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PROGNOSTIC BIOMARKERS AND MULTIMODAL TREATMENT OUTCOMES IN UROTHELIAL AND SMALL-CELL BLADDER CANCER

Ph.D. Thesis

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Budapest

2026

“You miss 100% of the shots you never take.”

attributed to Wayne Gretzky

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1 LIST OF ABBREVATIONS

CBMMT – Cystectomy-Based Multimodal Therapy

CI – Confidence Interval

CIS – Carcinoma in Situ

CT – Computed Tomography

CTX – Chemotherapy

CSS – Cancer-Specific Survival

ctDNA – Circulating Tumor DNA

DFS – Disease-Free Survival

DSS – Disease-Specific Survival

ECM – Extracellular Matrix

ELISA – Enzyme-Linked Immunosorbent Assay

ES – Extensive Stage

EAU – European Association of Urology

FGFR3 – Fibroblast Growth Factor Receptor 3

FDG-PET/CT – Fluorodeoxyglucose Positron Emission Tomography / Computed Tomography

HR – Hazard Ratio

I^2 – Higgins' Heterogeneity Statistic

IPD – Individual Patient Data

LND – Lymph Node Dissection

LN – Lymph Node

LS – Limited Stage

LS-SCBC – Limited-Stage Small-Cell Bladder Cancer

MIBC – Muscle-Invasive Bladder Cancer

MMP – Matrix Metalloproteinase

MMP-7 – Matrix Metalloproteinase-7

MRI – Magnetic Resonance Imaging

NAC – Neoadjuvant Chemotherapy

NCDB – National Cancer Database

NE – Neuroendocrine

NEUROD1 – Neurogenic Differentiation Factor 1

NMIBC – Non-Muscle-Invasive Bladder Cancer

OS – Overall Survival

PECO – Population, Exposure, Comparator, Outcome

PICO – Population, Intervention, Comparator, Outcome

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses

QoL – Quality of Life

RBMMT – Radiation-Based Multimodal Therapy

RC – Radical Cystectomy

RFS – Recurrence-Free Survival

ROC – Receiver Operating Characteristic

SCBC – Small-Cell Bladder Cancer

SE – Standard Error

SEER – Surveillance, Epidemiology, and End Results

SPSS – Statistical Package for the Social Sciences

TCGA – The Cancer Genome Atlas

TMT – Trimodality Therapy

TNM – Tumor, Node, Metastasis Classification

TURB – Transurethral Resection of the Bladder

TURBT – Transurethral Resection of Bladder Tumor

UC – Urothelial Carcinoma

UBC – Urinary Bladder Cancer

UTUC – Upper Tract Urothelial Carcinoma

VEGF – Vascular Endothelial Growth Factor

VI-RADS – Vesical Imaging-Reporting and Data System

WHO – World Health Organization

2 STUDENT PROFILE

2.1 Vision and mission statement, specific goals

My vision is to improve risk stratification and treatment selection in bladder cancer through biomarker-driven, evidence-based approaches. My mission is to evaluate the prognostic value of circulating biomarkers and to compare multimodal treatment strategies to support personalized management of bladder cancer.

2.2 Scientometrics

Number of all publications:	12
Cumulative IF:	19.563
Av IF/publication:	1.63
Ranking (Sci Mago):	D1:2, Q1:5, Q4:1
Number of publications related to the subject of the thesis:	2
Cumulative IF:	7.6
Av IF/publication:	3.8
Ranking (Sci Mago):	D1:1, Q1:1
Number of citations on Google Scholar:	93
Number of citations on MTMT (independent):	22
H-index:	4

The detailed bibliography of the student can be found on pages 56-57.

2.3 Future plans

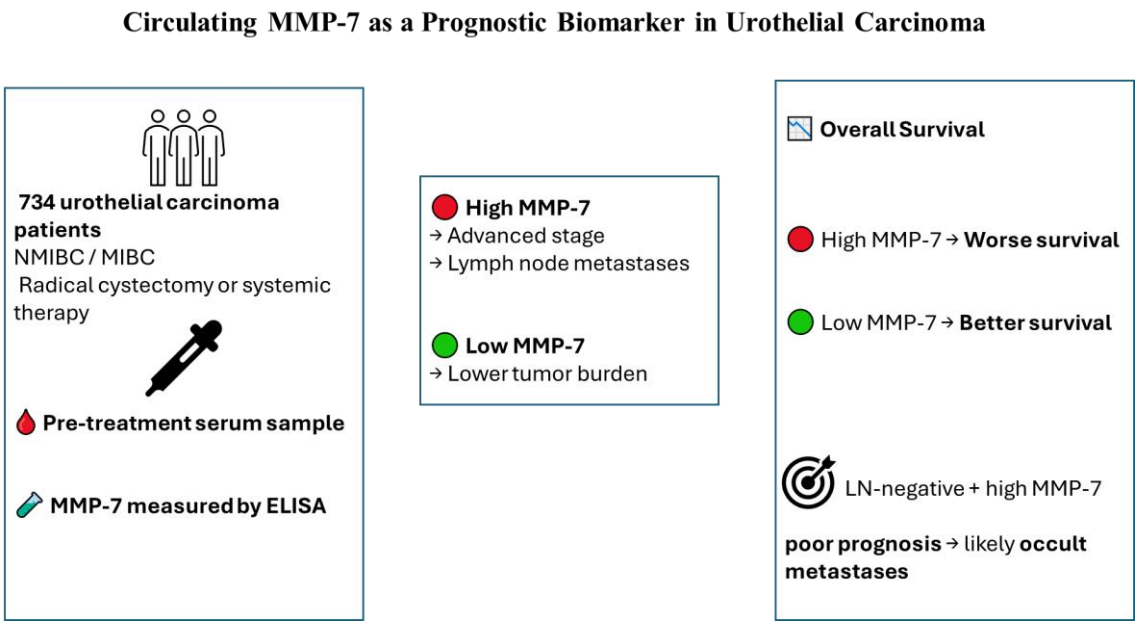
My future work will focus on urologic surgical oncology with an emphasis on uro-oncology, aiming to integrate biomarker research into clinical decision-making. Building on the MMP-7 and SCBC studies, I plan to advance translational research to improve risk stratification, treatment selection, and quality-of-life outcomes in patients with high-risk bladder cancer. By combining clinical practice, biomarker-driven research, and evidence synthesis, I aim to contribute to the development of personalized management strategies in bladder cancer.

3 SUMMARY OF THE THESIS

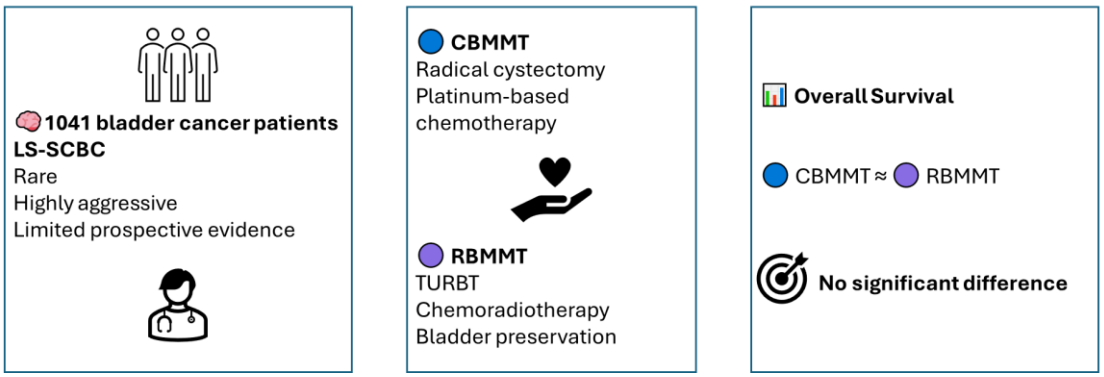
Bladder cancer is a biologically heterogeneous disease with variable clinical outcomes, and optimal risk stratification and treatment selection remain challenging in high-risk subgroups. This thesis investigates prognostic biomarkers and multimodal treatment strategies to support evidence-based and personalized management of bladder cancer. The first part of the thesis evaluates the prognostic value of circulating matrix metalloproteinase-7 (MMP-7) in urothelial carcinoma. Using two independent patient cohorts and a comprehensive systematic review and meta-analysis, elevated pretreatment serum MMP-7 levels were consistently associated with lymph node metastasis and significantly reduced overall survival. Notably, a subgroup of lymph node–negative patients with high MMP-7 levels showed survival outcomes comparable to node-positive cases, suggesting the presence of undetected metastatic disease. These findings support the role of circulating MMP-7 as a robust prognostic biomarker that may complement conventional staging and support individualized treatment strategies. The second part of the thesis focuses on limited-stage small-cell bladder cancer (LS-SCBC). A systematic review and meta-analysis comparing cystectomy-based and bladder-preserving multimodal treatment approaches demonstrated no significant difference in overall survival. These results support a bladder-preserving therapy approach as a viable alternative to radical surgery with similar oncological outcomes in selected patients. Overall, this thesis highlights the importance of integrating biomarker research and evidence synthesis into clinical decision-making to improve personalized care for bladder cancer.

4 GRAPHICAL ABSTRACT

4.1 Graphical abstract- Study I.



4.2 Graphical abstract – Study II.



5 INTRODUCTION

5.1 Overview of the topic

5.1.1 What is the topic?

Our research focuses on prognostic stratification and therapeutic decision-making in bladder carcinoma, with particular emphasis on two clinically relevant areas: the role of the circulating serum matrix metalloproteinase-7 (MMP-7) in urothelial carcinoma, and the comparative analysis of outcomes between radical surgery-based and bladder-preserving treatment approaches in limited-stage small-cell bladder cancer (SCBC).

5.1.2 What is the problem to solve?

Preoperative staging frequently fails to identify aggressive disease or occult metastases, limiting accurate prognostication. Reliable biomarkers that reflect underlying tumor biology are lacking. In parallel, optimal management of SCBC remains uncertain due to the scarcity of high-quality comparative data. These gaps affect treatment planning and clinical decision-making.

5.1.3 What is the importance of the topic?

Improved prognostic tools may refine risk assessment and support personalized treatment pathways in urothelial carcinoma. Establishing the prognostic relevance of MMP-7 and clarifying multimodal treatment outcomes in SCBC offer clinically meaningful information for the prognostication and management of bladder cancer.

5.1.4 What would be the impact of our research results?

Identifying markers of aggressive tumor biology can aid in selecting high-risk patients who may benefit from a more aggressive oncological therapy. Providing a systematic comparison of SCBC treatment modalities may support more evidence-based therapeutic choices in a rare entity where prospective trials are not feasible.

