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Bladder Cancer Epidemiology in Latvia and Machine Learning-Based Evaluation of Urinary Exosomal MicroRNAs as Diagnostic Biomarkers

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Abstract

Bladder cancer (BC) is the most prevalent malignancy of the urinary system and ranks as the tenth most common cancer worldwide. Recent data indicate a significant increase in incidence, with hundreds of thousands of new cases diagnosed annually. Based on GLOBOCAN data, bladder cancer ranked 7th among cancers in Latvia in 2022, with 521 new cases. BC was the seventh most prevalent cancer in the world. The global burden of bladder cancer is considerable, with millions of individuals living with the disease. The overall global five-year prevalence was estimated at 1 700 000 in 2020. There is a substantial number of deaths attributed to it each year, making it the 13th leading cause of cancer mortality. Due to the main risk factors – smoking and chemical exposures – associated with BC, it is considered a largely preventable and avoidable cancer. BC is a heterogeneous disease with highly variable clinical, anatomic, and molecular pathology features, along with a high mutation burden. In recent years, microRNAs (miRNAs) have emerged as crucial regulators of cellular activities, and their dysregulation has been associated with the onset and progression of cancer.

Although there are many studies on BC around the world, there is still no comprehensive analysis of the epidemiological trends and long-term survival rates of bladder cancer in Latvia, nor has there been an examination of the potential of exosomal microRNAs (miRNAs) as biomarkers for the diagnosis of BC.

The aim of my PhD thesis was to carry out a population-based study on the epidemiological trends of bladder cancer in Latvia from 1990 to 2017, and to evaluate the diagnostic potential of urinary exosomal miRNAs in combination with clinical and laboratory data using machine learning approaches. The study also aimed at improving the understanding of cell-to-cell communication between bladder cells in patients with low-grade (LG) and high-grade (HG) BC, and the possible impact of the urinary exosomal miRNAs on the oncotransformation of normal cells.

The results of the study showed that bladder cancer incidence, prevalence, and mortality increased in Latvia between 1990 and 2017. Findings revealed that the age-standardised incidence rate of bladder cancer increased from 6.8 per 100 000 in 1990 to 10.8 per 100 000 in 2017, with a statistically significant rise (APC 2.6 %) from 1990 to 2014, followed by a minor and statistically insignificant decline through 2017 (APC −3.6 %). The prevalence rates increased from 25.2 in 1990 to 69.7 in 2017, with an AAPC of 3.8 % (95 % CI 3.1 to 4.6) during the whole period. A statistically significant reduction was observed only in the ≥ 80-year age group, with an APC of −7.6% from 2013 to 2017. Mortality rates also increased from 3.9 to 4.4 per 100,000 during the same period, although a notable decrease was observed in the 40–59 age group, with an annual percentage change of −1.1%. Additionally,

relative survival rates improved from 55.0% during 1990–2000 to 59.0% in the years 2013–2017, suggesting advancements in treatment or early detection strategies. These epidemiological findings – particularly the rising incidence and the persistent burden in older age groups – underscored the need for more sensitive, non-invasive diagnostic tools, motivating the subsequent investigation of urinary exosomal miRNAs as candidate biomarkers.

Striking differences in the miRNA cargo of exosomes from the BC group as compared to the control group were identified. Among the up-regulated exosomal miRNAs, 7 miRNAs in the LG group and 3 miRNAs in the HG group were identified as directly targeting *TP53*. Twenty-two and 17 *PTEN*-targeting miRNAs were observed among the up-regulated miRNAs of the LG and HG groups, respectively. The target genes of these exosomal miRNAs and their interaction network predicted that *TP53* is the strongest hub gene in both BC exosomal miRNA networks. Further, the possible role of exosomal miRNAs in synchronous BC development was discussed. Building on these differentially expressed candidates, 11 miRNAs were selected based on statistical significance and fold regulation to test whether combining these miRNAs with routine clinical and laboratory data improves diagnostic accuracy compared with using either data type alone.

Machine learning was performed using the 11 specially selected miRNAs in 152 samples (89 bladder cancer patients and 63 controls), selected from an initial 210-participant cohort by applying the criterion of complete clinical data availability. Statistical analysis identified notable miRNAs, such as mir-455-3p, mir-30b-3p, and mir-429, as potential biomarkers. This study highlighted the potential of combining miRNA expression levels with laboratory tests and clinical data for improved BC diagnostics. The SVM classifier achieved the best performance on the combined miRNA and clinical dataset, with an AUC of 0.85, sensitivity of 0.76, and specificity of 0.76, demonstrating the effectiveness of the multimodal approach. Feature importance analysis, performed independently for all three classifiers (Random Forest, SVM, and XGBoost), consistently identified erythrocyturia in urine tests and patient age as the most discriminating clinical predictors, alongside important miRNAs such as miRNA-20a-3p, miRNA-324-5p, and miRNA-339-5p, which were ranked among the top features by all three models. These findings indicate that miRNA expression levels can complement traditional clinical tests and support the development of multimodal non-invasive diagnostic approaches for bladder cancer detection.

Overall, this comprehensive study highlights the alarming trends in bladder cancer epidemiology in Latvia and demonstrates the potential of urinary exosomal miRNAs combined with clinical and laboratory data as a multimodal non-invasive diagnostic approach. The findings suggest that the integration of liquid biopsy biomarkers with machine learning

may provide a broader insight into tumour-related molecular processes compared to a single-site biopsy and complement existing diagnostic approaches in bladder cancer.

Keywords: Bladder cancer, epidemiology, biomarkers, diagnostics, miRNAs

Anotācija

Urīnpūšļa vēža epidemioloģija Latvijā un urīna eksosomālo mikroRNS kā diagnostisko biomarķieru izvērtējums, izmantojot mašīnmācīšanās modeļus

Urīnpūšļa vēzis (UV) ir visizplatītākā ļaundabīgā slimība urīnceļos un ieņem desmito vietu pasaulē izplatītāko audzēju sarakstā. Jaunākie dati liecina, ka urīnpūšļa vēža incidence ievērojami pieaug, diagnosticējot simtiem tūkstošu jaunu gadījumu katru gadu. Saskaņā ar *GLOBOCAN* datiem 2022. gadā urīnpūšļa vēzis Latvijā ieņēma 7. vietu starp citiem ļaundabīgajiem audzējiem, reģistrējot 521 jaunu gadījumu. Globālā līmenī urīnpūšļa vēzis ir septītais visizplatītākais vēzis, ar kopēju piecu gadu prevalenci apmēram 1,7 miljonos gadījumu 2020. gadā. Katru gadu no UV mirst ievērojams skaits cilvēku, un tas ierindojas 13. vietā starp galvenajiem vēža izraisītajiem mirstības iemesliem. Ņemot vērā galvenos riska faktorus – smēķēšanu un ķīmisku vielu iedarbību –, kas saistīti ar UV, to uzskata par lielā mērā novēršamu ļaundabīgu audzēju.

UV ir heterogēna slimība ar ļoti mainīgām klīniskām, anatomiskām un molekulārās patoloģijas pazīmēm un izteiktu mutāciju slodzi. Pēdējos gados mikro-RNS (miRNS) ir atzītas par svarīgiem šūnu aktivitātes regulētājiem, un to disregulācija ir saistīta ar vēža attīstību un progresēšanu. Neskatoties uz plašo pētījumu skaitu par UV pasaulē, Latvijā joprojām nebija veikta visaptveroša analīze par epidemioloģiskajām tendencēm un ilgtermiņa izdzīvošanas rādītājiem, kā arī par eksosomālo mikro-RNS potenciālu kā biomarķieriem urīnpūšļa vēža diagnostikā.

Promocijas darba mērķis bija veikt populācijā balstītu pētījumu par urīnpūšļa vēža epidemioloģiskajām tendencēm Latvijā no 1990. līdz 2017. gadam un identificēt potenciālos biomarķierus slimības diagnostikai. Tāpat pētījums vērsts uz to, lai uzlabotu izpratni par šūnu savstarpējo komunikāciju pacientiem ar zemas pakāpes (*low-grade*, LG) un augstas pakāpes (*high-grade*, HG) urīnpūšļa vēzi, kā arī noskaidrotu eksosomālo miRNS iespējamo ietekmi naivo (normālo) šūnu onkotransformācijā.

Pētījuma rezultāti liecina, ka UV incidence, prevalence un mirstība Latvijā ir palielinājusies no 1990. līdz 2017. gadam. Tika konstatēts, ka urīnpūšļa vēža vecumstandartizētā incidence pieauga no 6,8 gadījumiem uz 100 000 iedzīvotāju 1990. gadā līdz 10,8 gadījumiem 2017. gadā ar statistiski nozīmīgu ikgadējo pieaugumu (APC 2,6%) no 1990. līdz 2014. gadam, kam sekoja neliels un statistiski nenozīmīgs samazinājums līdz 2017. gadam (APC −3,6%). Prevalences rādītāji palielinājās no 25,2 uz 100 000 iedzīvotāju

1990. gadā līdz 69,7 gadījumiem 2017. gadā vidējām gada procentuālajām izmaiņām (AAPC) visā periodā sasniedzot 3,8% (95% TI: 3,1 līdz 4,6). Tikai iedzīvotāju grupā vecumā virs 80 gadiem tika novērots statistiski nozīmīgs samazinājums ar ikgadējo procentuālo izmaiņu −7,6% no 2013. līdz 2017. gadam.

Mirstības rādītāji šajā periodā pieauga no 3,9 uz 4,4 gadījumiem uz 100 000, lai gan 40–59 gadu vecuma grupā tika novērots ievērojams samazinājums ar ikgadējo procentuālo izmaiņu −1,1 %. Turklāt relatīvās dzīvildzes rādītāji uzlabojās no 55,0 % (1990.–2000.) līdz 59,0% (2013.–2017.), kas norāda uz terapeitisko vai agrīnas diagnosticēšanas stratēģiju uzlabošanos. Šie epidemioloģiskie rezultāti – īpaši pieaugošā incidence un nemainīgi augstā slimības slodze vecāka gadagājuma cilvēku grupās – uzsvēra nepieciešamību pēc jutīgākiem un neinvazīviem diagnostikas rīkiem un pamatoja tālāko urīna eksosomālo miRNS kā potenciālo biomarķieru izpēti.

Salīdzinot eksosomas UV grupā ar kontroles grupu, tika konstatētas ievērojamas atšķirības miRNS komponentēs. Starp paaugstināti ekspresētām eksosomālajām miRNS tika identificēti 7 miRNS zemas malignitātes pakāpes audzēja (LG) grupā un 3 miRNS augstas maliginātes pakāpes audzēja (HG) grupā, kas ir tieši mērķētas pret *TP53* gēnu. LG grupā tika konstatēti 22 miRNS, kas mērķētas pret *PTEN*, bet HG grupā – 17 miRNS. Šo eksosomālo miRNS mērķa gēni un to mijiedarbības tīkls norādīja, ka *TP53* ir spēcīgākais mezgla gēns abu UV grupu eksosomālo miRNS tīklos. Turklāt tika izvērtēta eksosomālo miRNS iespējamā loma sinhrona UV attīstībā. No atšķirīgi ekspresētajām miRNS turpmākai analīzei tika atlasītas 11 statistiski nozīmīgākās, lai pārbaudītu, vai šo miRNS apvienošana ar klīniskajiem un laboratoriskajiem datiem nodrošina augstāku diagnostisko precizitāti nekā katrs datu veids atsevišķi.

Mašīnmācīšanās analīze tika veikta ar 11 izvēlētajām miRNS 152 paraugos (89 UV pacienti un 63 kontroles personas), kas atlasīti no sākotnējās 210 dalībnieku kohortas, pamatojoties uz pilnīgu klīnisko datu pieejamību. Statistiskā analīze identificēja šādus nozīmīgus miRNS kā potenciālos biomarķierus – miR-455-3p, miR-30b-3p un miR-429. Šis pētījums izcēla miRNS ekspresijas līmeņu potenciālo apvienošanu ar laboratorisko testu un klīniskajiem datiem, lai uzlabotu urīnpūšļa vēža diagnostiku. Apvienojot miRNS ekspresijas līmeņus ar klīniskajiem datiem, SVM klasifikators sasniedza AUC vērtību 0,85 ar jutīgumu 0,76 un specifitāti 0,76, demonstrējot multimodālās pieejas efektivitāti. Funkciju nozīmes (*feature importance*) analīze kā svarīgākos klīniskos prognozētājus identificēja hematūriju un eritrocitūriju, bet kā nozīmīgākās miRNS – miR-20a-3p, miR-429 un miR-30b-3p. Šie rezultāti norāda, ka miRNS ekspresijas analīze var būtiski papildināt tradicionālos klīniskos testus, nodrošinot spēcīgu neinvazīvu rīku vēža atklāšanai.

Kopumā šis visaptverošais pētījums atklāj satraucošas UV epidemioloģiskās tendences Latvijā un perspektīvu urīna eksosomālo miRNS lomu kā diagnostiskiem biomarķieriem. Šādas pieejas ieviešana ne tikai palielinētu diagnostikas jutīgumu un specifitāti salīdzinājumā ar pašlaik pieejamajām metodēm, bet arī ļautu precīzāk izvērtēt audzēja bioloģisko tipu, sniedzot visaptverošāku informāciju nekā viena punkta biopsija, tādējādi nodrošinot savlaicīgu un personalizētu ārstēšanu.

Atslēgvārdi: urīnpūšļa vēzis, epidemioloģija, biomarķieri, diagnostika, miRNS.

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Abbreviations used in the Thesis

AAPC	Average annual percentage change
ADAM12	ADAM metallopeptidase domain 12
AI	Artificial intelligence
AJCC	American Joint Commission on Cancer
AKT pathway	AKT serine/threonine kinase signalling pathway
AKT1	AKT serine/threonine kinase 1
APC	Annual percentage change
APP	Amyloid beta precursor protein
AR	Androgen receptor
ASR	Age-standardised rates
ATM	ATM serine/threonine kinase
AUC	Area under the curve
BC	Bladder cancer
BCG	*Bacillus Calmette-Guérin*
BCL2L1	BCL2 like 1
BDNF	Brain-derived neurotrophic factor
BRCA1	Breast cancer type 1 susceptibility gene
BTA	Bladder tumour antigen
CAFs	Cancer-associated fibroblasts
CASP3	Caspase 3
CCNA2	Cyclin A2
CCND1	Cyclin D1
CDC42	Cell division cycle 42
CDH1	Cadherin 1 (E-cadherin)
CDH2	Cadherin 2 (N-cadherin)
CDK1	Cyclin-dependent kinase 1
CDK2	Cyclin-dependent kinase 2
CDK6	Cyclin-dependent kinase 6
CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21)
CDKN2A	Cyclin-dependent kinase inhibitor 2A
CDPC	Centre for Disease Prevention and Control
CI	Confidence interval
CIS	Carcinoma in situ
COPS5	COP9 signalosome subunit 5
CREB1	cAMP responsive element binding protein 1

CSB	Central Statistical Bureau of Latvia
CSS	Cancer-specific survival
CT	Computed tomography
CTNNB1	Catenin beta 1
CUL1	Cullin 1
DCO	Death certificate only
DICER1	Dicer 1, ribonuclease III
DNA	Deoxyribonucleic acid
DNMT1	DNA methyltransferase 1
DOI	Digital object identifier
EAU	European Association of Urology
EFTUD2	Elongation factor Tu GTP binding domain containing 2
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay
EP300	E1A binding protein p300
EPC	Edge Percolated Component (CytoHubba)
ERBB2	Erb-B2 receptor tyrosine kinase 2 (*HER2*)
ESMO	European Society for Medical Oncology
ESR1	Estrogen receptor 1
EVs	Extracellular vesicles
exomiRs	Exosomal miRNAs
EZH2	Enhancer of zeste homolog 2
FGF2	Fibroblast growth factor 2
FGFR	Fibroblast growth factor receptor
FGFR3	Fibroblast growth factor receptor 3
FISH	Fluorescence in situ hybridisation
FN1	Fibronectin 1
FOXO1	Forkhead box O1
FOXO3	Forkhead box O3
FR	Fold regulation
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GCO	Global Cancer Observatory
GLOBOCAN	Global Cancer Observatory database
GRB2	Growth factor receptor bound protein 2
GSK3B	Glycogen synthase kinase 3 beta
H2BC21	H2B clustered histone 21

HDAC2	Histone deacetylase 2
HDI	Human Development Index
HG	High-grade
HGF	Hepatocyte growth factor
HIF1A	Hypoxia-inducible factor 1 alpha
HRAS	Harvey rat sarcoma viral oncogene homolog
HSP90AA1	Heat shock protein 90 alpha family class A member 1
HSPA8	Heat shock protein family A (Hsp70) member 8
IARC	International Agency for Research on Cancer
ICD-10	International Classification of Diseases, 10th Revision
IGF1	Insulin-like growth factor 1
IGF1R	Insulin-like growth factor 1 receptor
IL1B	Interleukin 1 beta
IL6	Interleukin 6
JUN	Jun proto-oncogene
KAT2B	Lysine acetyltransferase 2B
KDR	Kinase insert domain receptor (VEGFR-2)
KEGG	Kyoto Encyclopedia of Genes and Genomes
KIT	KIT proto-oncogene receptor tyrosine kinase
KRAS	Kirsten Ras oncogene homolog
LCR	Latvian Cancer Registry
LG	Low-grade
LNA	Locked nucleic acid
MAPK	Mitogen-activated protein kinase
MAPK1	Mitogen-activated protein kinase 1
MAPK14	Mitogen-activated protein kinase 14
MAPK8	Mitogen-activated protein kinase 8
MCC	Maximal Clique Centrality (CytoHubba)
MCC	MCC regulator of WNT signalling pathway (gene)
MCL1	MCL1 apoptosis regulator, BCL2 family member
MIBC	Muscle-invasive bladder cancer
miRNAs	Microribonucleic acids
MMP2	Matrix metallopeptidase 2
MNC	Maximum Neighborhood Component (CytoHubba)
MRI	Magnetic resonance imaging
mRNA	Messenger RNA

MTOR	Mechanistic target of rapamycin kinase
MVAC	Methotrexate, vinblastine, doxorubicin, cisplatin
MVB	Multivesicular body
MYC	*MYC* proto-oncogene
NCCN	National Comprehensive Cancer Network
NFKBIA	NFKB inhibitor alpha
NHS	National Health Service
NMIBC	Non-muscle invasive bladder cancer
NMP22	Nuclear matrix protein 22
NOTCH1	Notch receptor 1
NPV	Negative predictive value
OS	Overall survival
PABPC1	Poly(A) binding protein cytoplasmic 1
PCR	Polymerase chain reaction
PFS	Progression-free survival
PI3K	Phosphoinositide-3-kinase
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PIK3R1	Phosphoinositide-3-kinase regulatory subunit 1
POLR2A	RNA polymerase II subunit A
PPARG	Peroxisome proliferator-activated receptor gamma
PREDA	Registry of Patients with Particular Diseases
PTEN	Phosphatase and tensin homolog
PTGS2	Prostaglandin-endoperoxide synthase 2 (COX-2)
PTPN11	Protein tyrosine phosphatase non-receptor type 11
RAB5A	RAB5A, member *RAS* oncogene family
RACK1	Receptor for activated C kinase 1
RC	Radical cystectomy
RHOA	Ras homolog family member A
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
RPS3	Ribosomal protein S3
RPS6KB1	Ribosomal protein S6 kinase B1
RS	Relative survival
RT-PCR	Reverse-transcription PCR
RT-*q*PCR	Reverse transcription quantitative PCR
RUVBL1	RuvB like AAA ATPase 1

SD	Standard deviation
SIRT1	Sirtuin 1
SMAD2	SMAD family member 2
SMAD3	SMAD family member 3
SMAD4	SMAD family member 4
SMARCA4	SWI/SNF-related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4
SOX2	SRY-box transcription factor 2
SRC	SRC proto-oncogene, non-receptor tyrosine kinase
SRSF1	Serine and arginine rich splicing factor 1
STAT1	Signal transducer and activator of transcription 1
STAT3	Signal transducer and activator of transcription 3
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
SYBR	SYBR Green fluorescent dye
TERT	Telomerase reverse transcriptase
TGFB1	Transforming growth factor beta 1
TLR4	Toll-like receptor 4
TME	Tumour microenvironment
TNF	Tumour necrosis factor
TNM	Tumour, Node, Metastases (staging system)
TP53	Tumour protein p53
TURBT	Transurethral resection of bladder tumour
UBA52	Ubiquitin A-52 residue ribosomal protein fusion product 1
UBC	Ubiquitin C
UBC test	Urinary Bladder Cancer test (urinary protein marker)
UMAP	Uniform manifold approximation and projection
UTR	Untranslated region
VCP	Valosin containing protein
VEGFA	Vascular endothelial growth factor A
WHO	World Health Organization
YPLL	Years of potential life lost

Introduction

Bladder cancer (BC) is a significant health challenge and is the tenth most common cancer worldwide (Bray et al., 2018). In approximately 30 % of cases, BC is diagnosed at a late stage when the tumour has already grown into the muscle layer. It is characterised by a high metastatic risk and, despite new treatment strategies, correlates with high mortality rates (Kaufman et al., 2009; Xie et al., 2017). Moreover, Latvia ranks second after Poland in terms of male mortality among European countries (Antoni et al., 2017). Bladder cancer prognosis varies markedly with stage at diagnosis, with five-year survival decreasing from over 75 % in stage I disease to under 10 % in metastatic disease (Antoni et al., 2017).

