prognostic value of two serum biomarkers (MMP-7 and sPD-L1) and certain clinicopathological parameters via retrospective clinical data evaluation and prospective serum level analysis (pre-, on- and post-treatment) in UTUC patients.

In our clinical data analysis with 12 years follow-up, we found significantly shorter OS in elderly patients, in case of pulmonary disease as comorbidity, and in individuals with BC in their medical history. High-grade tumors, surgical margin positivity, tumor necrosis and vascular invasion were also significantly associated with worse OS. From these factors, advanced age, pulmonary disease, previous BC in medical history, high-grade tumor and necrosis were identified as independent prognostic factors of OS. In this cohort, bladder recurrence occurred in 23% of patients after 20 months, on average. The time until IVR was significantly shorter in patients with hypertension, while no other clinicopathological parameters showed a significant correlation with IVR.

In our serum biomarker studies, we found that individuals with LN or distant metastases had significantly elevated MMP-7 concentrations in the RNU and CTX cohorts. We found significantly shorter OS and PFS in case of high-stage and high-grade tumors and in patients present with LN or distant metastases in the RNU cohorts. Higher preoperative MMP-7 and sPD-L1 levels were also correlated with significantly shorter OS in the RNU cohort. In the CTX cohort, LN or distant metastases and higher MMP-7 and sPD-L1 concentrations showed a significant association with worse OS. MMP-7 levels decreased significantly after RNU, while changes of MMP-7 concentrations during therapy were not significant in the CTX or ICI cohorts. We also found a significant correlation between the concentrations of the two serum biomarkers in the RNU cohort, but not in the CTX and ICI cohorts.

According to our results, elderly and multimorbid patients need a closer follow-up regiment after RNU. Furthermore, both serum biomarkers showed significant correlations with prognosis and have a promising prognostic value.

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9. Bibliography of the candidate's publications

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1. Kovács PT, Juhász D, Módos O, Kocsmár I, Terebessy A, Lotz G, Szarvas T, Nyirády P, Riesz P. A húgyhólyag-recidíva jellemzői felső üregrendszeri daganatos betegekben radikális ureteronephrectomia után [Characteristics of bladder recurrence after radical nephroureterectomy in upper urinary tract cancer]. Orv Hetil. 2020 May;161(21):881-888.
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