5.2 Bladder cancer: diagnosis, treatment and disease progression

Urinary bladder cancer (UBC) is the second most common malignancy of the urinary tract, with an annual worldwide incidence of 614,000 cases and over 220,000 deaths (1), and 2,400 new cases and 1,200 deaths in Hungary (2). Approximately 10-15% of UBC patients are diagnosed with muscle-invasive bladder cancer, and nearly 10% of newly diagnosed patients are diagnosed with locally advanced and/or metastatic disease. For these patients, perioperative chemotherapy (CTX), radical cystectomy (RC) with pelvic lymph node dissection (LND), and urinary diversion are the gold standard treatments (3). Half of these patients benefit from radical surgery, while the other half of the patients progress rapidly and die within two years (4). For these patients, lymph node (LN) positivity is the most significant risk factor (5), occurring in approximately 20-25% of RC-treated UBC patients (6, 7). However, LN positivity is challenging to detect preoperatively, as the low sensitivity of imaging methods limits the detection of LN metastases, especially small metastases (8). LN metastases have proved to be the most reliable prognostic factor for survival in patients undergoing RC (9). The optimal extent of LND has been investigated in extensive prospective studies with conflicting results. Some of these studies showed that extended LND (also including the deep obturator, common iliac, presacral, paracaval, interaortocaval, and para-aortal nodes to the inferior mesenteric artery) may provide improved recurrence-free (RFS) and disease-specific survival (DSS) compared to limited LND (including only the obturator and internal and external iliac nodes) (10, 11). In contrast, more recent studies have not found an association between LND extent and patient outcome (12, 13). This suggests that the performing extended LND may improve prognosis only in a subgroup of RC-treated patients (14, 15).

5.3 Matrix metalloproteinase-7 (MMP-7) in urothelial carcinoma

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases involved in the degradation of extracellular matrix (ECM) and a variety of physiological and pathological processes. They comprise more than 25 enzymes categorized by structure and substrate specificity. Most MMPs contain a pro-peptide region, a zinc-binding catalytic domain, and a hemopexin-like C-terminal domain; the latter is absent in MMP-7. MMPs are

secreted as inactive proenzymes and become activated through proteolytic cleavage (16). Their activity contributes to ECM degradation, tissue remodeling, angiogenesis, and wound healing (17). Dysregulated MMP expression is associated with inflammatory (18), pulmonary (19), musculoskeletal (20), cardiovascular (21), and malignant diseases (22-25). In cancer, MMPs participate not only in invasion and metastasis but also in early carcinogenic events by cleaving of multiple cell-surface and ECM-associated proteins. MMP-7 is the smallest member of the family and belongs to the matrilysin subgroup. Unlike most MMPs, which are primarily produced by stromal cells, MMP-7 is also expressed by tumor cells. It can cleave proteins such as E-cadherin and the Fas receptor, thereby disrupting cell–cell adhesion and inhibiting apoptosis (26). Therefore, MMP-7 is involved in several tumor-supporting cellular processes, such as apoptosis, angiogenesis, and tumor-related osteolysis (27). In UBC, elevated tissue, serum, and urine MMP-7 levels were associated with the presence of LN metastases and an unfavourable prognosis (28).

5.4 Small-cell bladder cancer (SCBC): clinical features and treatment modalities

Urothelial carcinoma histology accounts for approximately 90% of all UBCs. In contrast, small-cell bladder cancer (SCBC), classified as pure non-urothelial carcinoma by the WHO in 2016, represents a rare and aggressive neoplasm, accounting for less than 1% of all UBCs (29). The etiology of SCBC is largely unknown; however, risk factors for UBC, such as male sex, advanced age, smoking, and occupational exposure to certain chemicals, are thought to play a role. SCBC is characterized by small, round cells with scant cytoplasm and highly invasive and metastatic potential (30). Diagnosis is most frequently established by histopathologic evaluation of transurethral tumor tissue, which must be confirmed by additional immunohistochemical staining for neuroendocrine markers such as chromogranin A, synaptophysin, and CD56 (31). Primary neuroendocrine tumors often arise from urothelial carcinoma precursors and show molecular overlap, with emerging subtypes and treatment strategies continuing to evolve (32). Patients with SCBC are frequently diagnosed with advanced diseases, and their prognosis is influenced mainly by tumor stage. SCBC is characterized by a clinical presentation closely resembling small-cell lung cancer, exhibiting poor prognosis and frequent metastasis to atypical sites for UBC, such as the bone and brain (33). The staging

categorization comprises limited stage (LS), which indicates tumor involvement of the bladder and/or pelvic lymph nodes (TxNxM0), and extensive stage (ES), with metastatic involvement of nonlocal LNs and distant anatomical sites (TxNxM1) (34). Our research focused on subgroups of patients with TxN0M0 disease to determine the optimal treatment based on median overall survival (OS). Management of LS-SCBC is particularly challenging because of its aggressive clinical behaviour, even in the early stages. Furthermore, owing to the rare occurrence of SCBC, no prospective clinical trials have been performed to identify the most effective treatment, and retrospective datasets are limited. According to current guidelines, the following potentially curative treatment options are recommended for LS-SCBC: (1) cystectomy-based multimodal therapy (CBMMT) with platinum-based perioperative chemotherapy and (2) bladder-preserving transurethral resection of the bladder tumor (TURBT) with radiation-based multimodal chemotherapy (RBMMT) (35, 36). According to the Surveillance, Epidemiology, and End Results (SEER) and National Cancer Database (NCDB), approximately 40% of patients with SCBC are diagnosed at an advanced stage, and the rate of CBMMT is only 16-31% (33, 34). Although better tolerated, RBMMT is used in only approximately 25% of patients (34). Emerging evidence suggests that molecular subtyping, including neuroendocrine differentiation markers (e.g. ASCL1, NEUROD1) and immune infiltration patterns, may guide treatment selection in SCBC. Patients with ‘luminal-like’ or inflamed immunophenotypes may respond more favourably to bladder-preserving approaches (37-39).

6 OBJECTIVES

6.1 Study I. – A comprehensive analysis of the prognostic value of circulating MMP-7 levels in urothelial carcinoma: a combined cohort analysis, systematic review and meta-analysis

Our research aimed to evaluate further the prognostic relevance of preoperative serum MMP-7 levels in two independent UC cohorts. Furthermore, we also assessed two additional new aspects of serum MMP-7: (1) its potential value for the decision-making regarding limited versus extended LND, and (2) its utility for therapeutic monitoring aimed at the early detection of disease progression. Finally, we performed a systematic review and meta-analysis to provide a comprehensive overview of the clinical value of circulating MMP-7 levels in UC.

6.2 Study II. – Radical Surgery Compared to Bladder-Preserving Approaches for Limited Stage Small-Cell Bladder Cancer: Systematic Review and Meta-Analysis

In this study, we conducted a systematic review and meta-analysis of retrospective studies to evaluate the efficacy of radical surgical intervention (CBMMT) compared with bladder-preserving RBMMT, with a particular focus on chemotherapy, in patients diagnosed with LS-SCBC.

7 METHODS

We performed a post hoc serum MMP-7 analysis in two independent patient cohorts for the first study, totaling 256 UBC patients. We performed two independent systematic reviews and meta-analyses. The first examined the prognostic significance of pretreatment circulating MMP-7 levels in patients with UC. The second compared oncological outcomes of radical surgical management (CBMMT) with bladder-preserving multimodal treatment (RBMMT) in SCBC patients. Both studies were reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) 2020 recommendations (40), and the Cochrane Handbook was followed (41). The protocols were registered on PROSPERO (Study I: CRD42022367152, Study II: CRD42023426822).

7.1 Literature search

The electronic databases PubMed, Scopus, EMBASE were screened for Study II. and we also included Cochrane Library and Web of Science for Study I. The dates of literature search and the search keys are included in the original articles. Two independent authors performed the systematic selection process. A third author resolved disagreements. References were screened using Endnote X9 (Clarivate Analytics, Philadelphia, PA, USA) and assessed by title, abstract, and full text.

7.2 Eligibility criteria, study selection and data extraction

7.2.1 Patient cohorts, eligibility criteria and data collection for Study I.

7.2.2 Serum MMP-7 ELISA analysis for Study I.

We used the enzyme-linked immunosorbent assay (ELISA) to determine serum MMP-7 levels using the Human Total MMP-7 Quantikine ELISA kit (R&D Systems, Wiesbaden, Germany, Catalog Number: DMP700), according to the product instructions. Colorimetric detection was performed by a Thermo Scientific™ Multiscan FC Microplate Photometer. The results were analyzed with the help of Skanlt 5.0 Software. For the data set achieved by ELISA, the predefined cut-off of 7.15 ng/ml serum MMP-7 level reported in a previously published study was used (28). The corresponding OS data are shown in Table 2 and Figure 2.

7.2.3 Serum MMP-7 cohorts for ELISA analysis of Study I.

For cohort 1, pretreatment serum samples were available for 188 UBC patients (137 males and 51 females) who underwent RC (n=87) or TURBT (n=101) at the Department of Urology, University Hospital of Essen, Germany, between August 2008 and November 2013. Cohort 2 included 68 UBC patients (43 males and 25 females) with pretreatment serum samples who underwent RC at the Department of Urology, Semmelweis University, Budapest, Hungary, between January 2014 and December 2018. In addition, for cohort 2, follow-up samples collected on postoperative days 2 and 5 were available for 57 and 46 patients, respectively. Additionally, for this cohort, LNs removed during RC were separately collected and evaluated, and LND was performed according to the anatomic template defined by Gschwend *et al.* (12). LNs were separated by location, thereby determining the extent of LN removal. Accordingly, LNs belonging to the limited versus extended LND were evaluated separately. Results of cohort 1 with cohort 2, which used preoperative UBC serum samples and the same MMP-7 assay, were included in Tables 1-3. The study was conducted in accordance with the ethical standards of the Declaration of Helsinki, and the study protocol was approved by the ethics committees of the University of Duisburg-Essen (15-6400-BO) and Semmelweis University (TUKEB 256/2014). The primary endpoint of this study was OS. Time to event (overall survival) was defined as the interval from surgery (RC or TURBT) to death or last follow-up (censored).

7.2.4 Eligibility criteria and data extraction for the systematic review and meta-analysis of the prognostic value of serum MMP-7

The PECO framework was applied to formulate our research question. We included original studies that investigated (P) UC patients treated with surgery or systemic therapy, and (E and C) compared the hazards of pretreatment high and low serum or plasma MMP-7 levels in terms of (O) OS. To define high and low levels of MMP-7, we used the included cut-off values for pretreatment serum and plasma levels as reported in each eligible article. We excluded studies with tissue-only analyses (without serum/plasma MMP-7 analysis), those reporting only postoperative MMP-7 levels, reviews, meta-analyses, conference abstracts, case reports, and case series. No restrictions were made based on cohort size and study design. Two independent authors extracted data from the selected articles. Data of interest included name of the first author, year of publication,

cancer type, stage, therapy received, country of sample/data collection, study type, cohort size, age and sex of patients, cut-off values for MMP-7 levels, method of determining cut-off values (e.g., median), assay method, follow-up time, OS and cancer-specific survival (CSS). In the muscle-invasive UBC cohorts, the primary outcome was OS, except for one study (42), and was assessed as OS in the meta-analysis, given the high mortality of the disease (Figure 6 A-C).