Effective diagnosis of bladder cancer is imperative for improving patient outcomes, yet the currently available non-invasive gold-standard methods for diagnosing and following-up BC are apparently insufficient and have limited sensitivity. Urinary cytology has high sensitivity in the high-grade tumours including *carcinoma in situ* but may be negative in low-grade tumours (European Association of Urology [EAU], 2026), which can lead to missed diagnoses. In contrast, cystoscopy is a relatively expensive and invasive diagnostic method, despite the development of new technologies such as fluorescence and *narrow-band* imaging. Furthermore, it is not usually used in daily practice in patients without lower urinary tract symptoms or haematuria (Hong, 2016). *European guidelines* (EAU, 2026) consider the use of some specific biomarkers like UroVysion (FISH), microsatellite analysis, nuclear matrix protein 22, fibroblast growth factor receptor (FGFR)3/telomerase reverse transcriptase (TERT), Cx-Bladder, ADX-BladderTM, Xpert Bladder® and EpiCheck™, CellDetect® for better diagnosis and disease recurrence (Crocetto et al., 2022; EAU, 2026). The main problem is that a number of non-invasive urine tests have failed to meet clinical expectations, because their diagnostic specificity is inferior to that of urinary cytology tests (Crocetto et al., 2022), and none of them has been accepted into routine practice by any clinical guidelines for primary diagnosis. Therefore, novel biomarkers for the diagnosis of BC need to be found and exosomal miRNAs (exomiRs) could fulfil this role.

Exosomes are small extracellular vesicles (EVs) ranging from 30 to 150 nm in diameter and produced by a variety of cells, including tumour cells, which can be found in a multitude of biological fluids (Mathieu et al., 2019; Wu et al., 2021). Although exosomes were recently considered to be a cellular waste bin, a growing number of studies now consider them to be important facilitators of intercellular communication and, thereby participants in the regulation of various biological processes, including cancer (Challagundla et al., 2015).

When exosomes are internalised by recipient cells, they can influence cellular behaviour by engaging in receptor-mediated interactions or by delivering their biologically active cargo,

which comprises proteins, transcription factors, messenger RNAs (mRNAs), and microRNAs (miRNAs) (Milane et al., 2015).

MiRNAs are small, non-coding RNAs that have been shown to play an important role in tumourigenesis (Weber et al., 2010). MiRNAs bind to the 3′-UTR of target mRNAs and, thus, post-transcriptionally regulate gene expression, which leads to the degradation of the target mRNA or inhibition of its translation (Shukla et al., 2011). Notably, miRNAs demonstrate greater stability than mRNAs in various samples, including urine, making them less prone to degradation by nucleases due to their small size (Tilki et al., 2011; Weber et al., 2010).

Moreover, exosomal microRNAs demonstrate significantly greater stability compared to microRNAs present in plasma. This inherent stability allows exosomal microRNAs to endure multiple freeze-thaw cycles, remaining practically indistinguishable from freshly obtained samples (Jacob et al., 2018). Considering the miRNA dual roles in tumour promotion and suppression, they are regarded as promising biomarkers for cancer diagnosis and prognosis (Ling et al., 2016).

Multiple studies have sought miRNA-based biomarkers for bladder cancer, but results rarely overlap across cohorts; most miRNAs are reported in only one or two studies (notable exception: miR-96-5p) (Grimaldi et al., 2022; Aveta et al, 2023). Despite this variability, a recent large mixed-cohort study developed a serum miRNA screening model using machine learning, achieving high accuracy for early BC detection (Lai et al, 2024). Similar pan-cancer work supports the diagnostic potential of circulating miRNAs (Wu et al, 2024). Beyond expression, miRNA editing events have been implicated in recurrent BC and may represent prognostic or therapeutic targets (Qin et al., 2022). Prognostic modelling has also used miRNA signatures to estimate survival (e.g., BLC-SVR with optimal feature selection), and several reviews highlight urinary miRNAs as promising diagnostic tools capable of discriminating cancer from non-cancer states (Grimaldi et al., 2022).

While blood sampling is invasive and resource-intensive, urinalysis is non-invasive, convenient, and requires no specialised personnel. As shown in this thesis, several urinary exosomal miRNAs were elevated in bladder cancer compared with matched controls. To better understand cell-to-cell communication in low-grade (LG) and high-grade (HG) disease and the potential impact of urinary exosomal miRNAs on the oncotransformation of normal cells, differentially expressed urinary exosomal miRNAs were profiled. Marked differences in exosomal miRNA cargo between cancer and control groups were identified. In this thesis, urinary miRNA levels were integrated with demographic, routine laboratory, and clinical data

using machine learning, demonstrating that multimodal data fusion can improve diagnostic performance.

Ongoing research aims to address the shortcomings of prior studies by incorporating machine learning and artificial intelligence. One effective approach is the evolutionary learning method, CancerSig, introduced by Yerukala Sathipati et al. (2023) , which couples the IBCGA feature-selection algorithm with a Support Vector Machine (SVM) classifier and a pan-cancer analysis of miRNA signatures to discriminate early- from advanced-stage disease across 15 cancer types with strong accuracy. Multimodal strategies have shown comparable promise specifically for bladder cancer: *Moisoiu et al.* combined urinary miRNA profiling with surface-enhanced Raman spectroscopy (SERS) in a liquid-biopsy setting, reporting improved point-of-care diagnosis and molecular stratification (Moisoiu et al., 2022). Building on this direction, the present study integrates urinary exosomal miRNA expression with demographic data, routine laboratory results, and clinical features in a machine-learning model, hypothesising that fusing these data sources improves the diagnostic accuracy of non-invasive bladder cancer detection.

Such an approach may enhance diagnostic performance and provide a broader view of tumour biology compared to single-site biopsy, thereby supporting more informed and timely clinical decision-making.

Aim of the Thesis

The aim of this study was to analyse epidemiological trends of bladder cancer in Latvia, and to evaluate the diagnostic potential of urinary exosomal miRNAs in combination with clinical and laboratory data using machine learning approaches, while also improving the understanding of cell-to-cell communication between bladder cells in patients with low-grade (LG) and high-grade (HG) bladder cancer and the possible role of urinary exosomal miRNAs in the oncotransformation of normal cells.

Objectives of the Thesis

1 To determine the dynamics of bladder cancer incidence, prevalence, and mortality in Latvia from 1990 to 2017, overall and by age and sex groups.
2 To determine the dynamics of overall and cancer-specific survival of bladder cancer patients in Latvia from 1990 to 2017.
3 To identify differentially expressed, clinically significant urinary exosomal miRNA biomarkers and evaluate their potential molecular targets.

4 To apply machine learning approaches for the evaluation of urinary exosomal miRNAs in combination with clinical and laboratory data as a multimodal, non-invasive diagnostic approach for bladder cancer.

Hypotheses of the Thesis

1 Between 1990 and 2017, bladder cancer incidence, prevalence, and mortality increased in Latvia, while survival outcomes improved and the proportion of advanced-stage bladder cancer cases decreased.
2 There is a significant difference in the expression of urinary exosomal miRNAs between bladder cancer patients and controls.
3 There is a significant difference in the expression levels of specific urinary exosomal miRNAs between patients with different tumour grades and stages.

Novelty of the Thesis

In this work, a large, population-based analysis of bladder cancer in Latvia from 1990 to 2017 was performed. The following epidemiological indicators of BC were evaluated:

- Incidence, prevalence, mortality rates overall and by age groups;
- Relative, overall, and cancer-specific survival, over the whole period and according to T-stage, including separate rates for early and advanced T-stages tumours, as well as by year of diagnosis.

These data complement and update the existing information on bladder cancer epidemiological trends in Latvia.

Differentially expressed urinary exosomal miRNAs were identified between the study and control groups in a Latvian population cohort, revealing potential associations with tumour-related molecular pathways and their possible involvement in cell-to-cell communication and oncotransformation processes.

Furthermore, this study integrated urinary exosomal miRNA expression profiles with clinical and laboratory data using machine learning approaches, demonstrating the potential of a multimodal, non-invasive diagnostic framework for bladder cancer detection.

Author's contribution

The author was responsible for developing the study concept and planning, exosome isolation from urine, miRNA profiling, statistical data analysis, interpretation of results, and writing this dissertation. The author led the collection of urine samples and clinical data at the Pauls Stradiņš Clinical University Hospital in collaboration with colleagues from Riga East University Hospital, who assisted with sample collection and the acquisition of clinical data.

The machine learning analysis was conducted in collaboration with researchers from Riga Technical University. This research was supported by the BioPhoT project LiqBio-BC: Liquid biopsy biomarkers for bladder cancer diagnostics (Project No. OSI_PIP_BioPhoT-2025/1-0038), the State Research Program in the field of biomedicine, medical technologies, and pharmacy (No. VPP-EM-BIOMEDICĪNA-2022/1-0001), the Latvian Council of Science (grant No. LZP-2020/2-0104), and an internal grant from Rīga Stradiņš University (No. RSU/PP/2020-07). The author assumes responsibility for the integrity of the work as a whole.

Artificial intelligent (AI) Disclosure / Large language model (LLM) statement

Given that the author is not a native English speaker, AI-based language models and editing tools were employed to refine the grammatical and stylistic aspects of this PhD Thesis. These technologies facilitated improvements in language use and readability but did not supplant essential authorial functions, such as the generation of scientific or medical insights and the derivation of conclusions. The author has thoroughly reviewed and revised the manuscript as required, ensuring the veracity and integrity of the research presented.

1 Literature review

1.1 Urinary bladder anatomy and embryology

The urinary bladder is an extraperitoneal hollow organ that lies under the pubic symphysis when empty and extends into the abdomen when filled with urine (Morton et al., 2018). The bladder develops following the craniocaudal division of the cloaca into the urogenital sinus anteriorly and the anorectal canal posteriorly by the the urorectal septum at 3 to 7 weeks of gestation. The caudal aspect of the urorectal septum forms the perineal body. The urogenital triangle is located in front of the perineal body and in the rear of the anal triangle (Adam et al., 2020).

The two main functions of the urinary bladder are urine storage and emptying. The normal bladder volume is about 500 mL (Sellers et al., 2018).

There are four layers of the bladder wall (Partin et al., 2020a):

1) mucosa, which in an empty bladder forms many folds;
2) submucosa (absent in the triangular region of the bladder, where the *tela mucosa* and *tunica muscularis* are tightly interconnected and do not form folds);
3) muscle layer – the bladder muscle (*m. detrusor*) is made up of mucous membrane musculature in three layers, which is necessary for the bladder to perform both the function of urine storage and urine elimination (contracting during voiding);
4) connective tissue sheath (*tunica adventitia* or *tunica serosa*).

1.2 Bladder cancer definition

Bladder cancer is malignant tumour that arises from the tissue of the inner wall of the urinary bladder due to abnormal growth of cells (National Cancer Institute, n.d.). Transitional cell carcinoma is the most common type of bladder cancer, accounting for 90 % of all cases. Less prevalent types are squamous cell BC (2 %–5 %), adenocarcinoma (0.5 %–2 %) and small cell carcinoma (< 1 %) (Chalasani et al., 2009; Shokeir, 2004; Ye et al., 2014).

1.3 Epidemiology of bladder cancer

Bladder cancer (BC) is the most common malignancy of the urinary system and is the tenth most common cancer globally. According to data from the Global Cancer Observatory (GCO), it is the tenth most common cancer worldwide (Teoh et al., 2020). During the past six years, the incidence of BC has increased to 549 393 cases annually. According to GCO, the estimated number of five-year prevalent cases of BC rose to 1 648 482 in 2018 worldwide. It ranks 13th in cancer-related mortality (199 922 estimated number of deaths in 2018) (Bray et al., 2018). Approximately 70 % of individuals diagnosed with bladder cancer are over the age of 65 (Bellmunt et al., 2014). BC predominantly affects men with average ratio 3–4:1

(men to women) (Antoni et al., 2017). A higher incidence, as well as higher cancer mortality rates, are found in countries with a high Human Development Index (HDI). The highest rates are in countries within East Asia, North America, and Western Europe, such as the United States of America, China, Japan and Germany (Wong et al., 2018). BC typically occurs in the older population, with a median age of 69 years in men and 71 years in women at the time of diagnosis (Scosyrev et al., 2009). The highest number of newly diagnosed bladder cancer cases occurred in individuals over the age of 70 years compared to those aged 60 to 69 years in 2013, representing 56.5% (226 612) and 24.0%, respectively (Dy et al., 2017). Current smoking is associated with younger age of onset compared to never smokers (Hinotsu et al., 2009). The predominant histological type of BC in Europe and the United States is urothelial or transitional cell carcinoma, accounting for approximately 90 % of bladder cancer cases. However, in countries with common schistosomiasis infections, squamous cell bladder cancer is more prevalent (Chalasani et al., 2009).

In addition, the overall increase of life expectancy is the current demographic trend in many Western European countries (Leon, 2011). Although life expectancy in Latvia also increased by almost 20 years between 1924 and 2018, it is still very low compared to the rest of Europe. In 2018, the life expectancy of men in Latvia was the lowest in the European Union (Centrālā statistikas pārvalde, 2020).

There are some articles on bladder cancer incidence and mortality both in Europe and worldwide, with some mention of Latvia (Antoni et al., 2017) (Wong et al., 2018) . However, there is still no comprehensive analysis of the epidemiological trends and long-term survival rates of bladder cancer in Latvia.

1.4 Bladder cancer classification

In the following sections, a detailed classification of bladder cancer (BC) will be provided. BC diagnosis predominantly relies on histological evaluation, which underpins the histological WHO and TNM (tumour, node, metastasis) classification systems (See Tables 1.1, 1.2 and 1.3.)

Table 1.1

AJCC/ TNM * Staging of Bladder Cancer

Stage	Tumour	Regional Lymph Node Metastasis	Distant Metastasis
Oa	Ta	N0	M0
Ois	Tis	N0	M0
I	T1	N0	M0
II	T2a, T2b	N0	M0
IIIA	T3A, T3B, T4a	N0	M0
	or		
	T1, T2, T3, T4	N1	M0
IIIB	T1, T2, T3, T4a	N2, N3	M0
IVA	T4b	Any N	M0
	or		
	Any T	Any N	M1a
IVB	Any T	Any N	M1b

AJCC = American Joint Commission on Cancer; T = primary tumour; N = regional lymph node metastasis; M = distant metastasis. Table adapted from (Amin et al., 2018)

* For AJCC/TNM definitions, see Table 1.2 TNM Definitions for Bladder Cancer.

Table 1.2

TNM Clinical classification of Bladder Cancer

Stage	Description	
T - Primary Tumour	The suffix (m) should be added to the appropriate T category to indicate multiple tumours. The suffix (is) may be added to any T to indicate presence of associated carcinoma in situ.	
	Tx	Primary tumour cannot be assessed
	T0	No evidence of primary tumour
	Ta	Non-invasive papillary carcinoma
	Tis	Carcinoma in situ: ‘flat tumour’
	T1	Tumour invades subepithelial connective tissue
	T2	Tumour invades muscularis propria
	T2a	Tumour invades superficial muscularis propria (inner half)
	T2b	Tumour invades deep muscularis propria (outer half)
	T3	Tumour invades perivesical tissue:
	T3a	microscopically
	T3b	macroscopically (extravesical mass)
	T4	Tumour invades any of the following: prostate stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall
	T4a	Tumour invades prostate stroma, seminal vesicles, uterus or vagina
	T4b	Tumour invades pelvic wall or abdominal wall
N - Regional Lymph Nodes	Nx	Regional lymph nodes cannot be assessed
	N0	No regional lymph node metastasis
	N1	Metastasis in a single lymph node in the true pelvis (hypogastric, obturator, external iliac, or presacral)
	N2	Metastasis in multiple regional lymph nodes in the true pelvis (hypogastric, obturator, external iliac, or presacral)
	N3	Metastasis in a common iliac lymph node(s)
M – Distant Metastasis	M0	No distant metastasis
	M1	M1 Distant metastasis
	M1a	Non-regional lymph nodes
	M1b	Other distant metastasis

Table adapted from (Amin et al., 2018)

1.4.1 Bladder cancer histological types

Bladder cancer is characterised by a heterogeneous array of histomorphological profiles and variable clinical patterns. While about 75 % of bladder tumours are classified as pure urothelial carcinoma (transitional cell carcinoma), the remaining 25 % encompass a variety of histological types. Numerous histological types have been systematically classified and identified over the years according to morphological characteristics (Moschini et al., 2017). Tumours of the urinary tract are summarised in Table 1.3.

Table 1.3

The 2022 WHO classification of urinary tract tumours (Amin, 2022)

Urothelial tumours	
Non-invasive urothelial neoplasms	
Urothelial papilloma	Solitary exophytic papillary neoplasm composed of slender fibrovascular cores covered by urothelium of normal thickness with normal cytological features.
Inverted urothelial papilloma	Benign neoplasm with an endophytic pushing growth pattern into the lamina propria, composed of a complex trabecular and anastomosing proliferation of sheets of urothelial cells.
Papillary urothelial neoplasm of low malignant potential (PUNLMP)	Urothelial neoplasm with papillary fibrovascular structures lined by thickened urothelium or increased cellularity, with mild cytological atypia.
Non-invasive papillary urothelial carcinoma, low-grade (LG)	Malignant urothelial neoplasm characterised by papillary structures that are confined within the basement membrane and lined by urothelial cells with mild architectural disorder and nuclear atypia.
Non-invasive papillary urothelial carcinoma, high-grade (HG)	Malignant urothelial neoplasm characterised by papillary structures lined by urothelial cells with marked architectural and cytological disorder. The neoplastic cells are confined within the basement membrane.
Urothelial carcinoma in situ (CIS)	Flat neoplastic proliferation of high-grade malignant urothelial cells of variable thickness without papillary formation. Urothelial dysplasia, an infrequently diagnosed and poorly reproducible category, encompasses changes that are thought to be neoplastic in nature but cytologically fall short of the diagnosis of carcinoma *in situ* (CIS).
Invasive urothelial neoplasms	
Invasive urothelial carcinoma	Malignant epithelial neoplasm with invasion, arising from the urothelial lining of the urinary tract.
Squamous cell carcinomas of the urinary tract	
Verrucous carcinoma of the bladder	Subtype of exophytic squamous cell carcinoma with multiple filiform projections covered by well-differentiated squamous epithelium, with an invasive front with a pushing margin.
Pure squamous carcinoma of the urothelial tract	Malignant neoplasm arising from the urothelium, showing a pure squamous phenotype on histology.

Table 1.3 continued

Urothelial tumours	
Glandular neoplasms	
Adenomas	
Villous adenoma	Rare benign neoplasm that is morphologically indistinguishable from its colonic counterpart.
Adenocarcinomas	
Adenocarcinoma not otherwise specified (NOS)	Arising from the urothelium is a malignant neoplasm showing a pure glandular phenotype on histology.
Urachal and diverticular neoplasms	
Urachal carcinoma	Malignant epithelial tumour that arises from urachal remnants. It is a diagnosis of exclusion.
Diverticular carcinoma	Carcinoma that is located in a diverticulum in the urinary tract.
Urethral neoplasms	
Urethral accessory gland carcinomas	
Littré gland adenocarcinoma	Carcinoma that is located in a diverticulum in the urinary tract.
Skene gland adenocarcinoma	Malignant tumour arising in female urethral accessory glands.
Cowper gland adenocarcinoma	Tumour of the periurethral bulbomembranous glands, with features of adenoid cystic carcinoma of the salivary glands.
Tumours of Müllerian type	
Clear cell adenocarcinoma of the urinary tract	Adenocarcinoma with cellular atypia; clear and eosinophilic cytoplasm; and tubulocystic, papillary, and solid growth patterns. It is thought to be of Müllerian origin.
Endometrioid carcinoma of the urinary tract	Adenocarcinoma of Müllerian origin with glands resembling normal endometrium.

Table adapted from (Amin, 2022)

1.5 BC pathogenesis

Bladder cancer pathogenesis involves a complex interplay of genetic, environmental, and cellular factors that lead to the development and progression of the disease. BC develops through two primary pathways (for an in-depth exploration, see Figure 1.1) (Sanli et al., 2017).

Superficial urothelial carcinoma typically arises from benign urothelium through a process of hyperplasia, which contributes only slightly (10–15%) to the progression towards high-grade non-invasive and subsequently invasive urothelial carcinoma (Lal, 2023; Zhao et al., 2016). The pathogenesis of papillary non-muscle-invasive bladder cancer (NMIBC) involves a loss of heterozygosity on chromosome 9 and mutations in oncogenes such as *FGFR3* and *TERT*, promoting hyperplasia and minimal dysplasia (Sanli et al., 2017).

In NMIBC, essential genetic changes are identified mainly in *FGFR-3*, *H-RAS*, and *PI3KCA*. *RAS* mutations typically activate the *MAPK* and *PI3K pathways*, leading to abnormal cell growth. Notably, *FGFR3* mutations, which seldom occur alongside *RAS* mutations, might share a common downstream oncogenic pathway with them. Additionally, the coexistence of

PIK3CA and *FGFR3* mutations may have a synergistic effect on tumour malignancy (Zhao et al., 2016).

Conversely, most invasive tumours likely evolve through a progression from dysplasia to flat carcinoma in situ (CIS) and onto high-grade non-invasive urinary carcinoma (Lal, 2023; Sanli et al., 2017). This process involves genetic instability that facilitates a build-up of genetic changes, pushing the progression to muscle-invasive bladder cancer (MIBC). Mutations predominantly found in tumour suppressor genes such as p53, p16, and Rb, which are crucial for regulating the cell cycle, fundamentally drive the progression to MIBC (Zhao et al., 2016). Additionally, low-grade papillary NMIBC may develop into MIBC as a result of *CDKN2A* loss (Sanli et al., 2017).

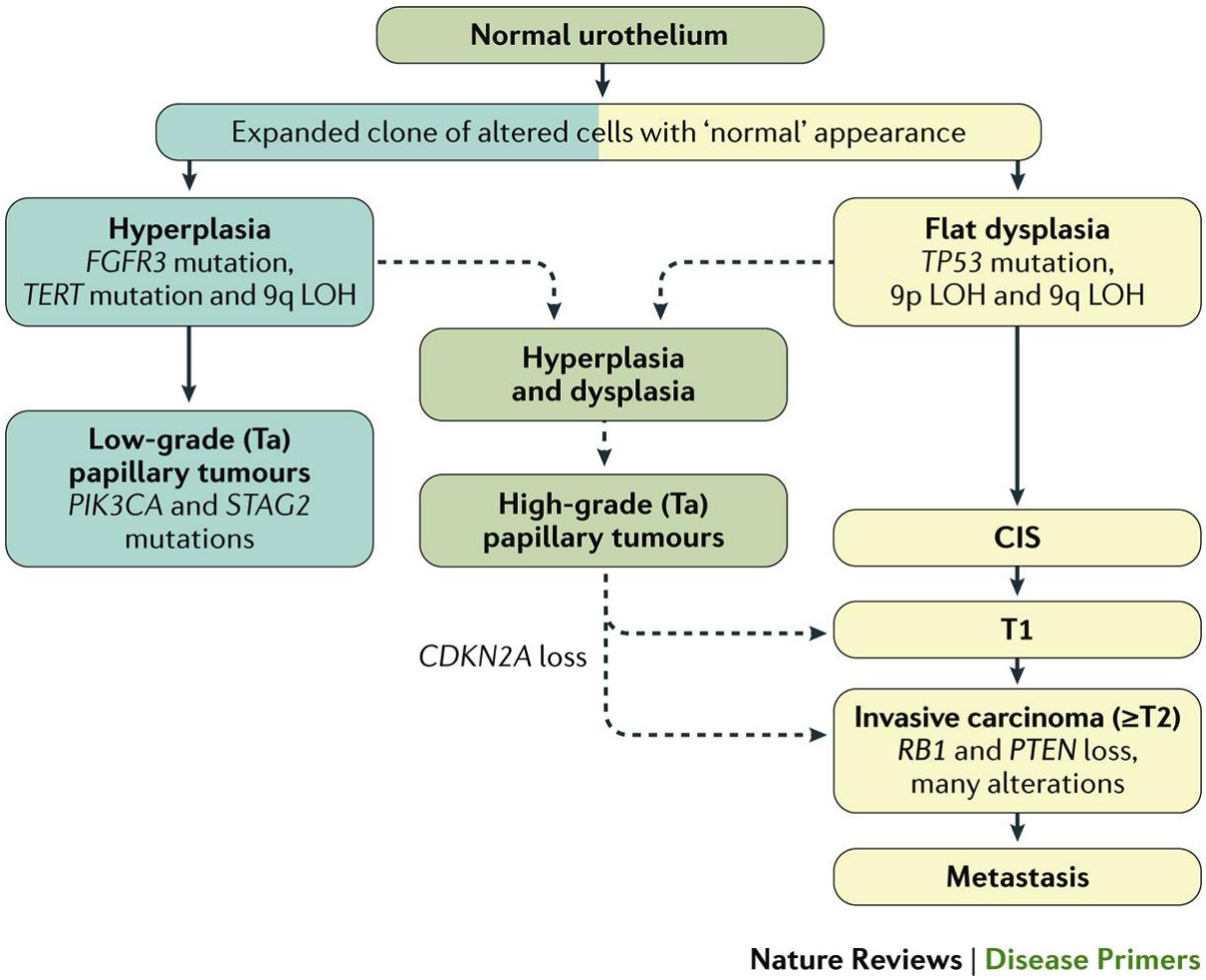


Figure 1.1 **Pathogenesis pathways**

*Two potential pathways of pathogenesis of papillary non-muscle-invasive bladder cancer (NMIBC) and solid muscle-invasive bladder cancer (MIBC) are shown.

Figure previously was adapted with permission from Knowles, M. A. & Hurst, C. D. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. *Nat. Rev. Cancer* 15, 25–41 (2015), Macmillan Publishers Limited. Reproduced with permission from Springer Nature from Sanli, O., Dobruch, J., Knowles, M. et al. Bladder cancer. *Nat Rev Dis Primers* 3, 17022 (2017) (Sanli et al., 2017).

1.6 Risk factors

In subsequent chapters examine the diverse array of factors associated with the risk of developing cancer. According to the *European Association of Urology (EAU) guidelines*, these epidemiological hazards include both modifiable and non-modifiable factors. The most important contributors are tobacco smoking and occupational exposure, followed by dietary habits, environmental exposure, genetics, pelvic irradiation, and other causes such as metabolic factors, certain drug use, and infection, such as schistosomiasis, etc (EAU, 2026).

The International Agency for Research on Cancer (IARC) has classified BC risk factors, divided into two distinct categories based on human epidemiological data: agents with sufficient evidence and those with limited evidence. which include such risk factors as tobacco smoking, various occupational and environmental exposures, medications, opium use and Schistosoma infection, while excluding genetics (see Table 1.4).

Table 1.4

BC risk factor summary according to IARC monographs

Cancer site	Carcinogenic agents with *sufficient evidence* in humans	Agents with *limited evidence* in humans
Urinary bladder	Aluminium production 4-Aminobiphenyl Arsenic and inorganic arsenic compounds Auramine production Benzidine Chlornaphazine Cyclophosphamide Firefighter (occupational exposure as a) Magenta production 2-Naphthylamine Opium consumption Painter (occupational exposure as a) Rubber manufacturing industry Schistosoma haematobium (infection with) Tobacco smoking ortho-Toluidine X- and Gamma-radiation	Acrylonitrile 4-Chloro-ortho-toluidine Coal-tar pitch Dry cleaning (occupational exposures in) Engine exhaust, diesel Hairdresser or barber (occupational exposure as a) 2-Mercaptobenzothiazole Outdoor air pollution Pioglitazone Printing processes (occupational exposures in) Soot (as found in occupational exposure of chimney sweeps) Tetrachloroethylene (Perchloroethylene) Textile manufacturing industry (work in)

Table adapted from (International Agency for Research on Cancer, 2024)

1.6.1 Tobacco smoking

Smoking is the cause of bladder tumours in around 50 % of cases. Moreover, a cohort study found that smoking has been associated with a two- to three-fold increase in the risk of bladder cancer development (Jubber et al., 2023). Compounding this, individuals who possess the *NAT2* slow acetylation polymorphism face an even greater danger due to their reduced

ability to detoxify aromatic amines, which are potent carcinogens in tobacco smoke (Max Kates & Trinity J. Bivalacqua, 2021; Partin et al., 2020b).

Adding to the complexity, the trend in nicotine consumption has shifted towards electronic cigarettes, which have gained popularity despite the overall decline in traditional smoking (Dai et al., 2022). The surge in the popularity of disposable e-cigarettes has led to a rise in smoking rates among adults, with the most significant increases observed in younger adults (Tattan-Birch et al., 2024). Several biomarkers associated with carcinogens, which are strongly linked to bladder cancer, have been detected in the urine of individuals using e-cigarettes (Bjurlin et al., 2021). Nonetheless, as e-cigarette usage is a relatively recent phenomenon, decades of systematic data collection and research are necessary to conclusively determine its correlation with cancer risks.

1.6.2 Occupational exposure

According to 2015 data from the United Kingdom, it is estimated that occupational exposure to carcinogens accounts for approximately 5.7 % of new bladder cancer cases (Brown et al., 2018). Occupations particularly susceptible to exposure to aromatic amines include those associated with manufacturing tobacco, rubber and dyes, along with professions such as leather working, hairdressing, and painting. Likewise, jobs involving polycyclic aromatic hydrocarbons, including chimney sweeping, aluminium processing, waiting, nursing, petroleum refining, and maritime activities, are also deemed at risk (Max Kates & Trinity J. Bivalacqua, 2021).

1.6.3 Genetics

Despite the identification of numerous somatic abnormalities in bladder cancer, there is a lack of evidence supporting a hereditary genetic aetiology for this malignancy. The loss of chromosomes 9p, 9q, 11p 13q, and 17q represents the most common cytogenetic abnormality observed in bladder cancer (Chan et al., 2009; Reynard et al., 2019). Significant alterations in oncogenes and tumour suppressor genes have also been observed. It has been reported that activation or amplification of oncogenes such as HRAS (formerly p21 ras), MYC, JUN, and ERBB2 plays a critical role in promoting cell proliferation and survival. On the other hand, the inactivation of tumour suppressor genes, particularly *TP53* mutations, which are associated with poor post-treatment survival, highlights the complexity of the TCC progression (Kuczyk et al., 2009). Other genes, such as retinoblastoma *(RB1)* and p16 kinase inhibitors, depend on the cyclic rate and are involved in regulating the progression and apoptosis of the cell cycle. Furthermore, a high expression of vascular angiogenic factors, in particular vascular endothelial

growth factors (*VEGFs*), has been recorded in TCCs, indicating a pro-angiogenic environment that supports tumour growth and dissemination (Reynard et al., 2019).

This information highlights a variety of genetic changes and molecular pathways that contribute to the pathogenesis and clinical outcomes of transitional cell carcinoma.

1.7 Bladder cancer diagnostic methods

The primary symptoms of bladder cancer include haematuria and symptoms of lower urinary tract issues, which typically manifest at more advanced stages of the disease. In approximately 30 % of the cases, the diagnosis is delayed, leading to a poor prognosis, because muscle-invasive BC rapidly progresses and metastasises; therefore, it correlates with a high mortality rate despite the new therapeutic strategies (Xie et al., 2017)

According to European Society for Medical Oncology (ESMO) guidelines, bladder cancer diagnostic includes a medical history and physical examination, cystoscopy or transurethral resection of bladder tumour (TURBT), urine cytology, blood tests, upper urinary tract imaging, and an additional work-up in high-risk patient groups (Powles et al., 2022).

The conventional approach to diagnosing bladder cancer involves invasive cystoscopy coupled with non-invasive urine cytology (EAU, 2026). Urine cytology is a valuable diagnostic aid for urinary tract cancers, especially for HG urinary cancers; however, it should not be utilised as the primary method for histological diagnosis, though positive results can indicate the presence of a tumour at any location within the urinary tract (Powles et al., 2022).

Bladder cancer staging is based on histological results from bladder cancer specimen obtained via invasive manipulations (biopsy or TURBT), and material from detrusor muscle should be included for accurate disease stage evaluation (Kim & Patel, 2020).

Computed tomography (CT) and magnetic resonance imaging MRI urography are recommended in screening for synchronous tumours in HG bladder cancer (Powles et al., 2022). Imaging techniques determine both the location and extent of the disease, such as potential involvement of the upper urinary tract, extravesical extension, hydronephrosis, lymph node involvement, or distant metastases. CT urography, performed with and without an intravenous contrast agent, is now the preferred method and has largely supplanted intravenous pyelography. In addition to CT urography, pelvic MRI, with or without intravenous contrast, can be employed for advanced local staging to assess the extent of bladder wall penetration. Multiparametric MRI, which provides superior soft tissue detail compared to CT, utilises the Vesical Imaging Reporting and Data System (VI-RADS) score to evaluate the probability of muscle invasion (Dyrskjøt et al., 2023; Powles et al., 2022).

1.7.1 Urine cytology

The assessment of bladder cancer includes the use of urine cytology, which functions as an additional diagnostic option. The National Comprehensive Cancer Network (NCCN) advocates for its use in conjunction with other diagnostic methods, such as cystoscopy, particularly for the ongoing surveillance of patients who fall into the categories of intermediate-risk, high-risk, or those with muscle-invasive disease (National Comprehensive Cancer Network [NCCN], 2024).

Various urine collection methods, including catheterisation, bladder and upper tract washing, and brush cytology, provide essential insights into the urinary system. Catheterised and washing samples yield higher cellularity despite potential instrumentation artifacts, while voided urine, though non-invasive, typically exhibits lower cellularity (Cibas & Ducatman, 2019; Zhu et al., 2019).

While urine cytology is recognised as an important non-invasive diagnostic tool for urinary tract cancers, it is not without limitations, notably its lower sensitivity compared to other diagnostic methods. However, it demonstrates higher specificity, particularly in identifying high-grade (HG) and grade 3 (G3) tumours, where specificity rates can reach up to 84 %. In contrast, the specificity for low-grade (LG) and grade 1 (G1) tumours typically falls below 20 %, underscoring the necessity of a multimodal approach (Planz et al., 2005; EAU, 2026; Yafi et al., 2015). Consequently, the standard diagnosis of bladder cancer (BC) requires a combination of cytology and cystoscopy, as reliance on cytology alone is insufficient for accurate diagnosis; together, they represent the gold standard method for bladder cancer diagnosis (EAU, 2026). Despite its limitations as a primary histological diagnostic method, positive findings from urine cytology may suggest the presence of tumours across various locations within the urinary tract (Powles et al., 2022).

When discussing factors that may influence the results of urine cytology, several critical elements related to sample quality must be considered. The timing of collection is particularly significant; voided urine specimens are optimally obtained in the mid-morning or early afternoon, as this timing enhances cytological yield (Mody et al., 2022; Sullivan et al., 2010). Early morning samples tend to be less favourable due to the presence of degenerative cells resulting from prolonged exposure to urine overnight, which may lead to cellular lysis and degradation, thus compromising cellular integrity (Murphy, 1990; EAU, 2026). To ensure robust diagnostic reliability, it is recommended that at least three mid-morning or random specimens be submitted for analysis, with clear communication to the laboratory regarding the specific method of collection employed. Furthermore, in female patients, interpretation can be adversely affected by contamination from cells of the lower genital tract. Inadequate storage

conditions, such as delays in laboratory processing or improper refrigeration, may further contribute to cellular degradation. When processing delays occur, the adequate fixation of specimens is advisable to maintain cytological integrity (Al-Abbadi, 2011; Mody et al., 2022; Sullivan et al., 2010; EAU, 2026).