7.2.5 Patient cohort for Study II.

The PICO framework was applied to formulate the research question. The included studies investigated (P-population) patients presenting with a histology of SCBC with LS and non-metastatic (M0) disease, who were treated with either RC-based multimodal therapy (CBMMT) (I-interventions) or bladder-preserving TURBT and radiation-based multimodal chemotherapy (RBMMT). The included studies provided survival data on subgroups of both radical surgery (RBMMT) and bladder-preserving (CBMMT) approaches to facilitate comparison (C-comparisons) and to assess their associations with OS (O-outcome). Studies that examined extensive stage (ES) and metastatic SCBC patients, single-modality or solely palliative therapy, or non-SCBC patients were excluded. Reviews, meta-analyses, and conference abstracts were also excluded. No restrictions were imposed based on cohort size and study design. Two independent authors extracted the data from the articles. The data of interest included the name of the first author, year of publication, number of patients with limited and extensive stage SCBC, number of patients receiving RBMMT or CBMMT, country of sample origin/data collection, study type, cohort size, age and sex of patients, follow-up time, and median OS. The primary outcome was median OS, extracted from the manuscript text or relevant Kaplan-Meier curves using Webplotdigitizer (43) and the methodology of Guyot *et al.* (44), followed by individual patient-level reconstruction based solely on time-to-event information. These pseudo-individual patient-level data (IPD) were then used to estimate pooled hazard ratios (HRs); no original IPD was available (Figure 9).

7.3 Quality assessment and serum MMP-7 ELISA analysis

7.3.1 Quality assessment for Study I. and Study II.

The risk of bias was assessed using the Quality in Prognostic Studies (QUIPS) tool (45) by two independent authors. The RobVisR tool was used to summarize the assessment results (46).

7.4 Data synthesis and analysis

7.4.1 Synthesis methods for Study I.

Random-effects models with the inverse-variance method were applied to pool log-transformed HRs with 95% confidence intervals (CIs). The restricted maximum-likelihood method (47) was used to estimate the variance component τ^2, and between-study heterogeneity was assessed using the Cochrane Q test and Higgins & Thompson's I^2 statistic (48). Forest plots were used to summarize the results graphically. Where applicable, we reported the prediction intervals of results according to IntHout *et al.* (49). Outlier and influence analyses were carried out following the recommendations of Harrer (47) and Viechtbauer and Cheung (50). Small-study effects were visually assessed in funnel plots. All statistical analyses were performed in the R (51) statistical environment and language, using the *meta* (52) and *dmetar* (47) packages.

7.4.2 Statistical analysis for serum ELISA analysis (Study I.)

In the study cohorts, both the Kaplan–Meier log-rank test and univariate Cox analysis were used to analyze OS. The nonparametric two-sided Wilcoxon rank-sum test (Mann–Whitney test) was used to compare the paired groups. In study cohort 2, nonparametric receiver operating characteristic (ROC) curves were used to determine the optimal cut-off value with the highest sensitivity and specificity for predicting patient death. In all tests, a p-value of at least 0.05 was considered significant. All statistical analyses were performed using the IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA).

7.4.3 Statistical analysis for Study II.

For time-to-event data, HR was used as the effect size measure, with 95% CI. To calculate the pooled HR, the logarithm of HR and its standard error (SE) were calculated from the extracted data following the methodology of Tierney *et al*. (53). IPD (reconstructed

pseudo-IPD) were generated from the digitized Kaplan–Meier curves and used in Cox proportional hazards models with a shared frailty term set to the Study ID. These reconstructed data reflect survival time and censoring information and do not include clinical covariates. To assess model robustness, sensitivity analysis was conducted by pooling individual HRs estimated from study-specific models based on these pseudo-IPD. To estimate the pooled median survival time, Kaplan–Meier models were separately fitted to each treatment group within each study, and the resulting medians were aggregated using a univariate random-effects meta-analysis. Given the rarity of LS-SCBC, a formal power calculation was not feasible. We included all known comparative studies to maximize available data.

8 RESULTS

8.1 Search and selection, characteristics of the included studies

8.1.1 Study I. – Investigating the prognostic role of serum MMP-7

In two independent cohorts, a total number of 256 UBC patients underwent post-hoc serum MMP-7 analysis. Patients' characteristics are shown in Table 1.

Table 1. [54] Patient characteristics and their correlation with preoperative MMP-7 levels. (PreOP: preoperative; UBC: urothelial bladder cancer; TURBT: transurethral bladder tumor resection)

		Cohort 1			Cohort 2		
		PreOP serum MMP-7 cc.			PreOP serum MMP-7 cc.		
Parameters		**n**	**median (range)**	**p**	**n**	**median (range)**	**p**
Whole UBC cohort		188	4.2 (1.0 - 75.2)	**<0.001**	68	5.21 (1.99 - 24.71)	-
Non-tumorous control		97	2.9 (1.7 - 5.7)	-	0	-	-
Age	**≤ 65**	51	3.6 (1.6 - 75.2)	**0.006**	28	5.26 (1.99 - 15.34)	0.866
	> 65	137	4.6 (1.9 - 62.0)	-	40	5.20 (2.09 - 24.70)	-
Sex	**male**	149	4.1 (1.6 - 62.0)	0.622	43	4.77 (2.09 - 24.00)	0.239
	female	39	4.4 (1.0 - 75.2)	-	25	6.11 (2.09 - 24.70)	-
Stage	**Ta**	81	3.9 (1.4 - 18.2)	-	0	-	-
	Cis	8	3.6 (2.4 - 2.6)	-	2	3.13 (2.83 - 3.43)	-
	T1	19	3.9 (1.0 - 15.9)	-	1	3.62	-
	T2	28	5.8 (1.9 - 75.2)	-	20	4.87 (1.99 - 9.28)	-
	T3	27	4.4 (2.3 - 26.2)	-	32	6.23 (2.98 - 24.71)	-
	T4	25	6.2 (1.9 - 62.0)	-	13	5.91 (2.09 - 17.09)	-

Non-inv.	**(Cis-Ta-T1)**	108	3.9 (1.0 - 18.2)	**0.006**	3	3.43 (2.82 - 3.62)	**0.030**
Invasive	**(T2-T4)**	80	5.3 (1.9 - 75.2)	-	65	5.31 (1.99 - 24.71)	
Grade	**G1**	37	4.1 (1.9 - 18.0)	-	0	-	-
	G2	93	4.4 (1.0 - 30.0)	-	9	6.11 (2.83 - 12.73)	-
	G3	58	4.1 (1.9 - 75.2)	-	47	5.31 (2.09 - 24.71)	-
	Unknown	0		-	12		-
Low-grade	**(G1-2)**	130	4.3 (1.0 - 30.0)	0.622	9	6.11 (2.83 - 12.73)	0.973
High-grade	**(G3)**	58	4.1 (1.9 - 75.2)	-	47	5.31 (2.09 - 24.71)	
Surgery	**TURBT**	101	3.9 (1.0 - 18.2)	**0.021**	0	-	-
	RC	87	4.7 (1.9 - 75.2)	-	68	5.21 (1.99 - 24.71)	-
Lymph node	**N0/Nx**	156	4.0 (1.0 - 75.2)	**0.015**	38	4.87 (1.99 - 24.00)	**0.021**
	N +	32	7.8 (1.9 - 75.2)		30	6.25 (2.17 - 24.71)	

8.1.2 Study I. - Cohort 1

In cohort 1 of 188 UBC patients, the median age was 71 years (range: 21-90) and the median follow-up time was 24 months. Of the 188 patients, 56 had passed away at the last follow-up. At the time of the diagnosis, 80 (42.5%) patients had muscle-invasive UBC, of whom 32 (17%) were LN-positive.

8.1.3 Study I. - Cohort 2

Cohort 2 included 68 patients with UBC with available pretreatment (n=68) and for 57 patients also postoperative serum MMP-7 levels as well as detailed RC-based LN status (n=45) were determined. The median age was 66.4 years (range: 41-83), the median follow-up time was 22.5 months. Of the 68 patients, 30 passed away at the last follow-up. At diagnosis, 65 (96%) patients had muscle-invasive UBC, of whom 30 (44%) were LN-positive.

8.1.4 Correlation of MMP-7 levels with clinicopathological parameters

In cohort 1, preoperative serum MMP-7 levels were not associated with sex or tumor grade. Significantly higher serum MMP-7 levels were detected in patients over 65 years of age (p=0.006), with muscle-invasive disease and LN metastases (p=0.006 and p=0.015) (Table 1). In cohort 2, preoperative serum levels of MMP-7 showed no association with age or tumor grade. However, significantly higher serum MMP-7 levels were found in patients with muscle-invasive disease and with LN metastases (p=0.030, p=0.021) (Table 1 and Figure 1).

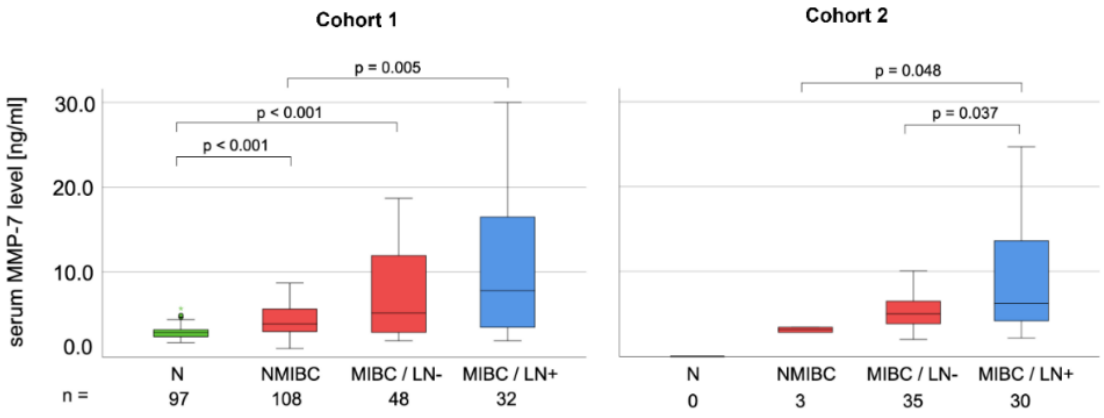


Figure 1. (54) Boxplot of preoperative serum MMP-7 levels at various stages of UBC. p-values were calculated using the Wilcoxon–Mann–Whitney U test. (NMIBC: non-muscle-invasive bladder cancer; MIBC: muscle-invasive bladder cancer; LN: lymph node).

8.1.5 Correlation of clinicopathological parameters and pretreatment serum MMP-7 levels with patient prognosis

Results of the univariate OS analysis are shown in Table 2. The presence of high-grade, muscle-invasive tumors and metastases, as well as elevated pretreatment serum MMP-7 levels, was associated with significantly shorter OS in cohort 1 (p=0.001, p<0.001 and p<0.001, p<0.001, respectively). Similarly, in cohort 2, patients with LN metastases and elevated pretreatment MMP-7 levels had poor OS (p=0.002 and p<0.001, Table 2).

Table 2. (54) Impact of clinicopathological parameters and preoperative serum MMP-7 levels on OS in cohorts 1 and 2. (Cis: carcinoma in situ; PreOP: preoperative; RC: radical cystectomy; TURBT: transurethral resection of bladder cancer; NMIBC: non-muscle-invasive bladder cancer; MIBC: muscle-invasive bladder cancer; OS: overall survival)

		Cohort 1				Cohort 2			
			OS				OS		
General data		**n**	**HR**	**95% CI**	**p**	**n**	**HR**	**95% CI**	**p**
Age	**≤ 65**	51	ref.			28	ref.		
	> 65	137	1.727	0.892 - 3.343	0.105	40	1.444	0.757 - 2.756	0.265
Sex	**female**	39	ref.			43	ref.		
	male	149	0.660	0.369 - 1.179	0.160	25	0.902	0.463 - 1.758	0.762
Stage	**NMIBC (Cis-Ta-T1)**	108	ref.			3	ref.		
	MIBC (T2-T4)	80	3.705	2.093 - 6.558	**<0.001**	65	2.085	0.286 - 15.206	0.469
Grade	**Low-grade (G1-2)**	130	ref.			9	ref.		
	High-grade (G3)	58	2.489	1.470 - 4.212	**0.001**	47	1.389	0.541 - 3.568	0.495
LN status	**N0/Nx**	156	ref.			38	ref.		
	N+	32	5.523	3.213 - 9.492	**<0.001**	30	2.721	1.427 - 5.189	**0.002**
PreOP MMP-7 whole cohort		188				68			
	≤7.15 ng/ml	139	ref.			49	ref.		
	>7.15 ng/ml	49	3.276	1.921 - 5.589	**<0.001**	19	3.699	1.913 - 7.153	**<0.001**
NMIBC (Ta-Cis-T1)		108				3			
	≤7.15 ng/ml	92	ref.			3	-		
	>7.15 ng/ml	16	2.743	0.885 - 8.502	**0.080**	0	-	-	-
MIBC (T2-T4)		80				65			
	≤7.15 ng/ml	47	ref.			46	ref.		
	>7.15 ng/ml	33	2.217	1.176 - 4.180	**0.014**	19	3.623	1.860 - 7.055	**<0.001**
RC		87				68			
	≤7.15 ng/ml	54	ref.			48	ref.		
	>7.15 ng/ml	33	2.455	1.291 - 4.669	**0.006**	19	3.699	1.913 - 7.153	**<0.001**
TURBT		101				0			
	≤7.15 ng/ml	85	ref.			0	-		
	>7.15 ng/ml	16	3.085	1.090 - 8.736	**0.034**	0	-	-	-

For patients who underwent RC, high serum MMP-7 levels were significantly associated with poorer OS (cohort 1: p=0.006, cohort 2: p<0.001, Table 2, Figure 2).

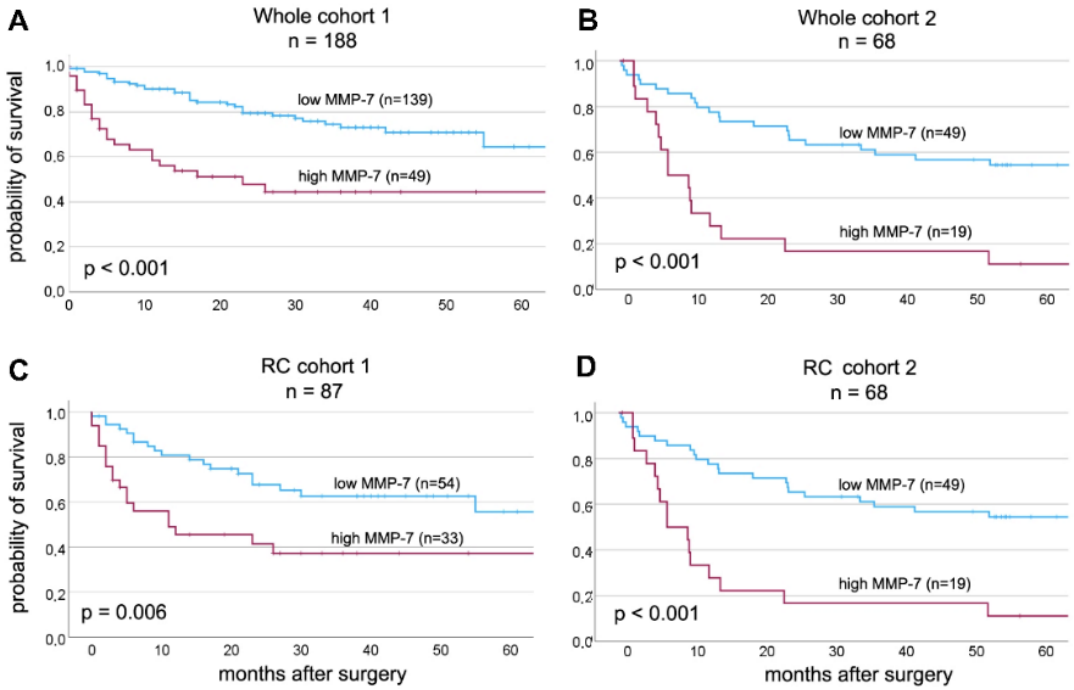


Figure 2. (54) Overall survival curves in cohort cohort 1 (A); cohort 2 (B) and in the subgroups of RC treated patients in cohort 1 (C) and cohort 2 (D). (RC: radical cystectomy)

In cohort 2, six patients had elevated pretreatment MMP-7 levels without LN metastases. This can be considered false positivity (biomarker positivity without LN positivity); however, these patients had a similarly poor prognosis (2-year survival 30%) as those with histologically detected LN positivity (30%) at RC, in contrast to those with LN negativity and low MMP-7 levels (2-year survival 75%). This may suggest that patients with high pretreatment serum MMP-7 levels and no LN positivity may have had undetected positive LNs (Figure 4C).

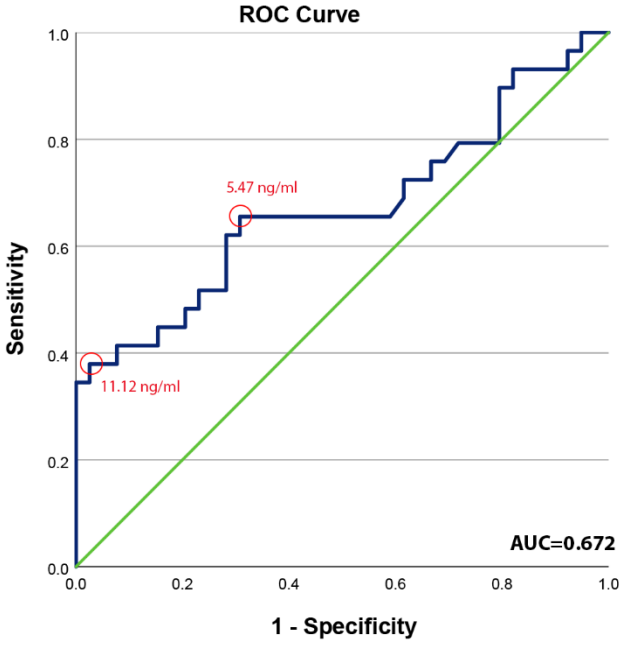
Multivariate analysis of patients who underwent RC in cohort 1 revealed high pretreatment serum MMP-7 levels as an independent risk factor for shorter OS (p=0.024; Table 3). These results were supported by data from cohort 2, similarly showing an independent prognostic value of high preoperative serum MMP-7 levels (p=0.004; Table 3).

Table 3. (54) Multivariate overall survival analysis. (OS: overall survival)

		Cohort 1			Cohort 2		
		OS			OS		
General data		**HR**	**95% CI**	**p**	**HR**	**95% CI**	**p**
Stage	**≤T2 vs. >T2**	4.253	1.764 – 10.252	**0.001**	1.272	0.493 – 3.281	0.619
Grade	**2 vs. 3**	1.424	0.664 – 3.054	0.364	1.256	0.484 – 3.261	0.640
Lymph node	**neg. vs. pos.**	2.316	1.115 – 4.810	**0.024**	1.434	0.685 – 2.999	0.339
MMP-7: ≤7.15 vs. >7.15 ng/ml		2.206	1.111 – 4.380	**0.024**	3.279	1.475 – 7.290	**0.004**

8.1.6 Correlation of pretreatment serum MMP-7 levels with the localization of lymph node metastases

To evaluate whether pretreatment MMP-7 levels could guide the decision between regional vs. extended LND, in cohort 2, pretreatment serum MMP-7 levels were assessed in relation to LN metastasis pattern; MMP-7 levels correlated with the presence of metastases in LNs corresponding to the regional vs. extended LND. The diagnostic



effectiveness of MMP-7 levels in predicting LN metastases at several cut-off values (Figure 3) was determined by ROC curve analysis - two of the three cut-off values were 5.47 ng/ml and 11.12 ng/ml based on the ROC curve analysis, and the third was 7.15 ng/ml, which was already used in a similar study (28).

Figure 3. (54) ROC curve of preoperative serum samples and optimal MMP-7 cut-off values to detect lymph node metastases. (ROC: receiver operating characteristic)

A total of 68 patients had preoperative serum samples and information on overall LN status. The relationship between high and low levels of MMP-7 and LN positivity was assessed using the $\chi 2$ test (Table 4), and a highly significant association was observed across all three cut-off values.

Table 4. (54) Correlations between lymph node status and preoperative MMP-7 levels at different cut-off values. (PreOP: preoperative)

PreOP MMP-7 concentration [ng/ml]	**Lymph node (n=68)**		**χ^2-test p-value**
	negative	positive	
≤5.47	26	11	0.009
>5.47	12	19	
≤7.15	32	17	0.012
>7.15	6	13	
≤11.12	37	19	<0.001
>11.12	1	11	

At a cut-off of 5.47 ng/ml, ~70% of patients without LN metastases had low (<5.47 ng/ml) preoperative MMP-7 levels, identifying 63% of LN-positive cases. As the cut-off value increased, the number of false-positive cases decreased: six LN-negative cases had high MMP-7 levels at 7.15 ng/ml, and only one LN-negative case at 11.12 ng/ml, but the proportion of false-negative cases rose from 37% to 57% and finally to 63%. It is of utmost importance to find the balance between the most accurate diagnosis of LN-positive cases and minimizing false-negative cases. The optimal cut-off value appears to be 5.47 ng/ml, as at this cut-point most patients with actual LN metastases can be classified as LN-positive based on high serum MMP-7 levels (63%). However, even with this cut-off value, many (37%) of patients with LN metastases would remain unidentified.

Twenty-three patients had detailed information on the status of both regional and extended LNs. In the group of patients who underwent extended LND, a retrospective analysis was conducted to determine whether an appropriate decision regarding the extent of LND could have been made based solely on preoperative serum MMP-7 levels (Table 5). Regional lymph node dissection (LND) was performed in all cases. The clinical relevance of decision-making was most pronounced in patients who had no positive regional lymph nodes but were later found to have non-regional lymph node metastases (four patients, highlighted in yellow in Table 5). Overall, none of the evaluated MMP-7 cut-off values demonstrated sufficient accuracy for predicting lymph node positivity in the extended dissection region. Using the lowest cut-off value (5.47 ng/mL), only one of the four patients with non-regional lymph node metastases had elevated pretreatment serum MMP-7 levels and would therefore have been correctly selected for extended LND based on MMP-7 alone. In contrast, when applying the two higher cut-off values, all four patients had low pretreatment serum MMP-7 levels, which would have indicated the need for regional LND only. Consequently, non-regional lymph node metastases would have been missed in all four cases (Table 5).