The initial attempt to examine urine for the diagnosis of bladder cancer dates back to the mid-nineteenth century, with a notable milestone occurring in 1856 when the Czech physician Vilém Lambl (1824–1895) became the first to observe cancer cells in the urine of patients with urinary bladder cancer (Diamantis & Magiorkinis, 2014). Since 1945, when Dr Papanicolaou reported the application of urine cytology in the diagnosis of urothelial malignancies, this method began gaining popularity as a diagnostic tool for evaluating patients with haematuria (Papanicolaou & Marshall, 1945). Subsequent to this, several other classifications for urine cytology were proposed, notably those developed by Papanicolaou, Koss, Murphy, Ooms & Veldhuizen, the Papanicolaou Society of Cytopathology, and the Johns Hopkins Hospital Template for Reporting Urine Cytology (Koss et al., 1978; Koss & Melamed, 2006; Layfield et al., 2004; Murphy, 1985; Ooms & Veldhuizen, 1993; Papanicolaou, 1947; VandenBussche et al., 2013)

However, none of these classifications was widely adopted in routine clinical practice due to various limitations, such as a lack of rigorous definitions for validated cytologic criteria within specific categories, a lack of consensus regarding atypical categorisation, and insufficient acceptance by the general pathology community (Owens et al., 2013; Xing et al., 2024).

Considering the limitations of previous methodologies, it became clear that a significant need existed for a standardised system. In 2016, The Paris System for Reporting Urinary Cytology was published to focus on the detection of high-grade urinary bladder carcinoma cells in urine samples, including diagnostic categories and diagnostic criteria (Barkan et al., 2016). The diagnostic categories include:

- Adequacy;
- Negative for high-grade urothelial carcinoma;
- Atypical urothelial carcinoma;
- Suspicious for high-grade urothelial carcinoma;
- High-grade urothelial carcinoma;
- Low-grade urothelial neoplasm;
- Other malignancies, including primary and metastatic lesions, as well as miscellaneous findings.

The diagnostic criteria outlined in this system include:

- A nuclear-to-cytoplasmic (N/C) ratio > 0.5;
- N/C ratio ≥ 0.7, hyperchromasia, irregular clumpy chromatin, and irregular nuclear membranes.

Nevertheless, this previous classification was also incomplete, which is why the second edition of The Paris System was introduced in 2022 (Wojcik et al., 2022), incorporating modifications to previously established diagnostic criteria and suggesting additional parameters for the diagnosis of high-grade urinary carcinoma, particularly in cases exhibiting hypochromatic nuclei. Importantly, it is advised that degenerated cells not be utilised in the criteria for diagnosis. Additionally, the second edition introduced two new categories: 'Cytopathology of the Upper Urinary Tract' and 'Risk of High-Grade Malignancy (ROHM)' (Xing et al., 2024).

When cytological findings raise suspicion, it is advisable to conduct a repeat urine cytology analysis, as these cases may carry an underlying risk of high-grade malignancy that can reach up to 53% (EAU, 2026; Wojcik et al., 2022) .

Several urinary biomarkers have been developed to enhance the diagnosis and monitoring of patients, aiming to improve clinical outcomes (Crocetto et al., 2022; EAU, 2026). These biomarkers encompass a variety of modalities, including mRNA detection, methylation analysis, biochemical assays for protein detection, cellular antigen assessment, and genetic alterations.

Nevertheless, the gold standard for the surveillance of non-invasive bladder cancer remains cystoscopy in conjunction with urine cytology (NCCN, 2024).

1.7.2 Urinary molecular markers

Urinary molecular marker tests have been developed in response to the low sensitivity of urine cytology for low-grade (LG)/G1 tumours, but none have yet become standard in clinical practice (EAU, 2026). These tests tend to have higher sensitivity but lower specificity than urine cytology, and their results can be influenced by benign conditions and previous *Bacillus Calmette-Guérin* (BCG) treatments (Batista et al., 2020; Heard & Mitra, 2024; Hermans et al., 2022; Max Kates & Trinity J. Bivalacqua, 2021; Murphy, 1990). The performance required of these markers varies with the clinical context, such as screening or follow-up for different risk levels.

European guidelines (EAU, 2026) consider the use of some specific biomarkers such as UroVysion (FISH), microsatellite analysis, nuclear matrix protein 22 (NMP22), fibroblast growth factor receptor (FGFR)3/telomerase reverse transcriptase (TERT), Cx-Bladder,

ADX-Bladder™, Xpert Bladder® and EpiCheck™, CellDetect® for better diagnosis and the detection of diseases recurrence (Crocetto et al., 2022; EAU, 2026). The main problem is that a number of non-invasive urine tests has failed to meet clinical expectations, because their diagnostic specificity is inferior to that of urinary cytology tests (Crocetto et al., 2022) and none of them have been accepted into routine practice by any clinical guidelines for primary diagnosis. In the surveillance setting, however, the EAU 2026 guidelines acknowledge that selected urinary molecular markers may serve as adjuncts to – but not replacements for – cystoscopy, particularly for reducing cystoscopy frequency in low- and intermediate-risk NMIBC or for enhancing the early detection of high-grade recurrence in high-risk patients (EAU, 2026). To better understand in greater detail the sensitivity and specificity of various biomarkers, please refer to Table 1.5.

When examining the individual urinary biomarkers in more detail, the well-known biomarker nuclear matrix protein 22 (NMP22) is of particular clinical significance, having been successfully isolated by Keesee and colleagues (Keesee et al., 1996). NMP22 is a common protein found in the nucleus of all cells. Ranging from DNA replication to gene expression, the multiple functions of nuclear matrix proteins play a critical role in maintaining the structure of the cell nucleus (Sugeeta et al., 2021). NMP22 is specifically engaged in mitosis by facilitating the precise allocation of chromatin to daughter cells, and this is reflected in the elevated levels of nuclear matrix proteins in urothelial tumours due to the turnover of cells associated with tumour apoptosis (Mbeutcha et al., 2016). For instance, Kumar and colleagues demonstrated that the NMP22 test is more sensitive than urinary cytology, with a sensitivity of 85 %, significantly surpassing the 41 % sensitivity of cytology. Notably, for low-risk tumours, NMP22 showed eight times greater sensitivity. However, its specificity was inferior to that of urinary cytology. Therefore, to improve diagnostic performance, both tests were combined, achieving an overall sensitivity of 91 % (Kumar et al., 2006).

It is important to note that despite negative cystoscopy results, positive findings in urine cytology or molecular tests such as UroVysion™ (FISH), NMP22®, *FGFR3*/*TERT*, and microsatellite analysis may indicate an increased risk of disease recurrence and progression (EAU, 2026)

Table 1.5

Commercial kits to detect and follow-up BC using liquid biopsy biomarkers

Commercial Kits	Biomarker	Assay Type	Sample Type	FDA Approved	Purpose	Predictive Capacity
Cytology	Sediment cells	Giemsa and HE staining	Urine	Yes	Diagnostic and surveillance (1)	Sensitivity = 38% Specificity = 98%
uCyt+	Sediment cells	Immunofluorescence	Urine	Yes	Surveillance in adjunct to cystoscopy	Sensitivity = 73% Specificity = 66%
UroVysion	Sediment cells	Multi-target FISH	Urine	Yes	Diagnostic	Sensitivity = 72% Specificity = 83%
UroMark (3)	Sediment cells	Bisulfite-based methylation assay	Urine	No	Diagnostic	Sensitivity = 98% Specificity = 97%
CellSearch	CTCs	Immunomagnetic enrichment	Plasma/ serum	Yes	Surveillance	Sensitivity = 48% Specificity = 98%
CxBladder	mRNA	RT-qPCR	Urine	No	Diagnostic	Sensitivity = 82% Specificity = 85%
CxBladder Monitor	mRNA	RT-qPCR	Urine	No	Surveillance	Sensitivity = 91% NPV = 96%
Xpert BC Detection	mRNA	RT-qPCR	Urine	No	Diagnostic	Sensitivity = 76% Specificity = 85%
Xpert BC Monitor	mRNA	RT-qPCR	Urine	No	Surveillance	Sensitivity = 84% Specificity = 91%
PanC-Dx	mRNA	RT-qPCR	Urine	No	Diagnostic	Sensitivity = 90% Specificity = 83%
UROBEST (4)	mRNA	RT-qPCR	Urine	No	Diagnostic and surveillance (5)	Sensitivity = 80% Specificity = 94%
NMP22	Protein	Sandwich ELISA	Urine	Yes	Surveillance	Sensitivity = 40% Specificity = 99%
NMP22 Bladder-Chek	Protein	Dipstick immunoassay	Urine	Yes	Diagnostic and surveillance (1)	Sensitivity = 68% Specificity = 79%

Table 1.5 continued

Commercial Kits	Biomarker	Assay Type	Sample Type	FDA Approved	Purpose	Predictive Capacity
BTA TRAK	Protein	Sandwich ELISA	Urine	Yes	Diagnostic and surveillance (1)	Sensitivity = 66%
						Specificity = 65%
BTA stat	Protein	Dipstick immunoassay	Urine	Yes	Diagnostic and surveillance (1)	Sensitivity = 70%
						Specificity = 75%
CYFRA 21.1	Protein	Immunoradiometric assay or ELISA	Urine	No	Diagnostic	Sensitivity = 82%
						Specificity = 80%
UBC test	Protein	Sandwich ELISA or dipstick immunoassay	Urine	No	Diagnostic	Sensitivity = 64%
						Specificity = 80%

*(1) Although these tests have been proposed for diagnosis and follow-up of BC, predictive values correspond to the detection of primary tumour. (2) DiagnoCure company was dissolved in 2016 and the uCyt+ test is not available at present. (3) The performance of the UroMark test is currently evaluated in Phase III studies. (4) UROBEST is not yet commercially available. (5) Biofina Diagnostics provides these predictive values for diagnostic and surveillance purposes together. NPV = Negative predictive value. Adapted from Lodewijk, I., Dueñas, M., Rubio, C., Munera-Maravilla, E., Segovia, C., Bernardini, A., Teijeira, A., Paramio, J. M., & Suárez-Cabrera, C. (2018). Liquid Biopsy Biomarkers in Bladder Cancer: A Current Need for Patient Diagnosis and Monitoring. *International journal of molecular sciences*, 19(9), 2514. (Lodewijk et al., 2018)

1.8 The tumour microenvironment (TME)

Tumours are composed of cancerous cells and the cell environment in which the tumour exists. It has been found that interactions between tumour cells and surrounding tissues are essential for tumour development (Liu et al., 2018; Valadi et al., 2007).

The tumour microenvironment (TME) is formed not only by tumour cells, but also by the components of the extracellular matrix and miscellaneous non-malignant cells (Jacob et al., 2018). This cellular environment includes fibroblasts (CAFs), endothelial cells, pericytes, and immune cells associated with cancer that form TME, as well as blood vessels, extracellular matrix and signal molecules (Arneth, 2019). Intercellular and stromal interactions in the tumour microenvironment contribute to cancer cell proliferation, survival, metastasis, and response to treatment (Jacob et al., 2018).

One of the most convincing components of TME signalling is the exosome, which are being investigated in many ongoing studies (Li & Nabet, 2019; Tan et al., 2022)

Urinary bladder cells share a common environment through urine. This suggests that the cancer microenvironment is extended via the urine, where normal and cancer cells share a common environment and possibly communicate through exosomes thus inducing heterogeneity and oncotransformation (Tan et al., 2020; Zhang et al., 2015).

1.8.1 Exosomes and MicroRNA (miRNA)

Exosomes are small extracellular vesicles (EVs) with diameters usually ranging from 30 to 150 nm, produced by various cell types including tumour cells, and are present in numerous biological fluids (Krylova & Feng, 2023; Mathieu et al., 2019; Wu et al., 2021).

Initially dismissed as cellular debris, exosomes are now recognised as pivotal agents in intercellular communication, influencing a broad spectrum of biological functions and playing significant roles in cancer development and progression (Challagundla et al., 2015).

They are formed through the inward budding of endosomal membranes, a process that aids in their release into the extracellular space. This mechanism results in the formation of multivesicular bodies (MVBs), which subsequently fuse with the plasma membrane, allowing exosomes to be released into the surrounding environment (Krylova & Feng, 2023). These vesicles contribute to each stage of cancer progression and metastasis (An et al., 2015), and when internalised by recipient cells through endocytosis, fusion, or phagocytosis, exosomes can exert effects via receptor-mediated interactions or by transferring their cargo – comprising proteins, transcription factors, mRNAs, and microRNAs (miRNAs) (Milane et al., 2015). Additionally, a hallmark of cancer-derived exosomes is their high microRNA content (Milane et al., 2015).

MiRNAs, short non-coding RNAs, are crucial in tumourigenesis (Weber et al., 2010). They function by binding to the 3′-UTR of target mRNAs, thereby regulating gene expression post-transcriptionally, either through mRNA degradation or by inhibiting translation (Shukla et al., 2011). Notably, recent findings indicate that precursor miRNAs (pre-miRNA) linked with the processing complex (such as Dicer, Ago2, and TRBP) are present in exosomes derived from breast cancer. Within these exosomes, pre-miRNAs are processed into their mature forms, highlighting a novel mechanism through which miRNAs are incorporated into exosomes (Sun et al., 2018).

Due to their small size, MiRNAs exhibit greater stability against nuclease degradation and maintain their integrity across various sample types, including urine (Tilki et al., 2011; Weber et al., 2010).

Exosomal miRNA exhibits remarkable stability compared to miRNAs in plasma, which begin to degrade at temperatures as low as 4 °C and −20°C. By contrast, the miRNA content in exosomes remains consistent even after repeated freeze-thaw cycles, closely matching that in freshly collected samples (Ge et al., 2014). This robustness can be attributed to the protective lipid bilayer of the exosomes (Jacob et al., 2018). Due to their roles in oncogenesis and tumour suppression, miRNAs are increasingly acknowledged as potential markers for cancer diagnosis and prognosis (Ling et al., 2016).

It has been reported that certain miRNAs (miR-182, miR-183 miRNA-143, miR-125b, miR-145, and miR-29a) are aberrantly expressed in cancer tissues. Finding these miRNAs in urine confirms that urinary miRNA composition partially reflects miRNA expression in cancer cells (Dyrskjøt et al., 2009; Han et al., 2011; Ichimi et al., 2009; Mengual et al., 2013; Puerta-Gil et al., 2012).

1.9 Bladder cancer treatment

Bladder cancer treatment has undergone considerable advancements over the years, incorporating a multifaceted approach that includes surgery, chemotherapy, and immunotherapy. Current standard treatments consist of transurethral resection of bladder tumour (TURBT), radical cystectomy, and intravesical or systemic chemotherapy and immunotherapy, which are all customised based on the bladder cancer stage, grade, and risk group (Powles et al., 2022).

1.9.1 *Bacillus Calmette-Guérin* (BCG) intravesical instillations

Intravesical therapy is considered a cornerstone in superficial NMIBC management. There are two types of instillations for BC treatment: immunotherapy and chemotherapy (EAU, 2026; Powles et al., 2022).

While BCG was initially recognised in the 1970s as a promising new cancer treatment, it has been steeped in controversy since its introduction in 1921 as a vaccine against tuberculosis. Ultimately, it was deemed ineffective after controlled trials in advanced bladder disease failed to demonstrate any benefits (Gandhi et al., 2013). Local immune responses are believed to be induced by BCG, which activates T-cells and macrophages to target any remaining cancer cells (Kates et al., 2017) .

Several meta-analyses have confirmed that TURBT alone or TURBT plus chemotherapy is inferior to BCG instillation following TURBT for preventing the recurrence of NMIBC (Böhle et al., 2003; Han & Pan, 2006; Malmström et al., 2009).

1.9.2 Radical cystectomy

Radical cystectomy (RC) with urinary diversion is regarded as the standard surgical treatment for MIBC and is also a crucial therapeutic option for very high-risk and treatment-refractory NMIBC (Aminoltejari & Black, 2020).

RC involves the complete removal of the bladder and surrounding tissues, which differs between sexes primarily due to anatomical considerations. In men, RC includes the *en bloc* removal of the bladder, distal ureters, prostate, membranous urethra, seminal vesicles and distal vasa deferentia, aalong with adjacent connective tissue, their lymphatic drainage paths, and

regional lymph nodes. Conversely, in women, RC typically involves the removal of the bladder along with the urethra, uterus, ovaries, part of the vaginal wall, fallopian tubes, round ligaments, surrounding connective tissue, lymphatic drainage paths, and regional lymph nodes. Total urethrectomy may be indicated during radical cystectomy for men, particularly when there is evidence of tumour involvement in the urethra (Biers et al., 2020). Urinary diversion can be reached by creation of a neobladder, ureterostomy, or the use of an ileal conduit (Partin et al., 2021).

Radical cystectomy may be performed as either an open procedure, a laparoscopic procedure, or a robot-assisted laparoscopic technique. Despite advances in technology and patient care, open RC still has high rates of perioperative complications. For example, while robot-assisted radical cystectomy generally requires a longer surgical duration and incurs greater costs, it is associated with shorter hospital stays and reduced blood loss when compared to traditional open surgery, nevertheless, the oncological outcomes for both methods are comparable (Aminoltejari & Black, 2020).

1.9.3 Systemic treatment of bladder cancer

Systemic treatment is the preferred approach in MIBC and the standard of care in metastatic BC. Systemic treatment of BC include chemotherapy, immunotherapy, targeted therapy, antibody-drug conjugates, and combinations of these agents (Powles et al., 2022, 2024).

Multiple clinical trials have shown that using neoadjuvant platinum-based combination chemotherapy in the treatment of MIBC in eligible patients significantly improves overall survival. The current standard of care is 3–6 cycles of DDMVAC including dose-dense methotrexate, vinblastine, doxorubicin and cisplatin (Grossman et al., 2003; Sternberg et al., 2001). A reasonable alternative perioperative regimen that has shown equivalence to conventional methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) in the advanced disease setting is a four-cycle regimen of cisplatin and gemcitabine (Dash et al., 2008; Maase et al., 2000). Both regimens can be used in the adjuvant treatment, but neoadjuvant setting is preferred duo to a higher level of clinical evidence (Dash et al., 2008; Grossman et al., 2003; Maase et al., 2000; Sternberg et al., 2001). In patients who have received platinum-based neoadjuvant therapy, adjuvant immunotherapy with nivolumab could be relevant (Bajorin et al., 2021).