Table 5. (54) Preoperative MMP-7 levels as a function of the extent of lymph node metastases. (PreOP: preoperative)

PreOP MMP-7 concentration [ng/ml]	**Regional and extended lymph node status (n=23)**			
	Regional & Extended negative	Regional negative & Extended positive	Regional positive & Extended negative	Regional & Extended positive
≤5.47	8	3	1	1
>5.47	5	1	3	1
≤7.15	12	4	3	1
>7.15	1	0	1	1
≤11.12	12	4	3	1
>11.12	1	0	1	1

8.1.7 Changes of MMP-7 levels after radical cystectomy

For cohort 2, serum samples from postoperative days 2 and 5 were available for 57 and 46 patients, respectively. Postoperative MMP-7 levels decreased significantly in patients with high preoperative MMP-7 levels (Figure 4A). Despite the sharp decrease in this

subgroup, MMP-7 levels remained high in LN-positive patients (Figure 4B), suggesting that MMP-7-producing tumor cells might persists after RC and LND in LN metastatic patients. Accordingly, serum MMP-7 levels at days 2 and 5 after RC were significantly associated with LN metastases (both p=0.002) as well as with positive surgical margins (p=0.003) (Table 6). Postoperative MMP-7 levels were also associated with poor OS (day 2: HR=2.674, 95% CI: 1.136 – 6.293, p=0.024 and day 5: HR=3.185, 95% CI: 1.113 – 9.111, p=0.031) (Table 7).

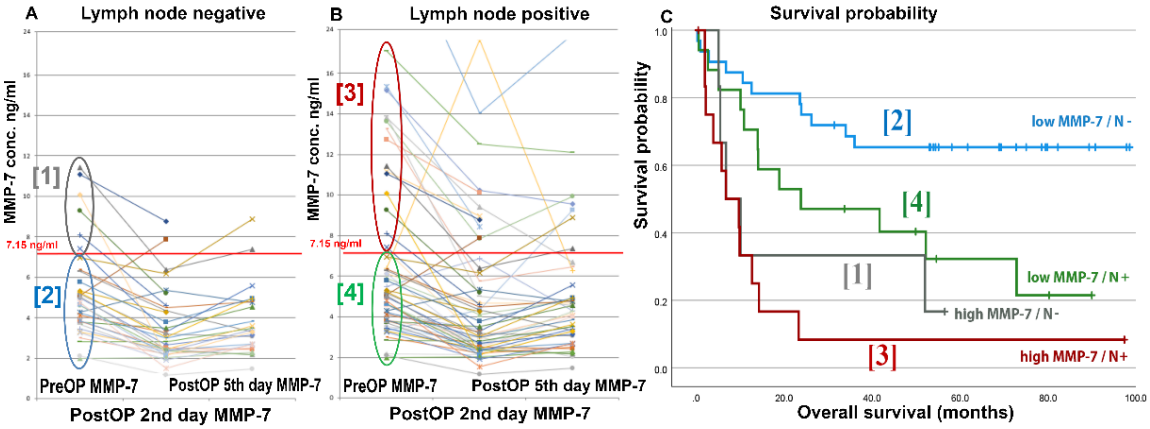


Figure 4. (54) Pre- and postoperative (days 2 and 5) serum MMP-7 levels in LN negative (A) and positive patients (B) and corresponding Kaplan-Meier survival curves (C). Note that patients with high preoperative serum MMP-7 levels and LN-negative histological findings (group [1]) have comparatively poorer survival than those with LN-positive tumors (group [3] and [4]). (PreOP: preoperative; PostOP: postoperative)

Table 6. (54) Univariate Cox regression analysis results for different clinicopathological parameters. (HR: hazard ratio)

Basic parameters	Overall survival		
	HR	95% CI	p
Age			
≤65 years	ref.		
>65 years	1.186	0.641 – 2.196	0.587
Sex			
Male	ref.		
Female	1.131	0.607 – 2.106	0.699
Stage			
pT0	ref.		
pT1-T4	5.958	0.819 – 43.635	0.078
Grade			
G2	ref.		
G3	1.322	0.554 – 3.154	0.529
Resection margin			
R0	ref.		
R1	2.677	1.395 – 5.137	**0.003**
Lymph node status			
N0/Nx	ref.		
N+	2.657	1.432 – 4.931	**0.002**
Smoking			
No	ref.		
Yes	0.648	0.247 – 1.696	0.376
Local instillation			
No	ref.		
Yes	0.100	0.145 – 1.183	0.067
Tumor stage at diagnosis			
Primary muscle-invasive UBC	ref.		
Primary non-invasive UBC to muscle-invasive form	1.200	0.592 – 2.433	0.612

Table 7. (54) Results of univariate Cox regression survival analysis for pre- and postoperative MMP-7 levels. (HR: hazard ratio)

	Overall survival		
MMP-7 concentration in serum	**HR**	**95% CI**	**p**
PreOP MMP-7 level			
≤7.15 ng/ml (low)	ref.		
>7.15 ng/ml (high)	3.381	1.610 – 7.100	**0.001**
PostOP 3rd day MMP-7 level			
≤7.15 ng/ml (low)	ref.		
>7.15 ng/ml (high)	2.674	1.136 – 6.293	**0.024**
PostOP 5th day MMP-7 level			
≤7.15 ng/ml (low)	ref.		
>7.15 ng/ml (high)	3.185	1.113 – 9.111	**0.031**

8.1.8 Systematic literature search and meta-analysis of the prognostic value of pretreatment serum MMP-7 levels in bladder cancer

Our systematic search yielded 6 eligible studies for data extraction, after retrieving 456 articles from the available databases (Figure 5).

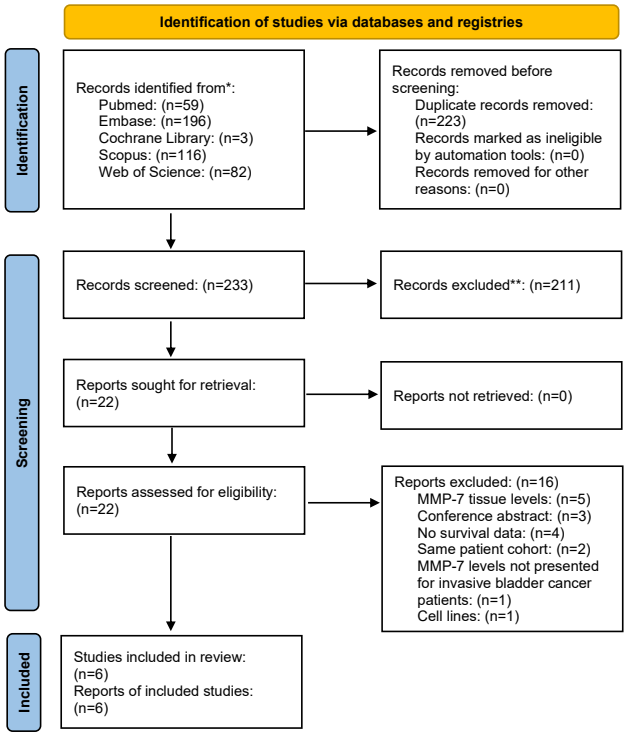


Figure 5. (54) PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, registries and other sources.

These were assessed together with the two patient cohorts presented above (cohorts 1 and 2). Overall, eight UC cohorts were included, totalling 734 patients who underwent surgical or systemic therapy and had available pretreatment serum or plasma MMP-7 levels. Due to the high mortality of patients with muscle-invasive UBC and the relatively homogenous population, the random-effects model was used ($p=0.44$, $I^2=0\%$), and a pooled analysis showed a significant association with poor OS for patients with high pretreatment serum MMP-7 levels (HR: 2.69; 95%CI: 2.15-3.36) (Figure 6A). A subgroup analysis of 593 patients with muscle-invasive high-grade UBC who underwent radical surgery (RC, RNU) or chemotherapy (CTX) showed that high pretreatment MMP-7 levels were significantly correlated with reduced OS (HR: 2.58; 95%CI: 2.04-3.26) (Figure 6B). Multivariate analysis using the random effects model revealed high serum MMP-7 levels to be predictive for poor OS (HR: 2.35, 95%CI: 1.81-3.05) (Figure 6C).

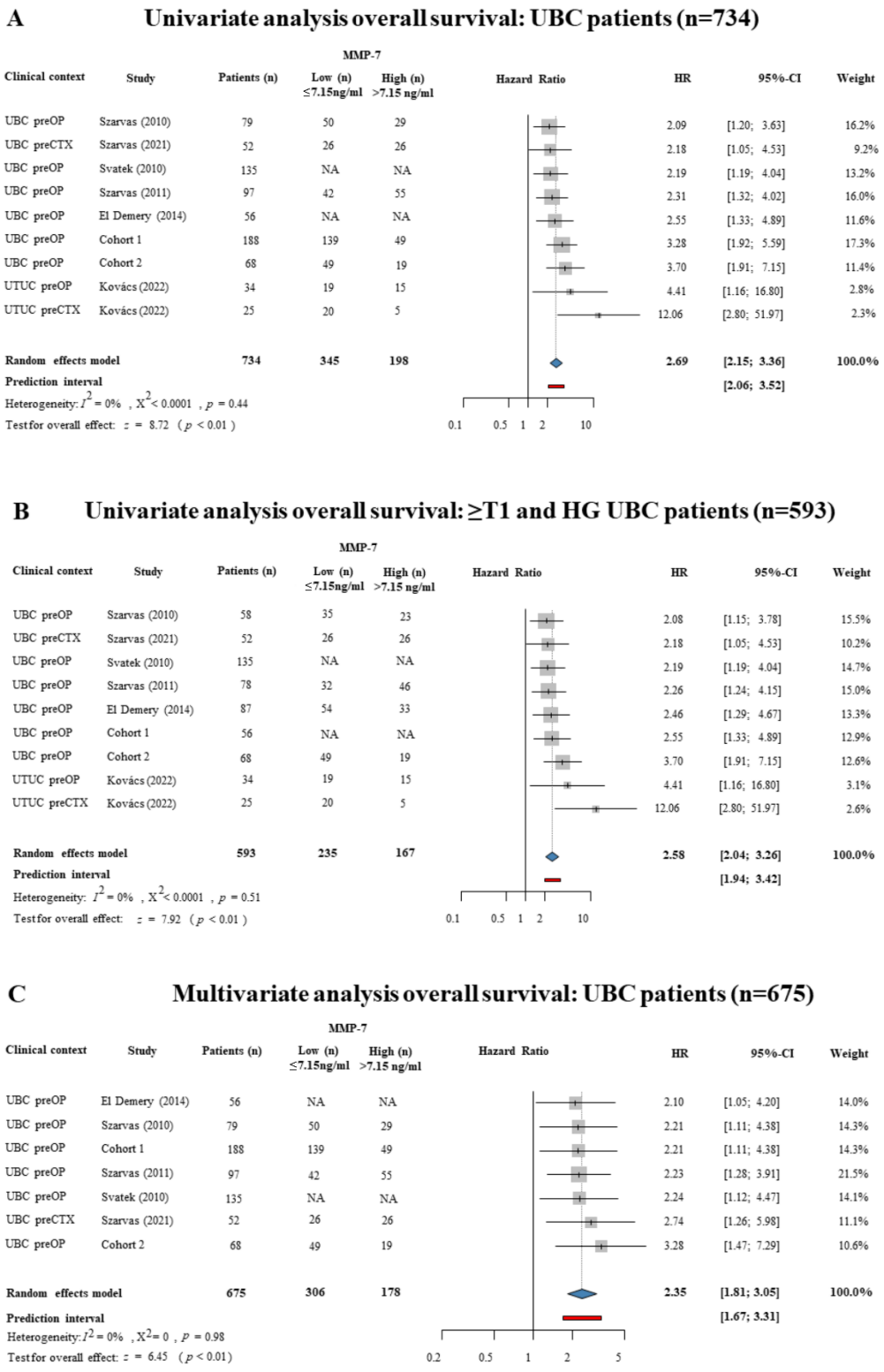


Figure 6. (54) Forest plots representing hazard ratios of (A) all urothelial cancer patients (n=734) for OS, (B) hazard ratios of ≥T1 and HG urothelial cancer patients (n=593) for OS using the univariate Cox regression analysis, and (C) hazard ratios of urothelial

carcinoma patients (n=675) for OS using multivariate Cox regression survival analysis in urothelial cancer patients with high pretreatment MMP-7 levels (cut-off value: 7.15 ng/ml).

8.1.9 Systematic search and meta-analysis of surgery-based and bladder sparing approches in limited-stage small-cell bladder cancer (study II)

The initial search identified 1661 records. After duplicate removal, 945 records remained (Figure 7, PRISMA flowchart). After screening titles and abstracts, a full-text review was performed on the remaining 115 articles. Finally, five studies comprising 1041 patients treated for LS-SCBC (55-59) were identified as eligible for qualitative and quantitative analyses.

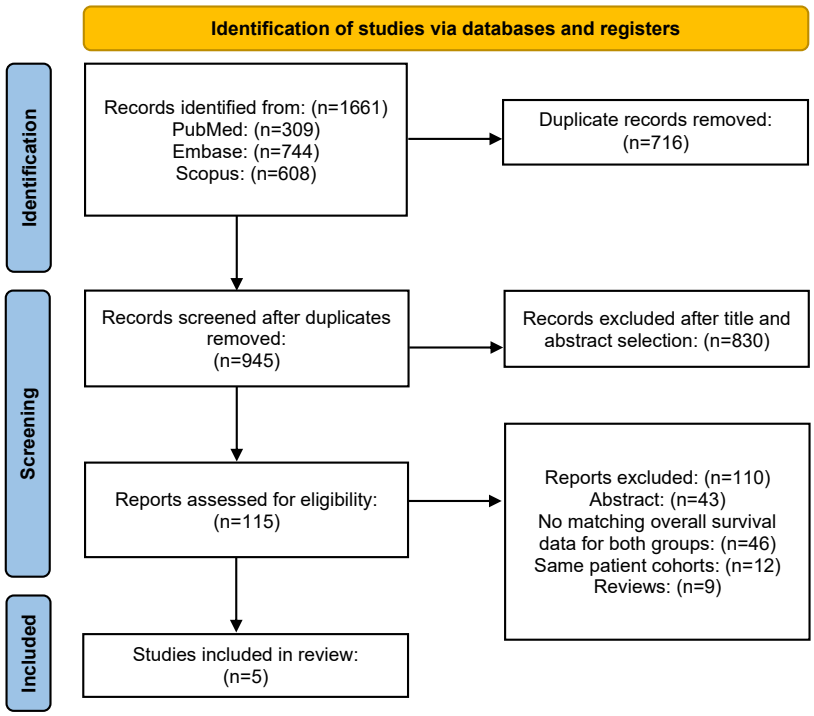


Figure 7. (60) PRISMA 2020 flow diagram for new systematic reviews, which included searches of databases, registers only.

The baseline characteristics of the patients and their outcomes are shown in Table 8. The median age and follow-up period ranged from 66 to 76 years and from 15 to 70 months, respectively. The pooled median OS for those who received RBMMT was 34.6 months (95% CI: 25.5-43.7), compared with 29.7 months (95% CI: 18.2-41.1) for those who received CBMMT.

Table 8. (60) Characteristics of included studies. (NA: not available; SCBC: small-cell bladder cancer; LS-SCBC: limited stage small-cell bladder cancer; RBMMT: radiation-based multimodal therapy; CBMMT: cystectomy-based multimodal therapy)

Authors	Year of publication	Study type	Study period	All SCBC patients (n)	LS-SCBC (n)	RBMMT (n)	CBMMT (n)	RBMMT median OS (months)	CBMMT median OS (months)
Chau [16]	2021	Multi-center case series	2006-2016	409	165	104	61	30.0	26.7
Fischer-Valuck [17]	2018	Registry based	2004-2013	856	404	203	201	34.1	32.4
Grigg [18]	2020	Single-center case series	2010-2019	30	30	17	13	36.8	30.6
Luzzago [27]	2020	Registry based	2001-2016	595	365	206	159	NA	NA
Oh [19]	2022	Registry based	1985–2018	109	109	44	33	33.6	26.4

As a sensitivity analysis, hazard ratios were re-estimated for each study using 60-month censored data and subsequently pooled (Figure 8). The concordance of these estimates with the frailty model supports the robustness of the primary analysis. The results showed a non-significantly better OS for RBMMT-treated compared to CBMMT-treated LS-SCBC patients (HR: 0.83, 95% CI=0.61-1.12, p=0.22) (Figure 9).

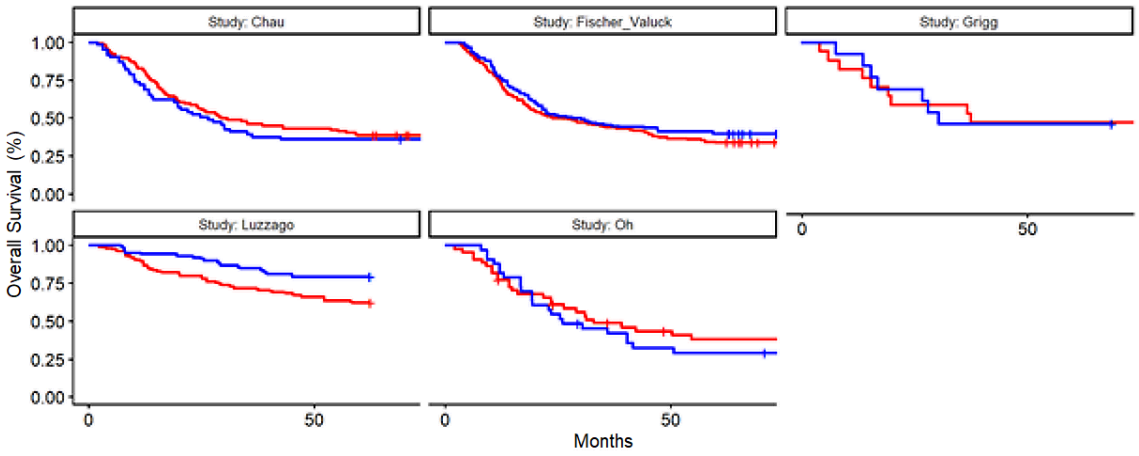


Figure 8. (60) Data extracted from individual studies' Kaplan-Meier curves using the frailty model and pooling for 5 years. Red: radiation-based multimodal therapy (RBMMT); blue: cystectomy-based multimodal therapy (CBMMT)

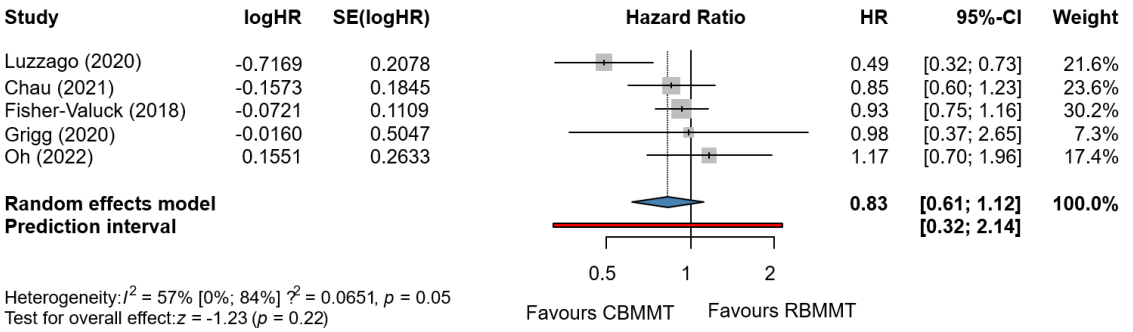


Figure 9. Sensitivity analysis based on study-wise reconstructed hazard ratios and pooled meta-analysis for overall survival.

9 DISCUSSION

9.1 Summary of findings, international comparisons

The management of aggressive urothelial and non-urothelial bladder neoplasms is rapidly evolving, which leads to increasingly complex decision-making. Therefore, identifying the most effective therapy for UBC subgroups is of great clinical importance. We addressed two clinically distinct but conceptually related questions: (1) whether pretreatment circulating MMP-7 levels can provide robust prognostic information and support therapeutic decision-making in UC; and (2) whether bladder-preserving, RBMMT offers oncological outcomes comparable to CBMMT in patients with LS-SCBC. We used quantitative meta-analysis to systematically analyze data from the literature, given that single-center retrospective studies had limited power to draw meaningful conclusions.

The first study focused on the prognostic value of serum MMP-7 in various therapeutic settings of UC. A special focus was placed on the potential clinical value of MMP-7, specifically to investigate whether preoperative serum MMP-7 could inform surgical decision-making, particularly regarding the extent of LND during RC. In addition, postoperative changes in serum MMP-7 levels were evaluated to assess their association with tumor removal, residual disease, and subsequent clinical outcomes in patients with muscle-invasive disease.

In line with previous reports, our present results showed that elevated pretreatment serum or plasma MMP-7 levels were consistently associated with poor OS in UC. The earliest study in this field demonstrated, in a German cohort, that high tumor tissue expression and elevated serum MMP-7 concentrations were significantly correlated with LN involvement and distant metastasis. A preoperative serum cut-off of 7.15 ng/ml yielded a sensitivity (82%) and specificity (71%) for predicting LN positivity and was independently associated with overall, disease-specific, and metastasis-free survival (28). Similar observations were reported in another independent case series. Svatek *et al.* demonstrated that high preoperative plasma MMP-7 levels were associated with shorter time to cancer-specific death in invasive UBC (42). At the same time, additional German and French cohorts confirmed the prognostic impact of circulating MMP-7 on overall and disease-specific survival in advanced disease (61, 62). Benoit *et al.* reported markedly

higher serum MMP-7 concentrations in muscle-invasive compared to non-muscle-invasive UBCs and non-cancer controls, suggesting a link with biologically aggressive disease (63). A large serum proteomics screen identified MMP-7 as one of the most significant prognostic markers in UC (64). Furthermore, elevated pretreatment MMP-7 levels were associated with shorter OS in Hungarian patients treated with platinum-based chemotherapy (65). Kovács *et al.* extended these findings to upper tract urothelial carcinoma, demonstrating that high preoperative MMP-7 predicted poor outcomes and may aid in preoperative risk stratification (66).

Our present data add two further cystectomy and systemic-therapy cohorts to this body of evidence. In cohort 1, which included 188 patients with preoperative serum samples and 97 non-tumorous controls, MMP-7 levels increased stepwise from controls (mean 2.9 ng/ml) through non-muscle-invasive UCs (3.9 ng/ml) and muscle-invasive UCs (5.3 ng/ml), reaching the highest concentrations in LN-positive patients (7.8 ng/ml). High preoperative MMP-7 (>7.15 ng/ml) levels were associated with significantly shorter OS in the entire cohort and in the muscle-invasive subgroup and remained independently prognostic for OS in multivariate analyses. These observations support the concept that circulating MMP-7 reflects both tumor burden and biological aggressiveness, complementing conventional clinicopathological risk factors.