For selected patients bladder preservation strategy can be applied. In stage II-IIIA disease, trimodality therapy – including maximal transurethral resection of bladder tumour

followed by concurrent chemoradiation – has shown similar oncological outcomes compared to radical cystectomy (NCCN, 2024; Powles et al., 2022; Zlotta et al., 2023).

Optimal candidates for bladder-sparing chemoradiotherapy are patients with tumours smaller than 6 cm, that does not invade the prostatic urethra, without associated hydronephrosis and concurrent extensive or multifocal Tis. Preferably application of visually complete or maximally debulking TURBT should be possible as well (NCCN, 2024; Powles et al., 2022).

In stage IIIB and non-metastatic IVA primary treatment options include systemic therapy or concurrent chemoradiotherapy. Cisplatin, low-dose gemcitabine or 5-FU with mitomycin is preferred systemic agents during radiotherapy due their radiosensitising activity (Network, 2024a).

Current standard of care for first-line systemic treatment in locally advanced and metastatic disease is immunotherapy and antibody drug-conjugate combined with pembrolizumab and enfortumab vedotin-ejfv, which has demonstrated significant improvement in progression-free survival (PFS) (12.5 months), overall survival (OS) (31.5 months) and an overall response rate (67.7 % with a complete response rate of 29.1 %) (Powles et al., 2024). If the above-mentioned treatment regimen is not possible, then the combination of nivolumab, cisplatin and gemcitabine or platinum-based chemotherapy followed by avelumab maintenance therapy are appropriate alternatives that also have demonstrated positive results on PFS and OS (Heijden et al., 2023; Powles et al., 2022, 2024).

2 Materials and methods

The study consists of two parts: 1) Description and analysis of BC epidemiology in Latvia; 2) Establishing the correlation between the relative ratio of several specific miRNAs in urine exosomes and bladder cancer in Latvia using molecular profiling and machine learning.

The research was conducted at the Oncology Institute of Rīga Stradiņš University. The first (epidemiological) part of the study was approved by the Ethics Committee for Clinical Research at the Development Society of Pauls Stradiņš Clinical University Hospital. The second (biomarker) part was conducted in accordance with the Declaration of Helsinki and approved by the Central Medical Ethics Committee of Latvia (protocol No. 1/19-02-12 of 12 February 2019, with supplement No. 01-29.1.2/1352 (Annexes 5 and 6). Additional institutional approvals were obtained from Rīga Stradiņš University (Annex 8) and Pauls Stradiņš Clinical University Hospital (Annex 9).

Patient consent was not required for epidemiological part of the study, as it used regularly collected, anonymous electronic data. For the second part of the study, every participant received a written explanation of the research alongside an informed consent statement (Annex 7), and subsequently provided written informed consent. The informed consent form and the research questionnaire are provided in Annex 10 and Annex 11, respectively.

2.1 BC epidemiology in Latvia

2.1.1 Study group

A retrospective cohort study was conducted using data from all patients diagnosed with bladder cancer (BC, n = 9589) categorised under the International Classification of Diseases (ICD-10, C67) between 1 January 1990 and 31 December 2017. The information was sourced from the Centre for Disease Prevention and Control (CDPC) of Latvia.

The following variables were included in the data array:

1 Gender;
2 Date of birth;
3 Date of death;
4 Cause of death code (according to ICD-10 classification).
5 ICD-10 diagnosis code;
6 Date of diagnosis;
7 Age at diagnosis;
8 Type of diagnosis;
9 Method of diagnostic confirmation;

10 Morphology code (according to ICD-10 classification);

11 Stage of disease;

12 TNM classification;

13 Date of recurrence;

14 Date of metastasis;

15 Location of metastases;

16 Date of removal from the patient registry;

17 Reason for removal from the registry;

Initiated in 1963, Latvia has implemented a nationwide system for cancer registration encompassing the entire population. The Latvian Cancer Registry (LCR) database, formally established in 1993, was transferred to the oversight of the CDPC in 2012. The LCR is integrated into the Registry of Patients with Particular Diseases (PREDA), capturing practically all diagnosed cancer cases. However, according to Paragraph 7 of the Cabinet of Ministers Regulation No 134 of 11 March 2014 "Regulations on the Unified Electronic Information System for the Health Sector", PREDA (hereinafter – the Register) was integrated in the unified electronic health information system (E-health system) and is linked to the National Health Service (NHS).

A total of 13 158 patient records were received from the CDPC of Latvia. Of these, 3569 (27.1 %) were identified as duplicate registrations and excluded. Consequently, 9589 unique records (72.9 %) of BC patients of all ages with the BC (ICD-10 topographical code C67) were selected for subsequent analysis.

The youngest patient diagnosed with BC was a newborn with a malignant rhabdoid tumour, and the oldest was 100 years old. In total, there were eleven patients aged 1 to 16 years – diagnosed with rhabdomyosarcoma, a malignant rhabdoid tumour, and bladder cancer without morphological verification – were excluded from further survival analysis.

Of the total available cases older than 20 years, 57.2 % were transitional cell carcinoma, 3.0 % squamous cell carcinoma, 5.8 % adenocarcinoma, and 6.5 % other tumor types, while 27.6 % of the cases had an unknown histological response.

BC patients were grouped and categorised in 20-year age cohorts to analyse age-specific incidence, prevalence, mortality, and survival. Because patients younger than 40 years accounted for only 145 cases, they were excluded from the age-specific trend analysis due to statistical insufficiency, leaving the following analytical groups: 40–59, 60–79, and 80+ years. Cases registered only by a death certificate (DCO) or diagnosed *post-mortem* were omitted from the survival analyses. The primary tumor stages included in the stage-specific incidence and survival trend analyses were T1, T2, T3 and T4.

Cancer-specific incidence, prevalence and mortality rates of the Latvian population were calculated as cases per 100 000 inhabitants. These calculations were derived from data sourced from the Central Statistical Bureau of Latvia (CSB) updated in 2018 (following the latest Population and Housing Census in 2011), which provided information on changes in the number and age structure of the Latvian population, as well as the annual total mortality rates, available on the website http://www.csb.gov.lv/dati/statistikas-datubazes-28270.html (Central Statistical Bureau of Latvia, 2011). Cancer rates were subsequently age-adjusted to the World Health Organization standard population in 2000 (Ahmad et al., 2001). To calculate the relative survival rate, the life expectancy table for the Latvian population was downloaded from the Human Mortality Database (Central Statistical Bureau of Latvia, n.d.)

2.1.2 Statistical methods

The statistical software package SPSS (version 22.0, 2013, USA: IBM Corp) was applied for all data analyses. In the descriptive analysis, categorical variables are reported as absolute numbers and percentages, while continuous variables are reported as means and standard deviations. Statistical indicators were calculated for the mean age of patients at the time of diagnosis and death.

In order to avoid year-to-year fluctuations in values for age and survival comparisons, the measures were grouped into three-year periods. The Mann-Whitney U test for quantitative data and chi-square test for qualitative variables were used for comparison.

Epidemiological metrics specific to bladder cancer, including incidence, prevalence and mortality rates, were calculated per 100 000 individuals using widely documented methodologies. For comparison analysis, age-standardised rates (ASR) were calculated using the WHO 2000 standard population (Ahmad et al., 2001) and the direct standardisation method.

The Joinpoint Regression Analysis programme (version 4.6.0.0, April 16, 2018, USA), available through the Surveillance Research Program of the US National Cancer Institute, was used to examine trends in ASR for incidence, prevalence and mortality rates of BC patients based on age and gender groups. The Joinpoint software identifies the number and location of joinpoints where a change in trend has occurred. Log-transformation was carried out for ASR for men, women and both; as well as for all age groups. The Monte Carlo Permutation method was used to select the number of joinpoints, which was set between 0 and 4. A significance level of 0.05 was used for the permutation test with 4499 randomly permuted datasets (Kim et al., 2000). The annual percentage change (APC) was also calculated for each separate linear segment to define time trends in BC incidence, prevalence and mortality. A significant

increase or decrease of a trend was defined as the slope of the curve being statistically significant ($p < 0.05$).

For the survival analysis, a cohort of BC patients with at least a 5-year follow-up was selected. Patients were stratified into seven time periods. Kaplan–Meier curves with the log-rank test were used for 5- and 10- year overall and cancer-specific survival (OS and CSS) rates. Over the said period, there were 392 BC cases diagnosed *post mortem* and not eligible for inclusion in the survival analysis and were excluded. The rest were followed-up from the date of diagnosis until death, censoring, or 31 December 2017. Survival was calculated as three-year average values to avoid year-to-year fluctuations. The Log-Rank test was employed to evaluate statistically significant differences in the survival curves.

The R statistical program (version 4.0.3, October 2020, Austria, R Foundation for Statistical Computing) and the package "*relsurv*" were used to calculate relative survival (RS). For survival analysis, a cohort of BC patients with a follow-up period of 5 years was selected. Patients were followed from diagnosis to death, censoring or 31 December 2017, and classified into three periods of 1990–2000, 2001–2012, and 2013–2017. The five-year RS for 1990–2012 data was calculated by cohort analysis of the Erler I method. The 5-year RS rates expected for the 2013–2017 data were calculated using the Ederer II method.

2.2 Urinary exosomal miRNAs biomarkers and their targets

2.2.1 Study group and inclusion criteria

From 2019 to 2024, urine samples were prospectively collected from bladder cancer patients and control subjects at Pauls Stradiņš Clinical University Hospital and Riga East University Hospital. All participants provided written informed consent prior to inclusion. Participants were categorised into three groups: low-grade bladder cancer (LG, histologically confirmed Ta or T1 tumour), high-grade bladder cancer (HG, T2 tumour), and healthy controls. Staging and grading were performed according to the TNM classification system and 2022 WHO criteria. Patients without a confirmed pathohistological diagnosis of bladder cancer had their urine samples excluded from the analysis. Inclusion and exclusion criteria for both groups are summarised in Table 2.1.

Table 2.1

Inclusion and exclusion criteria for the study

Criterion	Bladder Cancer Group	Control Group
Inclusion	Age ≥ 50 years; pathohistologically proven primary or recurrent bladder cancer after TURBT or cystectomy; without previously received systemic chemotherapy; signed informed consent; available medical records	Age-matched; no history of malignancy; urinalysis without signs of inflammation or infection; no significant blood test abnormalities; signed informed consent; available medical records
Exclusion	Any other oncological disease or previous upper urinary tract cancer; diabetes mellitus; inflammatory or autoimmune disease; non-urinary tract infection	Any other oncological disease or previous upper urinary tract cancer; diabetes mellitus; inflammatory or autoimmune disease; non-urinary tract infection

The discovery miRNA profiling cohort consisted of 7 healthy controls, 6 LG patients, and 6 HG patients. The differential expression analysis of urinary exosomal miRNAs was performed on an initial cohort of 210 participants (111 in the bladder cancer group and 99 in the control group) meeting the exclusion criteria listed in Table 2.1. For the machine learning analysis, only participants with complete clinical data were retained, resulting in a final analytical cohort of 152 participants (89 bladder cancer patients and 63 healthy controls). All three machine learning classifiers were trained and evaluated on this 152-participant cohort. A detailed flowchart of participant selection is provided in Figure 2.2.1. Missing blood creatinine values were imputed with the mean value of the respective group.

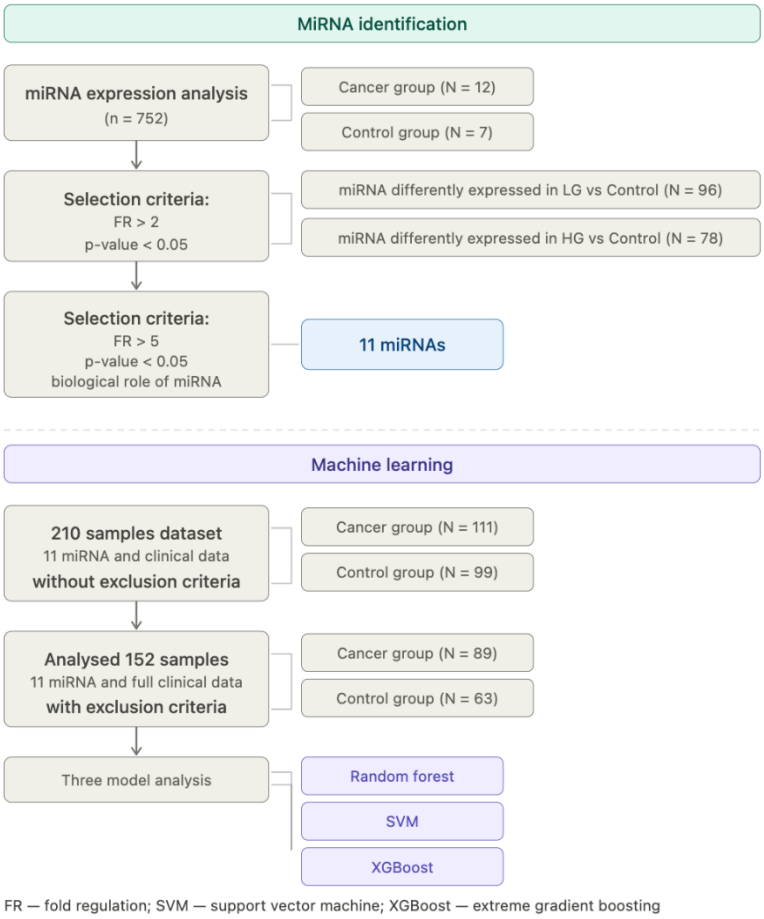


Figure 2.2.1. **Flowchart of data analysis**

2.2.2 Clinical data

All clinical data were prospectively obtained from the patients' medical records and entered into a dedicated database. The database included information on the patients' clinicopathological characteristics, surgical treatment received, chemotherapy, laboratory test results, family history, and smoking and occupational exposure history.

For Ta and T1 stage patients, the European Organisation for Research and Treatment of Cancer (EORTC) risk calculator was applied to predict recurrence and progression. A non-invasive urinary ultrasound was offered to control group participants before enrolment to exclude bladder tumour. Urine samples for urinalysis, urine cytology, and urinary miRNA profiling were collected from every bladder cancer patient before surgery; in the control group, urine samples were collected only for urinalysis and miRNA profiling.

2.2.3 Urine sample collection and processing

Spontaneous voided urine samples were collected from all participants. If immediate processing was not possible, samples were stored at 4 °C for up to 24 hours. Samples were centrifuged at 3.0 rcf (4.4 rpm) for 5 minutes 30 seconds at room temperature to remove cellular debris. The resulting supernatant was aliquoted and stored at −80 °C until analysis.

2.2.4 Exosome isolation and RNA extraction

Exosome isolation was performed according to the miRCURY Exosome Cell/Urine/CSF Kit protocol (Qiagen, Hilden, Germany; Cat. No. 76743). Stored samples were thawed on ice and subjected to a second centrifugation at 10,000 × g for 10 minutes at room temperature to remove residual cellular debris. Exosomes were then isolated from 1960 µL of urine supernatant divided into two tubes.

Total RNA, including miRNA, was extracted from urinary exosomes using the miRNeasy Mini Kit (Qiagen, Hilden, Germany; Cat. No. 17004) according to the manufacturer's protocol. A spike-in synthetic mixture of UniSp2, UniSp4, and UniSp5 RNA (Qiagen, USA; Cat. No. 339390) was added to each sample, and purified RNA was eluted in 50 µL of RNase-free water.

2.2.5 Reverse transcription and RT-qPCR

Reverse transcription and polyadenylation of RNA to complementary DNA (cDNA) were performed using the miRCURY LNA RT Kit (Qiagen, USA; Cat. No. 339340) according to the manufacturer's instructions, with a UniSp6 spike-in for process quality control.

Incubation was carried out using the peqSTAR thermal cycler: 42 °C for 60 minutes, 95°C for 5 minutes, held at 4°C. An increased RNA template volume of 4 µL (instead of the standard 2 µL) was used to improve sensitivity. Reverse transcription and subsequent amplification were carried out using the miRCURY LNA miRNA PCR Starter Kit (Qiagen, USA; Cat. No. 339320) together with the miRCURY LNA SYBR Green PCR Kit (Qiagen, USA; Cat. No. 339347). In the discovery phase, miRNA profiling was performed on Pre-designed miRCURY LNA miRNA miRNome PCR Panels (Qiagen, USA; Cat. No. 339322). For the machine learning phase, a custom subset of miRNAs was assayed on Pre-designed miRCURY LNA miRNA Custom PCR Panels (Qiagen, USA; Cat. No. 339330 and 339332; see Table 2.2). Quantitative PCR was performed on the Applied Biosystems® ViiA™ 7 Real-Time PCR System following the manufacturer's instructions, and CT values for all miRNAs were determined using automatically assigned threshold and baseline settings.

Table 2.2

miRCURY LNA ™ miRNA Custom PCR Panel YCA34464

Position	Assay Name
A01-A24	UniSp6
B01-B24	UniSp3
C01-C24	UniSp2
D01-D24	UniSp5
E01-E24	hsa-miR-30c-5p
F01-F24	hsa-miR-126-3p
G01-G24	hsa-miR-1260a
H01-H24	hsa-miR-20a-3p
I01-I24	hsa-miR-28-3p

Table 2.2 continued

Position	Assay Name
J01-J24	hsa-miR-28-5p
K01-K24	hsa-miR-30b-3p
L01-L24	hsa-miR-324-5p
M01-M24	hsa-miR-339-5p
N01-N24	hsa-miR-429
O01-O24	hsa-miR-455-3p
P01-P24	hsa-miR-502-5p

2.2.6 RT-qPCR data analysis

To analyse the data obtained from miRNA panels, the miRCURY miRNA PCR Data Analysis programme (version 1.0) was used. Data normalisation was performed using the global mean normalisation method for discovery phase analysis. The p-value of differences in miRNA expression levels between controls and cancers was calculated based on the Student's t-test of replicate values of 2 ^ (−ΔCT) for each miRNA in both the control and cancer groups. The p value calculation was based on a parametric, two-sample, equal-variance test with a two-tailed distribution. The GeNorm algorithm, which is an integrated module of the miRCURY miRNA PCR Data Analysis programme (version 1.0), was used to identify internal control miRNAs with expression levels corresponding to global mean CT values and demonstrating the highest similarity to the average CT value. The miRNAs expressed differently between LG and HG were compared to the control group and filtered by the condition of fold change and p-values.

The sample size for miRNA panels was calculated based on the reference article (Lee & Whitmore, 2002) to identify differentially expressed miRNAs in urine. Statistical power was set at 90 % to detect a two-fold difference in 700 miRNAs, with a 0.05 significance level. The minimum sample size calculated was 5 vs. 5.