In cohort 2, we prospectively assessed perioperative MMP-7 kinetics in 68 patients undergoing RC with extended LND. Baseline MMP-7 levels were again higher in LN-positive disease and associated with inferior OS. Importantly, we observed a significant reduction in serum MMP-7 on postoperative days 2 and 5 among patients with high preoperative levels, supporting tumor tissue as the primary source of circulating MMP-7. Nonetheless, nearly three-quarters of LN-positive patients with elevated baseline MMP-7 remained above the 7.15 ng/ml threshold postoperatively, which may indicate residual microscopic disease or persistent systemic tumor activity despite RC. This pattern suggests a potential role for postoperative MMP-7 monitoring as an early, minimally invasive indicator of incomplete tumor eradication or early recurrence, analogous to the emerging role of ctDNA in post-cystectomy surveillance (67-69).

When evaluating the potential of preoperative MMP-7 to guide the extent of LND at RC, we found that only approximately half of histologically LN-positive patients had elevated

preoperative MMP-7 (false negatives). In contrast, 15–20% of LN-negative patients showed high MMP-7 values. Thus, preoperative MMP-7 alone is insufficient to reliably discriminate between patients who would benefit from extended vs. regional LND. This is concordant with contemporary guideline statements that, despite extensive investigation of nodal yield, template definition, and nodal density, no single biomarker or clinical parameter can yet reliably guide the extent of LND at RC (70).

More importantly, the group of technically "false-positive" patients – those with high serum MMP-7 levels but histologically negative LNs – warrants particular attention. These patients exhibited OS outcomes similar to LN-positive cases and significantly worse than LN-negative patients with low MMP-7, suggesting that MMP-7 may detect occult (micro)metastatic disease beyond the resolution of conventional imaging or histopathological assessment. Current guidelines emphasize that a relevant proportion of patients with muscle-invasive UBC harbor micrometastatic disease at the time of RC despite negative standard staging, which underpins the routine recommendation of cisplatin-based neoadjuvant chemotherapy and, in selected cases, adjuvant systemic therapy (7, 71, 72). In this context, preoperative MMP-7 might help identify apparently node-negative patients at higher systemic risk, who could benefit from intensified systemic treatment and closer follow-up, similar in concept to ctDNA-guided adjuvant approaches (67).

A biological rationale supports the prognostic relevance of MMP-7 in UC. MMP-7 has broad substrate specificity and has been implicated in multiple signalling pathways relevant to invasion and metastasis. It cleaves cell adhesion molecules, such as E-cadherin, thereby disrupting epithelial integrity, promoting epithelial–to–mesenchymal transition, and increasing motility. It also degrades Fas/FasL and other pro-apoptotic factors, contributing to resistance against apoptosis (73). MMP-7 can process growth factors and cytokines, including TNF-α and TGF-β, and generates biologically active fragments such as endostatin, angiostatin, and RANKL, influencing angiogenesis, bone remodeling, and the tumor microenvironment (74). By activating other MMPs, MMP-7 may amplify proteolytic cascades and facilitate matrix remodeling (19). Collectively, these processes provide a molecular explanation for the close relationship between high MMP-7 levels, nodal involvement, and systemic spread (28).

Our meta-analysis, which integrated six independent studies with the two current cohorts (a total of 734 patients), confirmed the robustness of MMP-7 as an adverse prognostic factor in UC. HRs for high *vs.* low MMP-7 ranged from approximately 2 to over 12 across different populations and treatment settings, with relatively narrow prediction intervals and low between-study heterogeneity, suggesting robust and consistent prognostic value for MMP-7. Notably, HRs were highest in UTUC (66), a disease in which adequate preoperative staging remains notoriously difficult, and risk-adapted decision-making between kidney-sparing and radical approaches is challenging. Although our present work focused on UBC, this pattern suggests that MMP-7 captures aspects of tumor biology that are particularly relevant in settings where conventional staging is limited. Taken together with guideline-based evidence, these data highlight an important gap in current UBC management: despite refined pathological staging, improved imaging (*e.g.,* VI-RADS MRI, FDG-PET/CT), and emerging molecular classifications (75-77), no circulating biomarker is yet sufficiently validated for routine risk stratification or treatment selection (35). The EAU guidelines explicitly state that, aside from specific actionable alterations such as *FGFR3* mutations for erdafitinib, most molecular and immune markers remain investigational (35, 67, 78). In this landscape, MMP-7 is one of the few circulating proteins with consistent prognostic validation across multiple independent cohorts and disease locations.

Our study also has implications for the broader development of biomarker-driven perioperative strategies. While ctDNA is rapidly emerging as a promising tool to guide postoperative immunotherapy and surveillance (67-69), protein biomarkers such as MMP-7 might be easier to implement in routine laboratories. They may provide complementary information about the proteolytic and stromal compartments of the tumor microenvironment. Integrating MMP-7 into multimodal prognostic models that already include clinical stage, comorbidity, histology, and molecular subtype, as well as radiographic risk features, could refine stratification between standard and intensified perioperative treatment strategies (79, 80).

The translational potential of MMP-7 extends beyond prognostication. Preclinical studies have shown that selective inhibition of MMP-7 reduces invasive capacity of UC cell lines, and that genetic or pharmacologic modulation of MMP-7 can attenuate bone metastases in prostate cancer models (81, 82). Although broad-spectrum MMP inhibitors such as

batimastat and *marimastat* have failed in phase III trials, likely due to off-target effects and interference with physiological MMP functions (83), the development of highly selective MMP-7 inhibitors and MMP-targeted imaging probes is advancing (66, 84-87). Selective radiolabelled or fluorophore-conjugated MMP inhibitors could enable early detection of micro-metastases using SPECT or PET, while synthetic MMP-7-specific inhibitors may, in the future, allow for tailored anti-protease therapies, in contrast to earlier broad-spectrum MMP inhibitors that failed in phase III clinical trials due to off-target effects and the pleiotropic roles of MMPs (88-90). These approaches allow MMP-7 to serve as both a marker and a therapeutic target, particularly in patients with MMP-7-driven micrometastatic disease that is inadequately captured by current TNM staging.

In study II, we investigated the optimal treatment strategy for LS-SCBC, a rare and highly aggressive neuroendocrine carcinoma of the bladder. Historically, RC combined with platinum-based chemotherapy has been regarded as the standard of care for LS-SCBC, primarily derived from small-cell lung cancer protocols (35). However, the morbidity of RC and the lack of prospective comparative data have raised the question of whether bladder-preserving, RBMMT could offer similar oncological outcomes with potentially better quality of life (QoL) (32-37). In this context, our systematic review and meta-analysis provide a structured comparison of CBMMT and bladder-preserving, RBMMT in LS-SCBC, focusing on OS as the primary endpoint. Across the retrospective series by Fischer-Valuck *et al.*, Grigg *et al.*, Chau *et al.,* and Oh *et al.,* consistently reported similar OS between CBMMT and RBMMT (55-57, 59), supporting the multimodal bladder-preserving strategy with complete TURBT, systemic chemotherapy, and consolidative radiotherapy as an oncologically reasonable option in selected cases. The only study reporting a clear oncologic advantage for CBMMT was the extensive registry-based analysis by Luzzago *et al.*, who observed lower cancer-specific mortality and longer OS in patients undergoing RC compared with those receiving RBMMT (58). Contemporary EAU guidelines recognize RC with bilateral LND as the reference standard for localized muscle-invasive UBC, with 5-year survival of approximately 50% (4, 7, 91). However, they also acknowledge trimodality therapy (TMT) – complete TURBT followed by chemoradiotherapy – as an oncologically acceptable alternative in selected patients, who are either unfit for RC or wish to preserve their bladder function, provided that close cystoscopic surveillance and prompt salvage RC are ensured (92-96). In the absence of

dedicated randomized trials in LS-SCBC, it is reasonable to extrapolate these principles, particularly given that SCBC is classified by the WHO as a pure neuroendocrine carcinoma or a neuroendocrine variant of UC, which typically presents with muscle-invasive or more advanced disease and requires upfront systemic treatment (97, 98). EAU–ESMO consensus statements specifically recommend that bladder tumors with a small-cell neuroendocrine component be managed with platinum-based chemotherapy followed by consolidative local therapy (99, 100). In practice, this can be implemented either as neoadjuvant chemotherapy followed by RC and LND (CBMMT) or as induction chemotherapy followed by TMT (RBMMT) (101). Our meta-analysis indicates that, when combined with adequate systemic therapy, both approaches yield similar survival in LS-SCBC. This is consistent with the broader guideline message that systemic therapy is central to the management of neuroendocrine histology subtypes. In contrast, the choice of local therapy can be individualized according to performance status, comorbidities, and patient preference (102).

QoL considerations are critical in LS-SCBC, given that the disease often presents in older patients with significant comorbidities and limited physiological reserve. Data from muscle-invasive UBC cohorts show that long-term health-related QoL after either RC with urinary diversion or bladder-preserving chemoradiation often returns to baseline, but the profiles differ (103). RC with ileal conduit or neobladder is associated with permanent stoma care or continence issues, potential sexual dysfunction, and the risks inherent to major pelvic surgery. In contrast, TMT avoids urinary diversion but entails long-term bladder and bowel toxicity and a need for intensive surveillance (92, 93, 95, 104, 105). Bladder-preserving strategies can maintain body image, avoid a stoma, and in some series provide better emotional and social functioning, which may be especially relevant for patients who place a high value on organ preservation and autonomy. Our meta-analysis therefore supports a shared decision-making model in LS-SCBC, in which patients are informed that both CBMMT and RBMMT can provide similar survival, while the QoL trade-offs differ.

The outlier in the literature is the study by Luzzago *et al.*, which reported lower cancer-specific mortality and longer OS in LS-SCBC patients treated with CBMMT compared to RBMMT (58). Significantly, this difference was primarily driven by higher other-cause mortality in elderly, higher-stage patients within the RBMMT cohort, raising the

possibility of selection bias and confounding by indication. Moreover, this is the only study to use cancer-specific survival as its primary endpoint, and it does not necessarily contradict the observation that OS – the clinically most relevant composite of oncologic outcome and treatment-related harm – may remain similar between modalities. Given that RC carries non-negligible perioperative risks, particularly in frail or multimorbid patients, any incremental cancer-specific benefit may be offset by higher treatment-related morbidity and non-cancer deaths. This is aligned with guideline recommendations to carefully evaluate comorbidity, frailty, and cognitive status when selecting candidates for RC, and to consider bladder-preserving strategies in patients with high surgical risk (105, 106).

Understaging and undertreatment remain major concerns in LS-SCBC, particularly when organ-preserving strategies are employed. Accurate staging relies on high-quality TURBT, cross-sectional imaging (CT or MRI), and, where appropriate, FDG-PET/CT to detect occult nodal or distant metastases. Guideline-based evidence in UBC indicates that modern imaging modalities – including multi-parametric MRI using VI-RADS and FDG-PET/CT – can improve staging accuracy and influence treatment planning, although micrometastatic disease still frequently escapes detection (76, 107-110). Given the exceptionally high metastatic potential of small-cell histology, meticulous staging and restaging after systemic therapy are crucial if bladder preservation is chosen. Patients should also be counseled that a proportion will ultimately require salvage RC for non-response or intravesical recurrence, as noted in the TMT series for conventional UC (104, 111).

Taken together, the two projects converge on several key concepts. First, both highlight the inadequacy of conventional staging alone in fully capturing the biological aggressiveness of bladder malignancies. In UC, elevated MMP-7 identifies a high-risk subgroup – including histologically node-negative patients – who harbour micrometastases and may benefit from early, intensified systemic therapy. In LS-SCBC, limitations of preoperative imaging and histology contribute to staging uncertainty and complicate the selection between CBMMT and RBMMT. Second, both studies propose a framework in which molecular biomarkers and refined imaging tools are used to complement, rather than replace, traditional clinical and pathological staging. MMP-7 and potentially other circulating markers, such as sPD-L1, NLR, CRP, or ctDNA, may in

the future help identify patients at particularly high risk of systemic progression who should not be offered bladder preservation despite apparent limited-stage disease. Conversely, patients with favourable biomarker profiles, good performance status, and node-negative staging could safely undergo RBMMT to optimise functional outcomes without compromising survival.

Finally, both analyses underline the methodological challenges and opportunities inherent in rare and aggressive malignancies. The retrospective design, heterogeneity in treatment regimens, and limited availability of biomarker data constrain the strength of causal inference. However, the use of rigorous systematic review methods, reconstructed pseudo-IPD, and robust meta-analytic techniques allows for more precise effect estimation than single-centre series alone. Future research should prioritise prospective, multicentre cohort studies or pragmatic trials that systematically integrate molecular biomarker assessment, advanced imaging, and standardised QoL endpoints into treatment algorithms for both UBC and LS-SCBC. Such efforts could move the field from empirically derived patterns of care towards genuinely personalised, biology-guided management in these high-risk UBC subtypes.

9.2 Strengths - MMP-7 Study I.

A major strength of the MMP-7 study is the integration of two independent institutional cohorts with a comprehensive systematic review and meta-analysis, which allows us to evaluate the biomarker from both a translational and an evidence synthesis perspective. The use of preoperative and postoperative serum samples provides unique insight into MMP-7 kinetics and supports the biological relevance of the marker in reflecting tumor burden. Consistent results across international studies and a pooled sample of 734 patients support the generalizability of the observed associations. Our analysis also included a dedicated high-risk surgical subgroup, providing clinically relevant information for RC candidates. A narrow prediction interval and similar HRs across independent cohorts support the prognostic robustness of MMP-7 in UC. Finally, inclusion of both LN-positive and LN-negative subgroups allowed assessment of MMP-7 in preoperative staging and identified a small but clinically relevant subset of node-negative patients with elevated MMP-7 and occult metastases.

9.3 Strengths – SCBC Study II.

This is the first meta-analysis directly comparing CBMMT with RBMMT specifically in LS-SCBC. By pooling five eligible studies with a high number (>1,000) of SCBC patients this analysis provides the most comprehensive comparative evaluation available in the literature. The reconstruction of pseudo-IPD from published Kaplan–Meier curves allowed harmonized OS comparisons across heterogeneous studies. Including both median OS and confidence interval estimations strengthened internal validity. Importantly, the analysis incorporated contemporary QoL considerations, recognising that functional outcomes are central to treatment decisions in LS-SCBC. The ability to demonstrate oncologic noninferiority for bladder preservation in selected patients provides valuable guidance for daily clinical decision-making.

9.4 Limitations - MMP-7 Study I.

The MMP-7 analysis carries limitations typical of retrospective biomarker studies. Serum sampling protocols, assay platforms, and cut-off values varied across included cohorts, which may influence comparability. Postoperative MMP-7 measurements were available for only a limited number of cases, limiting conclusions about early postoperative dynamics. Furthermore, the relatively high false-negative rate for predicting LN metastasis limits the usefulness of MMP-7 for surgical decision-making regarding the extent of LND. Finally, while the meta-analysis demonstrated consistent prognostic associations, potential publication bias and between-study clinical heterogeneity cannot be entirely excluded. Prospective trials using standardized biomarker platforms are needed to refine thresholds and clarify integration into clinical pathways.

9.5 Limitations - SCBC Study II.

The SCBC meta-analysis is limited by the retrospective nature of all included studies. Treatment selection is confounded by age, comorbidities, tumor stage, and institutional preference, which may influence survival outcomes. Considerable heterogeneity exists regarding chemotherapy regimens, radiation doses, surgical techniques, and staging strategies, reflecting changes in clinical practice over several decades. A key issue is the potential for understaging. Standard diagnostic pathways relying on TURBT with CT or

MRI may not detect low-volume nodal or micro-metastatic disease, which could lead to inappropriate allocation to bladder-preserving therapy. Because cancer-specific survival was reported only in one study, the ability to separate oncologic risk from other-cause mortality is limited. Additionally, no uniform QoL metrics were available across studies, despite QoL being a central consideration when offering bladder preservation. The inability to perform detailed subgroup analyses – such as by N-stage, T-stage, or chemotherapy protocol – also restricts interpretation. Prospective standardized datasets are needed to refine patient selection for RBMMT *vs.* CBMMT.

10 CONCLUSIONS

We demonstrated that circulating MMP-7 is a strong and consistent prognostic biomarker in UC. High pretreatment serum concentrations were associated with adverse pathological features, LN involvement, and significantly shorter OS. The postoperative decline in MMP-7 supports the tumor as a primary source of the circulating marker, while persistently elevated postoperative levels in some patients may reflect residual microscopic disease. Notably, a subset of LN-negative patients with high MMP-7 showed survival outcomes comparable to LN-positive cases, indicating possible occult metastases that escape conventional staging methods. The systematic review and meta-analysis confirmed these observations across eight independent cohorts, highlighting the reproducibility of MMP-7 as an adverse prognostic marker. These findings suggest that MMP-7 has potential clinical utility in preoperative risk stratification and may help identify patients who would benefit from intensified systemic therapy. Future studies are required to validate cut-off values, clarify integration into clinical practice, and explore its role in biomarker-guided treatment algorithms.

The SCBC project showed that bladder-preserving multimodal therapy (RBMMT) provides OS outcomes comparable to RC-based multimodal therapy (CBMMT) in patients with LS-SCBC. Across more than 1,000 patients, no significant survival difference was detected between the two treatment modalities. These results support considering bladder preservation in appropriately selected patients, particularly when QoL and functional outcomes are important. Overall, the aggregated evidence indicates that RBMMT is a viable, oncologically sound alternative for selected patients with LS-SCBC. This is particularly relevant given the morbidity of RC and the rarity of the disease, which makes randomized trials unlikely. Future efforts should focus on improving staging accuracy and refining patient selection criteria.

11 IMPLEMENTATIONS FOR PRACTICE

The MMP-7 study holds the potential to influence clinical practice by improving preoperative risk stratification for patients with UC. Elevated pretreatment MMP-7 levels could help clinicians identify individuals at higher risk for metastatic progression, including those with apparently LN-negative disease. If incorporated into standard diagnostic workflows, MMP-7 could support decisions on early systemic therapy, adjuvant treatment, or intensified follow-up schedules. Furthermore, postoperative MMP-7 dynamics may offer additional information regarding residual disease. With appropriate validation, MMP-7 testing could complement imaging and pathology in therapy planning, helping reduce undertreatment in high-risk patients and avoid unnecessary overtreatment in low-risk individuals. The advantages of a blood-based biomarker – availability, reproducibility, and minimal patient burden – make it an attractive tool for routine clinical use.

The SCBC findings have immediate clinical relevance for urologists and oncologists managing LS-SCBC. Evidence supporting the oncologic non-inferiority of RBMMT allows clinicians to confidently offer bladder preservation to suitable patients, especially those with LN-negative disease and adequate functional status. Integrating RBMMT into treatment discussions may improve QoL without compromising survival. Moreover, understanding that survival outcomes are comparable between RBMMT and CBMMT encourages shared decision-making, considering patient preferences, comorbidities, and anticipated treatment tolerance. The data also highlight the importance of thorough staging and appropriate systemic therapy in both treatment pathways. Incorporating these insights into multidisciplinary tumor boards may enhance consistency and quality of care for this rare malignancy.

12 IMPLEMENTATION FOR RESEARCH

Future research should prioritize prospective validation of MMP-7 in large, biomarker-driven clinical cohorts. Establishing standardized assay platforms, sampling protocols, and clinically relevant cut-off values would enable broader clinical adoption. Investigating the interaction between MMP-7 and systemic therapies – including neoadjuvant chemotherapy, adjuvant therapy, and immunotherapy – could refine its role in personalized treatment strategies. Additional studies may clarify the biological link between MMP-7 and metastatic dissemination, supporting its potential use as a target for molecular imaging or therapeutic inhibition. Integrating MMP-7 into multi-parameter prognostic models alongside genomic, radiologic, and clinical variables may further improve risk stratification in UC.

Given the rarity of LS-SCBC, future research should focus on multicentre collaborations, prospective registries, and harmonized data collection. Improved staging methods – such as advanced PET imaging or circulating biomarkers – should be evaluated to reduce the risk of understaging and improve patient selection for bladder preservation. Comparative effectiveness studies including patient-reported outcomes are needed to understand better functional and QoL differences between CBMMT and RBMMT. Additionally, identifying biological or molecular predictors of treatment response may support a more individualized approach to LS-SCBC, mirroring advances in other uro-oncologic diseases.

13 IMPLEMENTATION FOR POLICYMAKERS

The findings support consideration of MMP-7 testing as part of preoperative diagnostic pathways in UC. From a health-system perspective, reimbursement frameworks and the availability of validated assays would be important to enable broader clinical use. Improved risk stratification may allow more appropriate treatment selection, potentially reducing unnecessary or ineffective interventions. Incorporating of biomarker-guided decision-making into national bladder cancer guidelines could promote more consistent, evidence-based care and align with value-based healthcare principles.

The SCBC findings support access to multimodal bladder-preserving treatment, including radiotherapy and multidisciplinary care. Bladder-preserving approaches may be considered in selected patients with LS-SCBC when survival appears comparable to radical surgery. Ensuring access to both surgical and radiation-based treatments is essential for patient-centred care.

14 FUTURE PERSPECTIVES

My future work will continue to focus on laparoscopic urologic oncology, with particular emphasis on uro-oncology. A central aim is to integrate biomarker research into clinical decision-making to improve risk stratification and treatment selection further. Building on the MMP-7 and SCBC studies, I plan to advance research that links surgical practice with translational investigation. An important future direction will be the optimization of treatment strategies for patients with muscle-invasive bladder cancer, including the systematic evaluation of QoL outcomes alongside oncological results. In the longer term, I seek to contribute to more personalized management approaches for high-risk bladder cancer by investigating molecular predictors, markers of treatment response, and mechanisms of metastatic progression. By integrating clinical experience, biomarker-driven research, and evidence synthesis, I aim to support the continued development of precision oncology in bladder cancer care.

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16.1 Publications related to the thesis

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16.2 Publications not related to the thesis

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APPENDICES

The full texts of the publications underlying this thesis are provided as Appendices. These publications form the scientific basis of the present dissertation and are attached in their original published format.

Appendix I

Full text of Publication I

Appendix II

Full text of Publication II