2.2.7 miRNA target analysis and pathway analysis

The list of the statistically significant, differentially expressed miRNAs was submitted to the miEAA tool (Kern et al., 2020) (https://ccb-compute2.cs.uni-saarland.de/mieaa2/) for miRNA enrichment analysis. The results were filtered by the FDR and the subcategories capturing the top 5 highest numbers of observed miRNAs were selected. Prediction of the miRNA targets was performed by miRTargetLink 2.0 (https://ccb-compute.cs.uni-saarland.de/mirtargetlink2) (Kern et al., 2021). The interaction of the predicted targets was analysed by the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; https://string-db.org/) (Szklarczyk et al., 2021). The hub genes were analysed by CytoNCA and CytoHubba (Tang et al., 2015), which are the plugins of Cytoscape (Shannon et al., 2003) with the network centrality of degree, betweenness, closeness, and eigenvector centralities using

CytoNCA, as well as the edge percolated component (EPC), maximal clique centrality (MCC), maximum neighbourhood component (MNC) and stress (CytoHubba).

2.2.8 Pilot diagnostic model

Nine differentially expressed (DE) miRNAs (screening criteria: fold change > 3 of either LG or HG, p-value < 0.05) and 3 reference miRNAs (expressed equally in BC patients and controls by using NormFinder) were selected to develop the diagnostic model. To test whether the selected miRNAs were suitable for model development, their performance was evaluated through a pilot calculation. A total of 23 control samples and 32 cancer patient samples were subjected to RT-qPCR to evaluate these specific miRNAs. The delta CT was obtained by subtracting the CT of the target miRNA from the CT of the normalisation miRNA. The delta CT values for each miRNA were then utilised for further analysis.

For the pilot analysis, machine learning classification model was built using neural networks within the Caret package of the R statistical programme. Samples were separated into the training set (15 controls and 24 cancer patients) and the test set (8 controls and 10 cancer patients). Training was optimised by various combinations of the selected DE miRNAs (miR-429, miR-30b-3p, miR-126-3p, miR-28-5p, miR-324-5p, miR455-3p, miR-20a-3p, miR-28-3p, and miR-30c-5p) and control miRNAs (miR-1260a, miR-502-5p, miR-339-5p).

These led to following results:

Accuracy: 0.8889

95% CI: (0.6529, 0.9862)

No Information Rat: 0.5556

P-Value [Acc > NIR]: 0.00288

Sensitivity: 0.8000

Specificity: 1.0000

One of the best results was obtained by combining miR-429, miR-30b-3p, miR-126-3p, miR-28-5p, miR-324-5p, and miR-455-3p as DE miRNAs, and miR-1260a as the normalisation miRNA. The pilot results confirmed the diagnostic potential of the selected miRNAs and informed the design of the main machine learning analysis (Section 2.2.9), which extended this approach to a larger cohort using three complementary classifiers and an expanded miRNA and clinical feature set.

2.2.9 Machine learning

Dataset and features

Initially, the dataset consisted of 210 participants (111 bladder cancer patients and 99 controls) who met the exclusion criteria (autoimmune disease, diabetes mellitus, any infections other than urinary tract infections related to bladder cancer, any history of malignancy, urine analyses with signs of inflammation or infection, and no significant abnormalities in blood tests). This 210-participant cohort was used for the differential expression analysis of the 11 candidate miRNAs. For the machine learning analysis, participants with complete clinical data were selected from this cohort, resulting in a final analytical dataset of 152 participants (89 bladder cancer patients and 63 controls). The combined feature set comprised 24 variables: 11 miRNA expression levels and 13 clinical and demographic variables. The clinical variables included demographic parameters (age, sex, weight, height), urinalysis findings (erythrocyturia, leukocyturia, nitrite test), risk factors (smoking history, aromatic amine exposure), symptoms and history (dysuria, chronic urinary tract infection, family oncological history), and an exclusion criteria indicator as a binary covariate (Figure 2.1).

Missing blood creatinine values were imputed using the mean value of the respective group (cancer or control); advanced imputation methods were considered but not applied due to the risk of overfitting in smaller datasets.

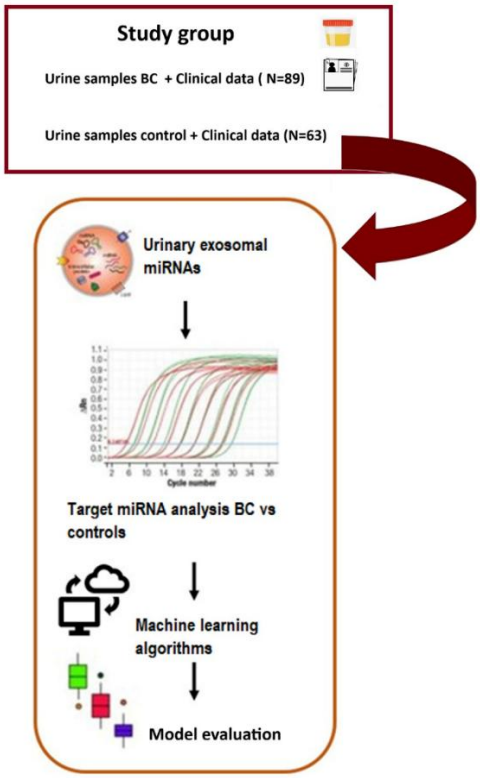


Figure 2.1 **Workflow of machine learning applied in this Doctoral Thesis**

Machine learning algorithms and hyperparameter optimisation

Three complementary machine learning classifiers were evaluated, each with distinct methodological strengths suited to biomedical data of moderate size. Random Forest (RF) is an ensemble of decision trees using bootstrap aggregation (bagging) to reduce overfitting; it captures non-linear interactions and provides inherent feature importance ranking. The RF algorithm was selected for its robustness and capability to handle high-dimensional data. The Support Vector Machine (SVM) identifies the optimal hyperplane that maximises the margin between classes; it is effective with high-dimensional data and smaller sample sizes due to its focus on the most informative support vectors, making it less prone to overfitting. XGBoost (Extreme Gradient Boosting) builds an ensemble of decision trees sequentially, with each tree correcting the errors of the previous one; it handles missing values natively and achieves high accuracy through gradient-based optimisation. To maximise model performance, hyperparameter tuning was performed: n_estimators and max_depth for Random Forest and XGBoost, and C and gamma for SVM. To enhance model performance, hyperparameter tuning was performed, focusing on the number of trees in the forest (n_estimators), ranges from 5 to 105 were tested in increments of 20 during an initial coarse search, and then refined to 80–90 in increments of 1 for fine-tuning. For the maximal depth of the trees (max_depth), ranges from 3 to 50 were tested in increments of 10, then refined to 3–10 in increments of 1. For SVM, the tested ranges were C [0.1–10] and gamma [0.001–1].

Feature importance and dimensionality reduction

Feature importance was assessed for each classifier to identify the most discriminative variables. For the Random Forest, the mean decrease in Gini impurity was used; for the SVM, feature weights derived from the linear kernel coefficients were applied; and for XGBoost, gain-based feature importance scores were calculated. Results were compared across all three classifiers to identify consistently important predictors. To visualise the separability of cancer and control groups in a two-dimensional space, Uniform Manifold Approximation and Projection (UMAP) dimensionality reduction was applied to the combined dataset, using the Jaccard distance metric (Li et al., 2022).

Model evaluation and validation

Leave-one-out cross-validation (LOOCV) was employed to evaluate model performance for each hyperparameter combination. LOOCV is particularly appropriate for smaller datasets, as it maximises the amount of data used for training while providing an unbiased estimate of generalisation performance by testing on each individual observation in turn. Parallel processing was implemented to reduce computational time across multiple

parameter combinations. To ensure result stability and independence from random initialisation, each model was trained and evaluated 20 times with different random seeds; performance metrics were averaged across all 20 repetitions. Performance metrics included accuracy, sensitivity (recall), specificity, F1-score, and the area under the receiver operating characteristic (ROC) curve (AUC). Three separate model configurations were evaluated for each classifier on the same 152-participant analytical cohort: (1) miRNA expression data only; (2) clinical and demographic data only; and (3) combined miRNA and clinical data. This design allowed a direct comparison of the individual and additive diagnostic contributions of molecular and clinical information while keeping the cohort constant across configurations. All machine learning analyses were conducted in Python. All AI-generated contributions were thoroughly reviewed and approved by medical experts to ensure accuracy and conformity to scientific standards.

3 Results

Overall, 9589 patients with newly diagnosed BC were identified between 1990 and 2017. Almost 75 % (7188 cases) were men and 25 % (2401 cases) were women, giving a gender ratio 3:1 (men to women; see Figure 3.1). The characteristics of the patient cohort are presented in Table 3.1.

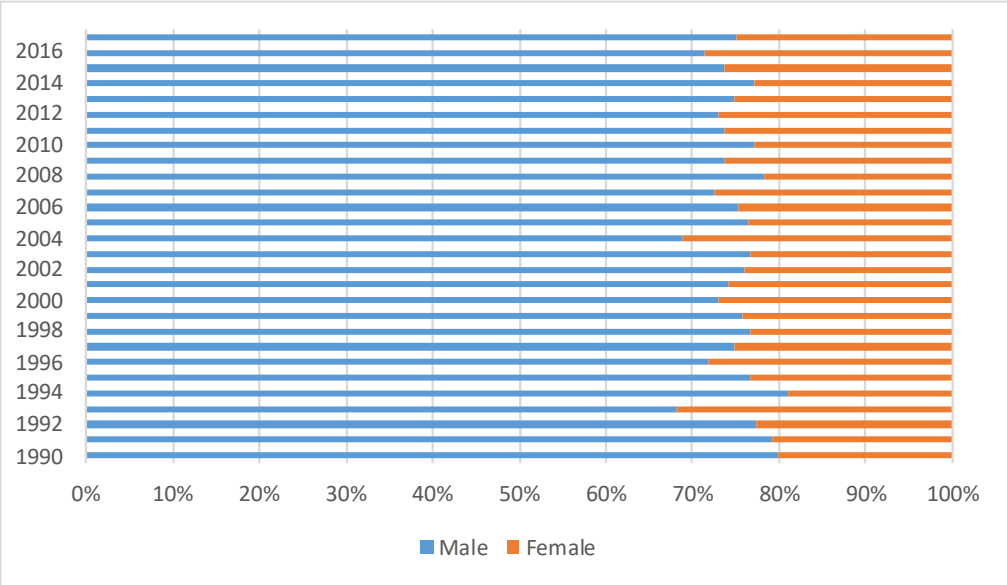


Figure 3.1 **Bladder cancer male and female ratio at different time periods**

Table 3.1

Bladder cancer patients' cohort characteristics

	Total 1990–2017		**Years 1990–2000**		**Years 2001–2012**		**Years 2013–2017**	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Total	9589	100	0	100	0	100	0	100
Gender								
Male	7188	75.0	2277	75.7	3233	74.7	1678	74.5
Female	2401	25.0	729	24.3	1098	25.3	574	25.5
Age group								
0–19	14	0.2	9	0.3	3	0.1	2	0.1
20–39	121	1.3	38	1.3	54	1.2	29	1.3
40–59	1711	17.8	589	19.6	796	18.4	326	14.5
60–79	6085	63.4	1919	63.8	2754	63.6	1412	62.7
80+	1658	17.3	451	15.0	724	16.7	483	21.4
T stage								
1	2858	29.8	422	14.0	1412	32.6	1024	45.5
2	2063	21.5	797	26.5	833	19.2	433	19.2
3	1526	15.9	675	22.5	630	14.5	221	9.8
4	546	5.7	177	5.9	249	5.8	120	5.3
Unknown	2596	27.1	935	31.1	1207	27.9	454	20.2

T stage – Tumour stage

The average age at diagnosis was 68.8 years (SD 11.5); it was 67.8 years (SD 11.3) for men and 72.0 years (SD 11.7) for women (Figure 3.2). Comparing the initial period (1990–1992) with the final period (2015–2017), the mean age of patients at the time of diagnosis increased significantly from 67.6 to 71.2 ($p < 0.001$). Comparing these two periods, the mean age in men significantly increased from 66.2 to 70.2 years ($p < 0.001$), whereas a statistically significant increase was not observed in women (72.7 to 73.9 years).

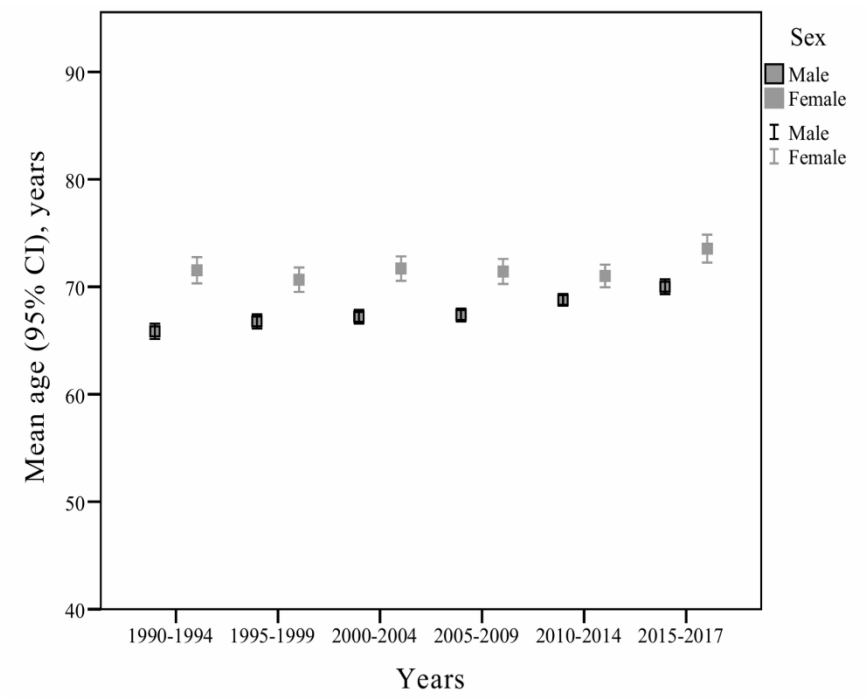


Figure 3.2 **Mean age with 95% confidence interval at bladder cancer diagnosis**

A total of 7182 BC patients died over the period under review, and 67 % (n = 4790) of them died from BC-related causes. The mean age of death was 72.8 years (SD 10.4) Significant changes occurred in the mean age of death between the beginning and the end of the reporting period, increasing from 71.3 years (SD 11.0) to 76.0 years (SD 9.4) (Figure 3.3).

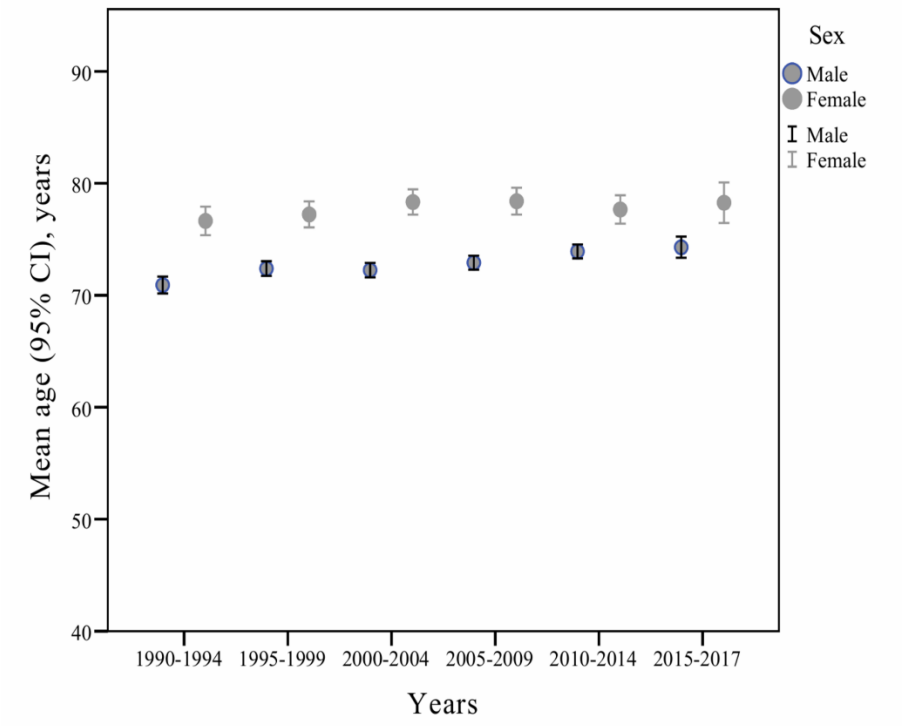


Figure 3.3 **Mean age with 95% confidence interval at bladder cancer deat**

3.1 Incidence

The overall ASR incidence rates (per 100 000) of BC increased gradually from 6.8 in 1990 to 10.8 in 2017, from 15.1 to 21.5 in men and 1.9 to 3.9 in women. There was one joinpoint observed indicating one period with a statistically significant APC: 2.6 % from 1990 to 2014 followed by a statistically non-significant reduction −3.6% from 2014 to 2017 (Table 3.2). Joinpoint analysis showed a significantly increasing trend in the standardised incidence rate for both sexes (Figure 3.4). As far as BC incidence rates by age groups are concerned, APC for all age groups remained stable during the entire review period. The greatest APC of 2.6% was registered in the > 80 years group from 1990 to 2017 (Figure 3.5).

Concerning BC incidence rates by T stage, the largest APC occurred in the T1 stage group, at APC 8.0% during the whole period, and one joinpoint was observed in 2013. Meanwhile, only the T3 stage BC incidence rates significantly decreased, with an APC of −2.3% between 1995 and 2017 (Figure 3.6).

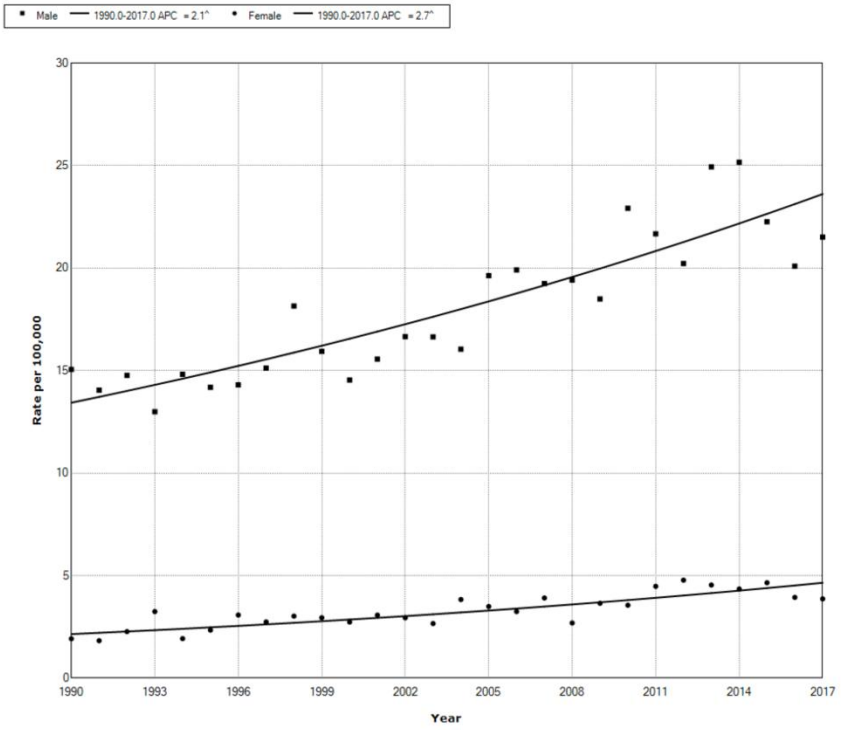


Figure 3.4 **Gender-specific bladder cancer incidence trends using joinpoint analysis**

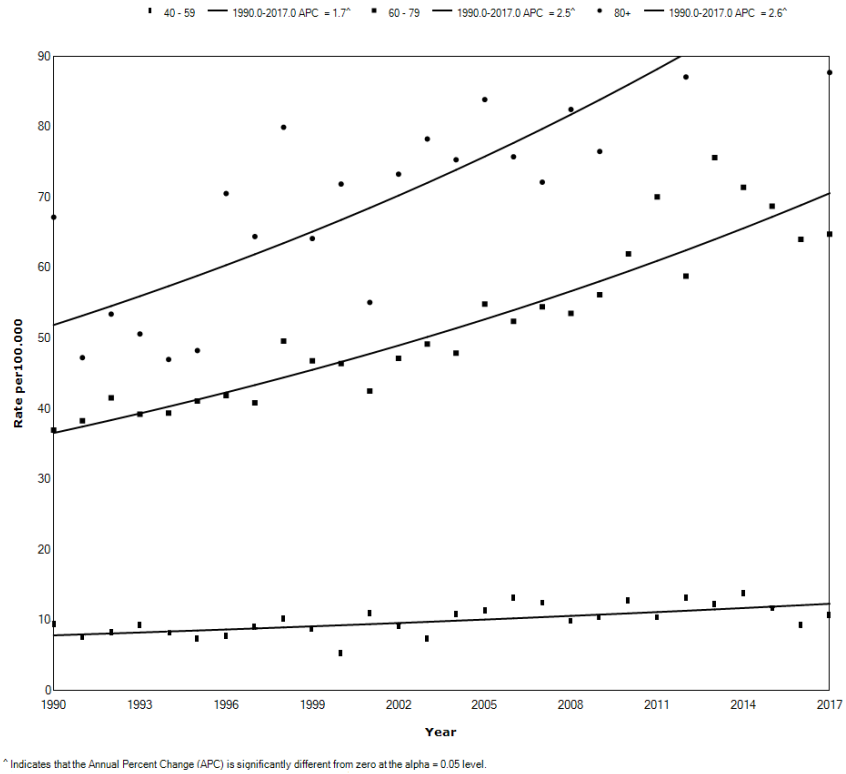


Figure 3.5 Age-**specific bladder cancer incidence trends using joinpoint analysis**

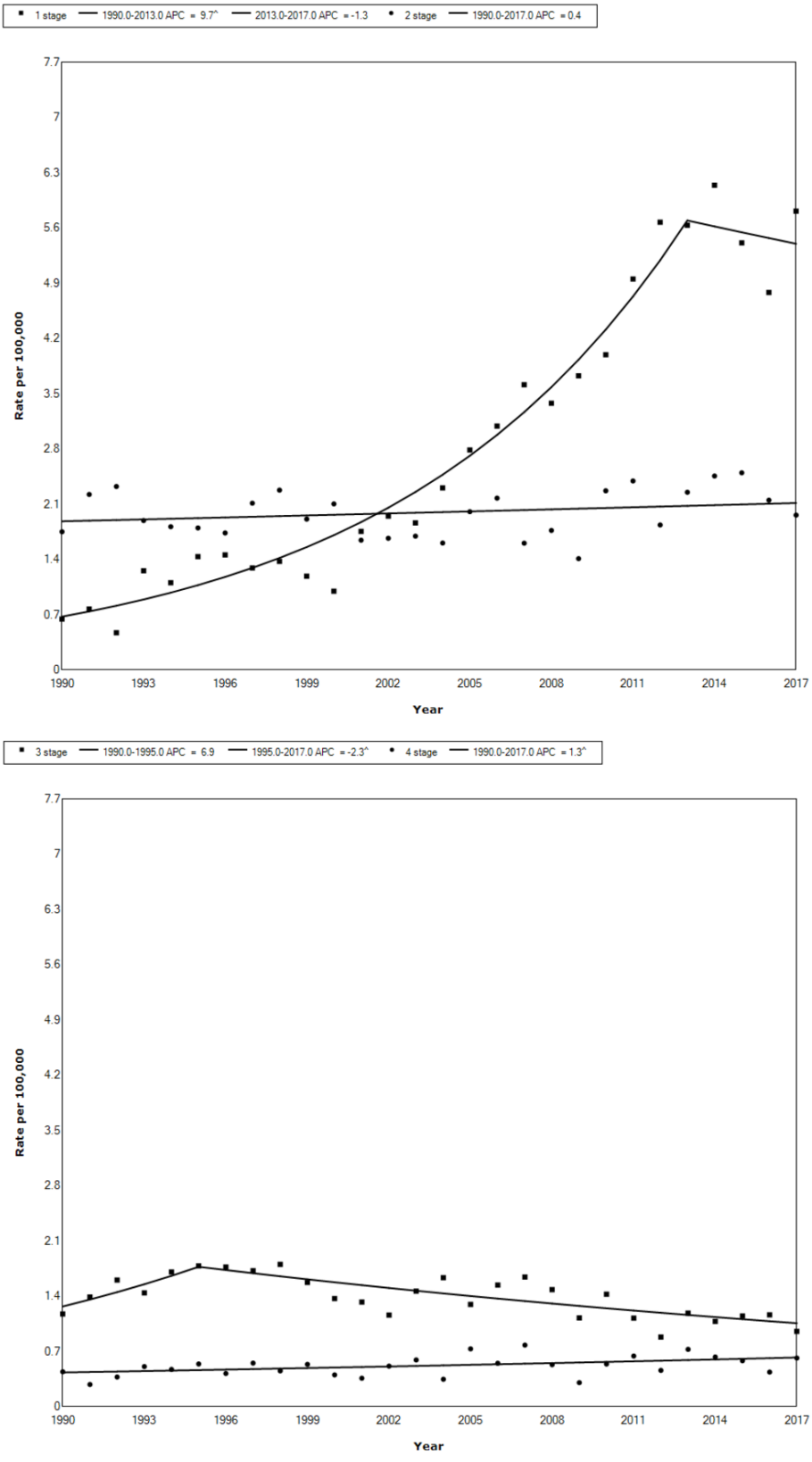


Figure 3.6. **Stage-specific bladder cancer incidence trends using joinpoint analysis**

The standardised incidence trends can be seen in Table 3.2.

Table 3.2

Bladder cancer incidence and mortality trends in Latvia 1990–2017
(joinpoint analysis: age-standardised, by age, gender and stage groups)

	Trend 1		Trend 2		AAPC (95% CI)
	Years	**APC**	**Years**	**APC**	**1990-2017**
Incidence	1990–2014	2.6*	2014–2017	−3.6	1.9* (0.9 to 2.9)
Gender					
Male	1990–2017	2.1*			2.1* (2.5 to 10.1)
Female	1990–2017	2.7*			2.7* (2.0 to 3.5)
Age group, years					
40–59	1990–2017	1.7*			1.7* (0.8 to 2.5)
60–79	1990–2017	2.5*			2.5* (2.1 to 2.8)
80+	1990–2017	2.6*			2.6* (2.0 to 3.1)
T stage					
1	1990–2013	9.7*	2013–2017	−1.3	8.0* (6.9 to 9.7)
2	1990–2017	0.4			0.4 (−0.3 to 1.1)
3	1990–1995	6.9	1995–2017	−2.3*	−0.7 (−2.2 to 0.9)
4	1990–2017	1.3*			1.3* (0.2 to 2.5)
Mortality	1990–2017	0.5*			0.5*(0.1 to 0.9)
Gender					
Male	1990–2017	0.5*			0.5* (0.1 to 1.0)
Female	1990–2017	0.2			0.2 (−0.8 to 1.3)
Age group, years					
40–59	1990–2017	−1.1*			−1.1* (−2.2 to −0.1)
60–79		0.7*			0.7* (0.2 to 1.3)
80+		1.9*			1.9* (1.2 to 2.5)

APC, annual percent change; AAPC, average annual percent change; ASR, age standardised rate per 100,000.

*P < 0.05

3.2 Prevalence and years of potential life lost

At the beginning of the period under review, the number of bladder cancer patients did not exceed one thousand, with 969 cases. Each year the number of patients increases deliberately and at the end of the period under review in 2017 it approximately increases three times and reaches 2 thousand 834 patient cases. When the largest numbers of patients by age group were counted during the reference period, in 2017 they were in the 80+ age group with 720 cases (25%) and in the 60–79 age group with 1689 cases (60%). Dividing patients into gender groups, in 2017 the highest number of cases was in the male group with 2030 (72%) bladder cancer patients, while in the female group only 804 (28%) cases occurred.

The standardised prevalence rates (per 100,000) increased from 25.2 in 1990 to 69.7 in 2017 with an average annual percent change (AAPC) of 3.8 %(95 % CI 3.1 to 4.6) during the whole period, and 3 join points were observed in 1999, 2002 and 2015 (Figure 3.7 and Table 3.3). The prevalence of BC in 1990 was 25.2 (per 100 000 inhabitants per year), which is the lowest value over the considered time period. A statistically significant increase in prevalence was observed between 1990 and 1999 (APC 4.6) reaching a prevalence of 38.1

(per 100 000 inhabitants per year) in 1999. Subsequently, no statistically significant increase in prevalence was observed between 1999 and 2002. From 2002 until 2015, a statistically significant increase in prevalence was noted reaching a prevalence of 68.6 (per 100 000 inhabitants per year) in 2015 with an APC of 4.3. After that no statistically significant increase was observed until 2017. Figure 3.7 provides more detailed information on the prevalence of bladder cancer.

Table 3.3

Bladder cancer prevalence trends in Latvia 1990–2017
(joinpoint analysis: age-standardised, by age, gender groups)

	Trend 1		Trend 2		Trend 3		Trend 4		Trend 5		AAPC (95% CI)
	Years	APC	Years	APC	Years	APC	Years	APC	Years	APC	1990–2017
Prevalence	1990–1999	4.6*	1999–2002	2	2002 – 2015	4.3*	2015–2017	0.4	–	–	3.8* (3.1–4.6)
Gender											
Male	1990–1996	3.5*	1996–1999	6.2*	1999–2002	1.2	2002–2014	4.1*	2014–2017	0.1	3.4* (2.6–4.3)
Female	1990–2017	4.4*	–	–	–	–	–	–	–	–	4.4* (4.3–4.6)
Age group, years											
40–59	1990–2003	2.8*	2003–2017	4.5*	–	–	–	–	–	–	3.7* (3.3–4.2)
60–79	1990–1995	3.3*	1995–1999	5.7*	1999–2002	1.3	2002–2015	4.2*	2015–2017	1.6	3.7* (3.0–4.5)
80+	1990–2000	7.2*	2000–2009	3.6*	2009–2013	7.1*	2013–2017	−7.6*			3.7* (2.7–4.7)

APC, annual percent change; AAPC, average annual percent change; ASR, age standardised rate per 100,000.

*P < 0.05

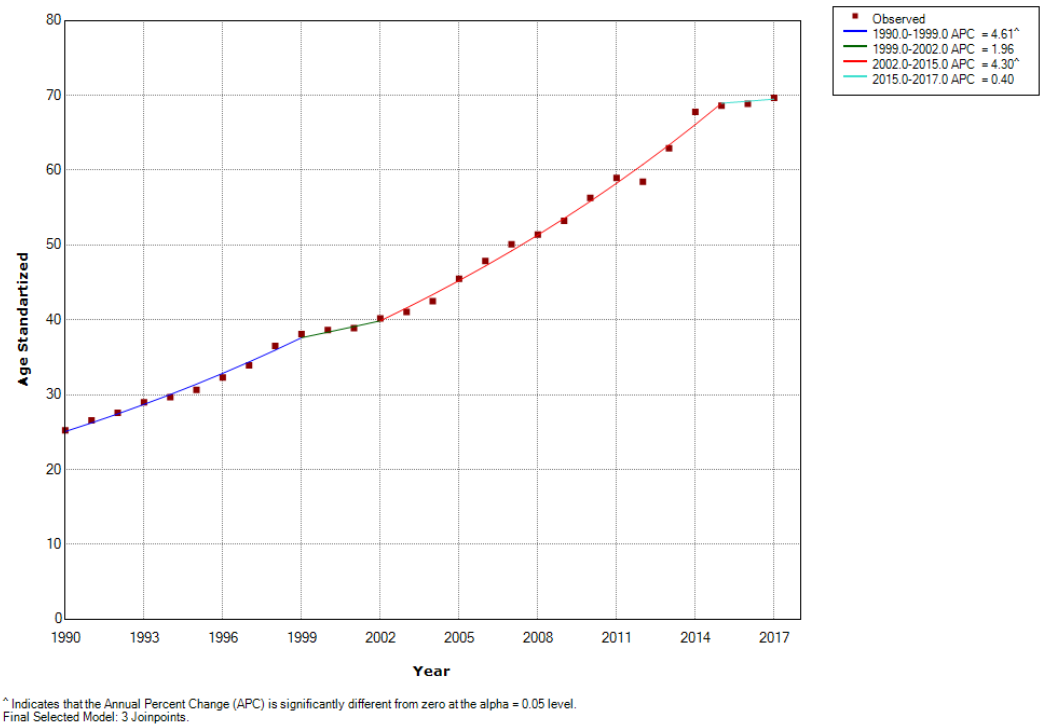


Figure 3.7 **Bladder cancer prevalence trends using joinpoint analysis**

Regarding BCa prevalence rates by sex, the largest fluctuations occurred in men, while the largest AAPC was observed in women (Table 3.3). Both sexes experienced an increase (per 100 000) in the standardised prevalence (rising from 53.4 in 1990 to 132.5 in 2017 in men, compared to a rise from 8.9 in 1990 to 29.2 in 2017 in women) throughout the study period. Only in the 80+ year-old age group was a statistically significant reduction observed, with an APC −7.6% from 2013 to 2017 (Table 3.3)

The total number of years of potential life lost (YPLL) due to BC in Latvia over 28 years was 7450. Comparing the YPLL by period, 959 years (12.1 per 100 000 general population) were lost between 1990 and 1992, and then decreased to 474 years (7.9 per 100 000) between 2015 and 2017. Every person who died due to BC in the beginning of the period, lost an average of 8 years of life, while in the end of the period, this factor fell to 6.5 years.

3.3 Mortality

The period began with an annual mortality rate of 186 deaths among bladder cancer patients. Over time, this rate increased moderately, reaching 360 deaths per year by 2017. A more detailed overview of the mortality rates is provided in Figure 3.8.

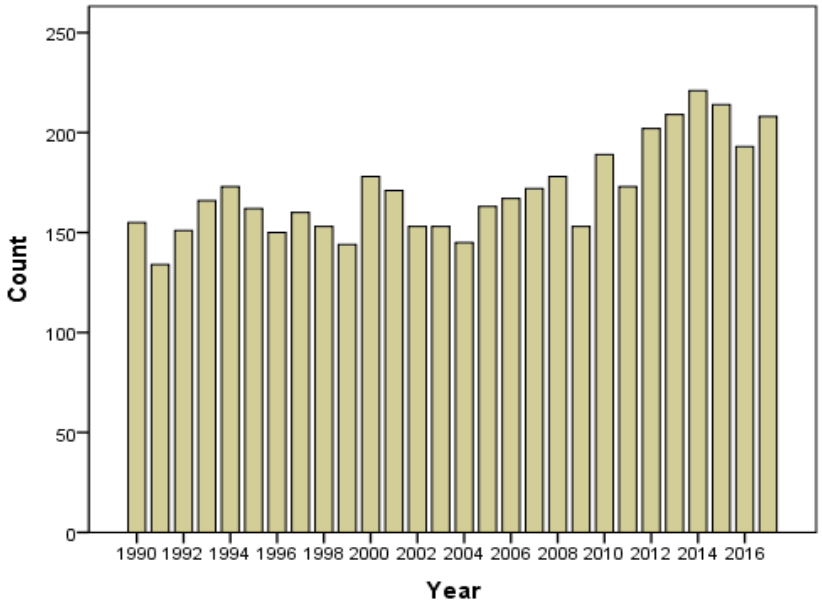


Figure 3.8 **The number of deaths among PC patients**

Meanwhile, the number of deaths due to bladder cancer was 155 per year in the beginning of the review period, increasing up to 208 bladder cancer-related deaths per year in the end of the period considered. A detailed representation of the mortality data is presented in Figure 3.9.

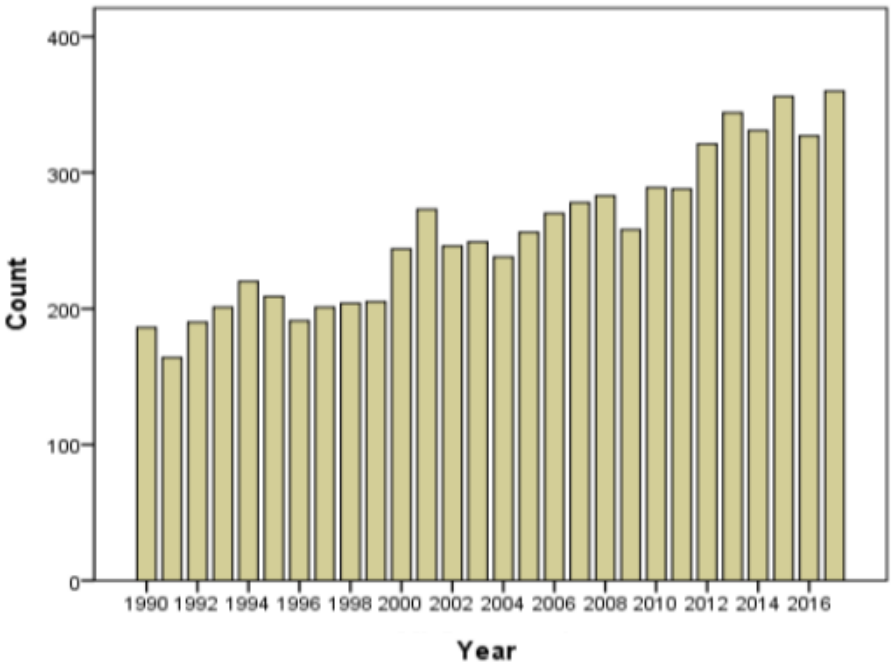


Figure 3.9 **Number of deaths due to BC**

Age-standardised overall mortality rates (per 100 000) also increased from 4.6 in 1990 to 7 in 2017 for women and the 40–59-year age group was the only one showing a statistically insignificant minimal increase in the mortality (Table 3.2).

Overall cancer-specific age-standardised BC mortality rates (per 100 000) rose from 3.9 (8.5 for men, 1.3 for women) in 1990 to 4.4 (9.7 for men, 1.2 for women) in 2017. These rates slightly increased, with APC 0.5 % (Table 3.2 and Figure 3.10), but statistically significantly decreased in the 40–59-year age group, with an APC of −1.1 % from 1990 to 2017. However, BC mortality rates significantly increased in the age group > 80 years, with an APC of 1.9 %, (95% CI 1.2 to 2.5) (Table 3.2 and Figure 3.11).

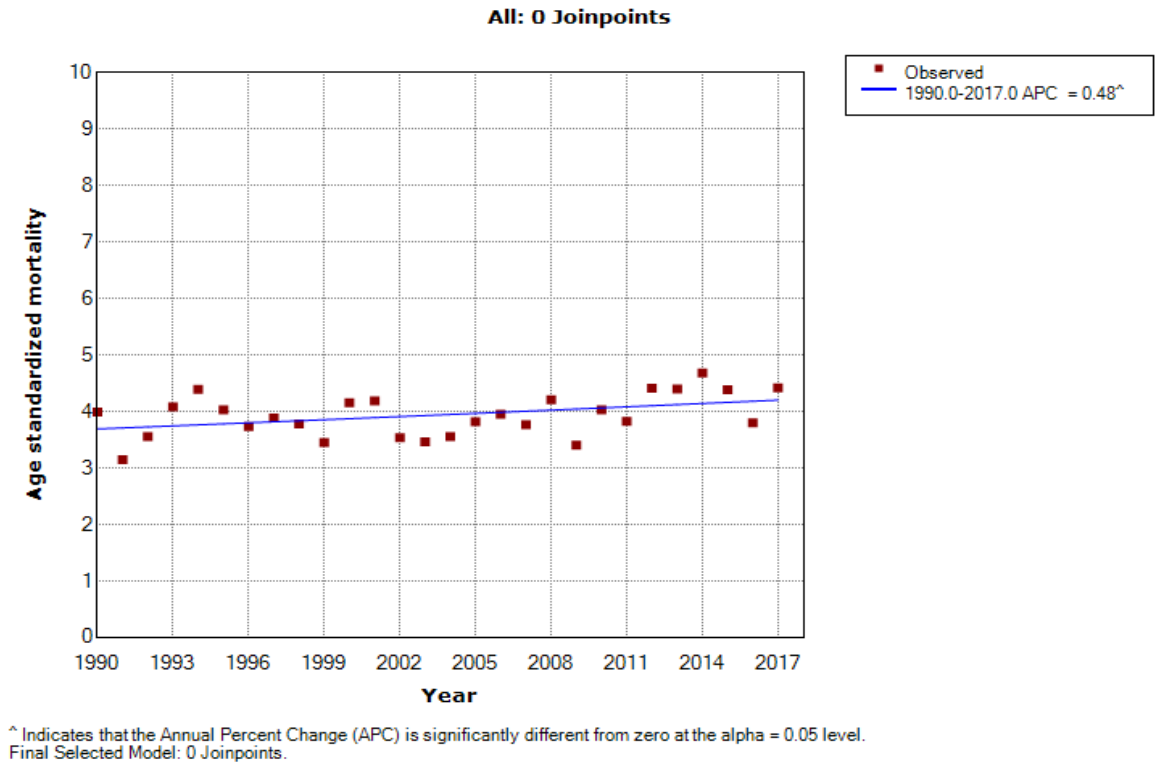


Figure 3.10 **Bladder cancer mortality trends using joinpoint analysis**

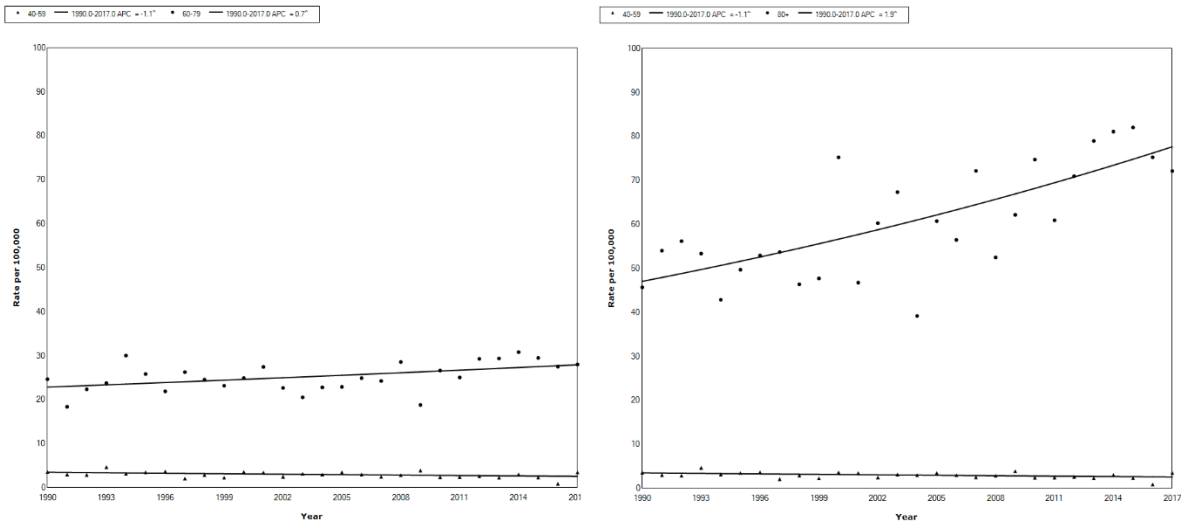


Figure 3.11. **Age-specific bladder cancer mortality trends using joinpoint analysis**

3.4 Survival

3.4.1 Relative survival

Over the whole period, the 5-year relative survival was 59.9% (95% CI 58.4 to 61.3), see Figure 3.12 and Table 3.4 The 5-year RS improved significantly when comparing the beginning (1990–2000) with the end of the study period (2001–2012) and (2013–2017) (55.0% vs. 61.9% vs. 59.0%; p = 0.006) (Figure 3.13 and Table 3.4).

Table 3.4

5-year Relative survival (RS) rates

Survival rates		1990–2000	2001–2012	2013–2017	1990–2017
5-year (CI 95%)	RS	55.0 (52.6 to 57.5)	61.9 (59.9 to 64.0)	59.0 (53.4 to 65.2)	59.9 (58.4 to 61.3)

RS - relative survival, CI - confidence interval

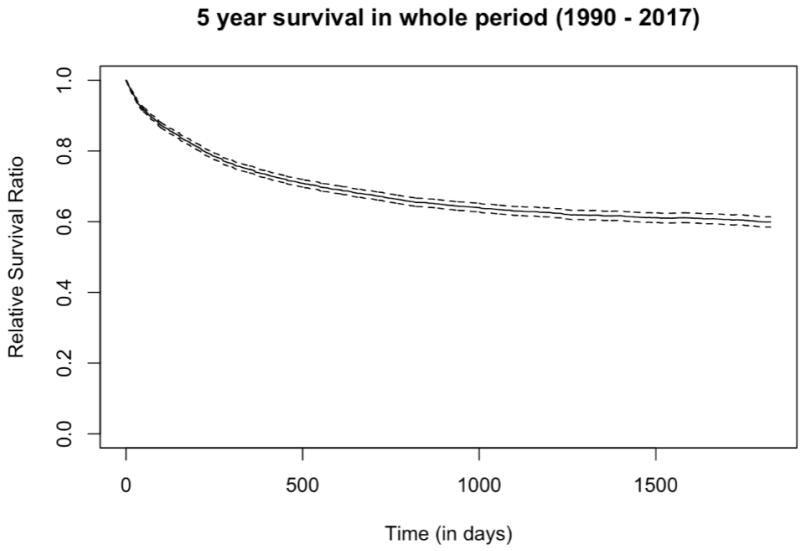


Figure 3.12. **Bladder cancer relative survival over the whole period**

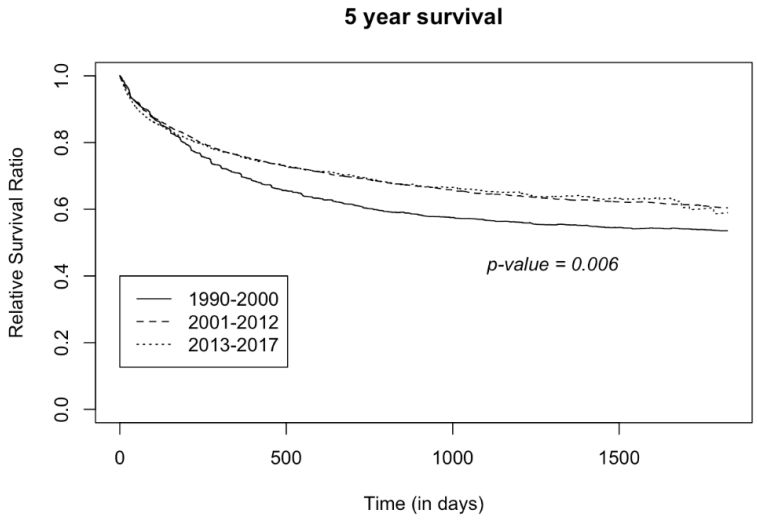


Figure 3.13. **Bladder cancer relative survival by three time periods**

Although women have higher 5-year RS rates than men, they are not statistically significant (Figure 3.14). For women, 5-year RS has increased over 28 years, while it remains fluctuating for men (Table 3.5).

Table 3.5

5-year survival rates in different gender groups

Survival rates		1990–2000	2001–2012	2013–2017
Men (CI 95%)	RS	54.9 (52.1 to 57.8)	60.5 (58.1 to 62.9)	55.6 (48.1 to 64.3)
Female (CI 95%)	RS	55.3 (2.5 to 50.6)	66.0 (62.2 to 70.1)	66.2 (58.8 to 74.6)

RS - relative survival, CI - confidence interval

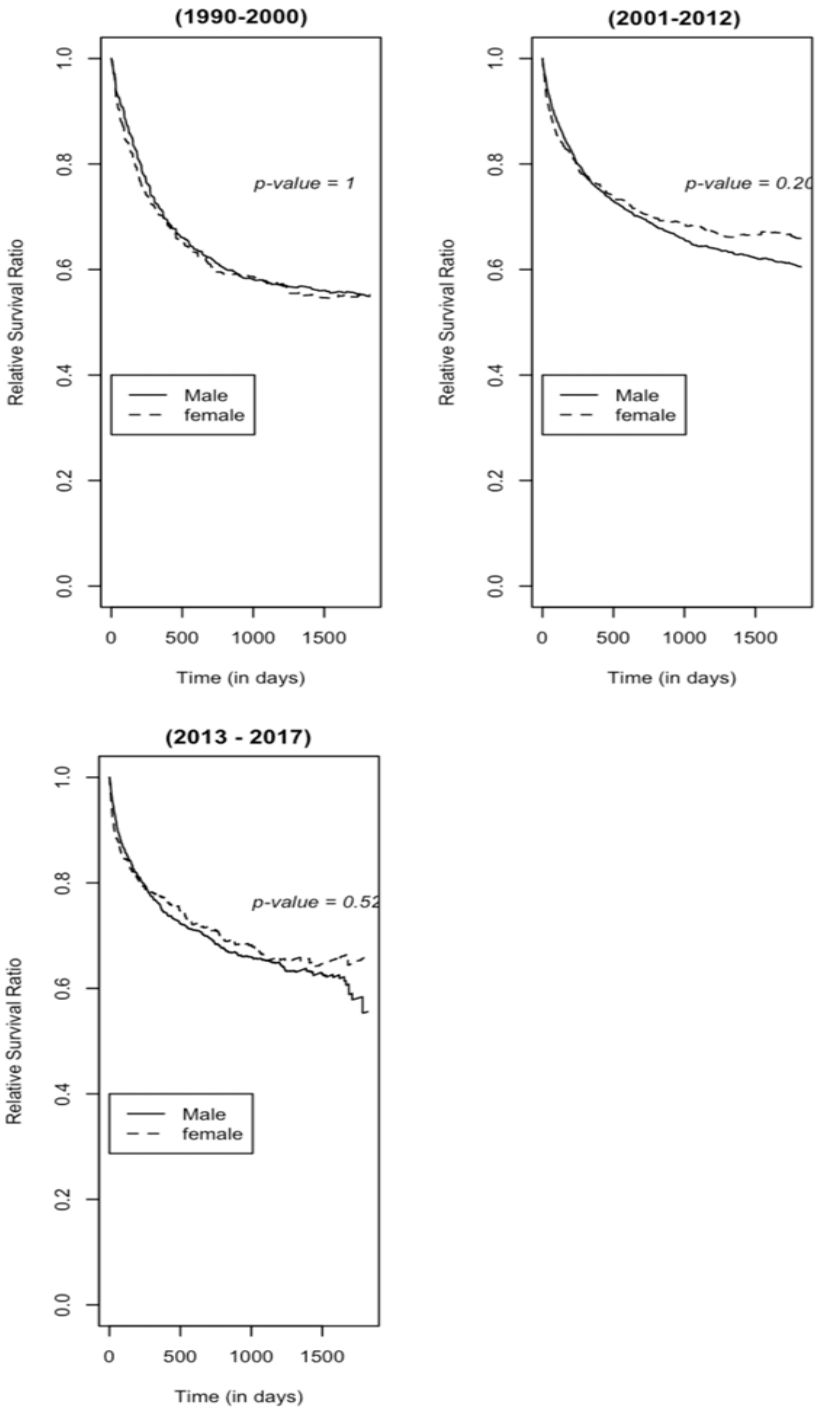


Figure 3.14 **Gender-specific 5-year relative survival of bladder cancer by three time periods**

Analysing survival by age groups, younger patients have a better survival compared to older ones (Table 3.6, Figure 3.15)

Table 3.6

Age-specific 5-year relative survival of bladder cancer during three time periods

Survival rates		1990–2000	2001–2012	2013–2017
4059 (CI 95%)	RS	63.2 (58.8–67.9)	72.4 (68.9–76.1)	76.9 (69.4–85.2)
60–79 (CI 95%)	RS	52.4 (49.5–55.4)	59.0 (56.6–61.6)	57.0 (49.8–65.3)
80+ (CI 95%)	RS	43.4 (35.1–53.5)	50.2 (43.6–57.8)	47.8 (34.3–66.5)

RS - relative survival, CI - confidence interval

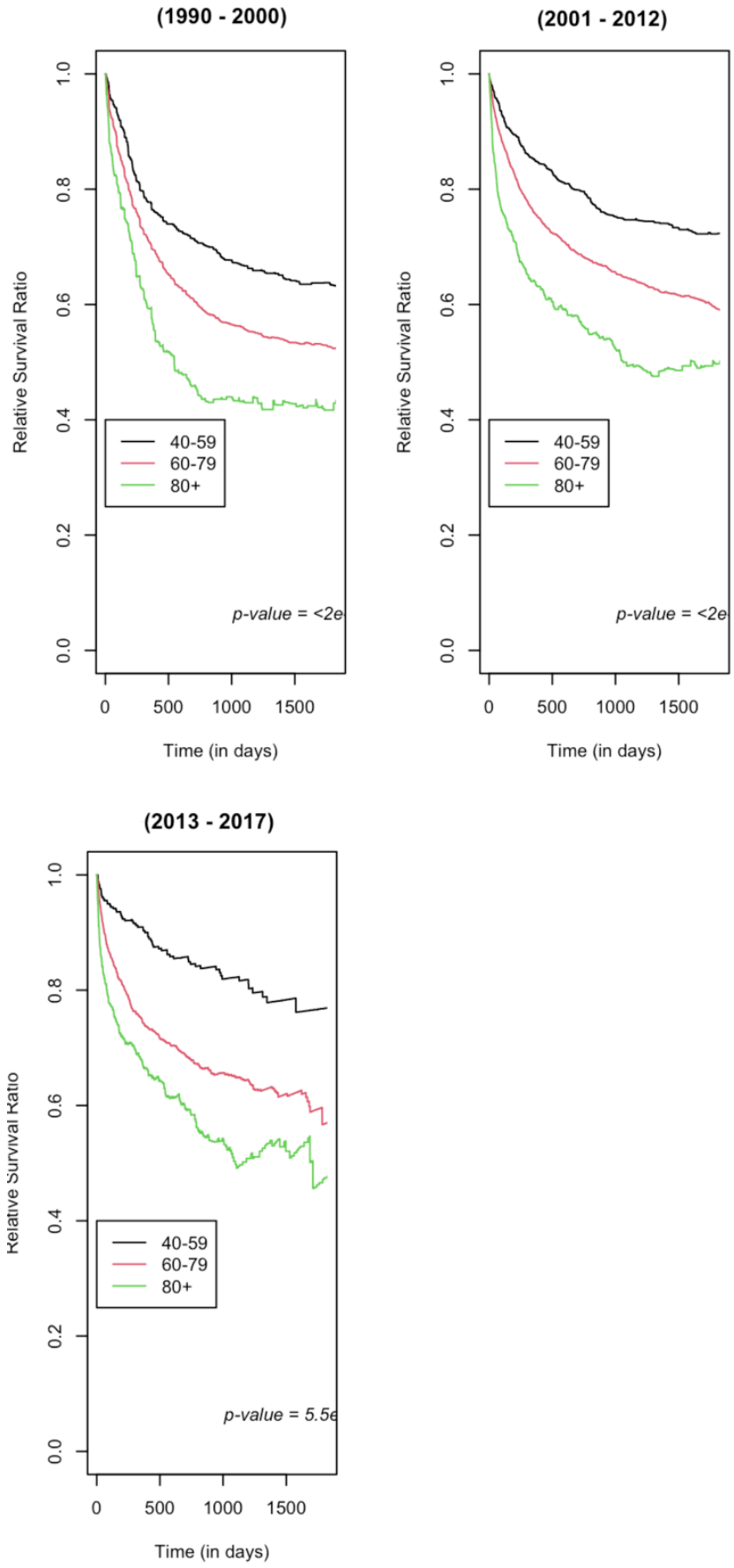


Figure 3.15 **Age-specific 5-year relative survival of bladder cancer by three time periods**

The 5-year RS rates by T stage over the whole study period are shown in Figure 3.16 and Table 3.7. The 5-year RS in the T2 and T3 stage groups has significantly deteriorated compared to 1990–2000 and 2001–2012 and 2013–2017 (Figure 3.17).

Table 3.7

Stage-specific 5-year relative survival of bladder cancer during three time periods

Survival rates		1990–2000	2001–2012	2013–2017
1 (CI 95%)	RS	95.8 (90.6–100)	92.2 (89.2–95.3)	85.2 (73.9–98.1)
2 (CI 95%)	RS	75.6 (71.1–80.4)	61.1 (56.7–65.8)	47.2 (38.1–58.5)
3 (CI 95%)	RS	30.5 (26.4–35.2)	33.1 (28.8–38.0)	10.7 (4.8–23.7)
4 (CI 95%)	RS	10.9 (6.68–17.8)	15.4 (10.9–21.8)	17 (10.3–28.2)

RS – relative survival, CI – confidence interval

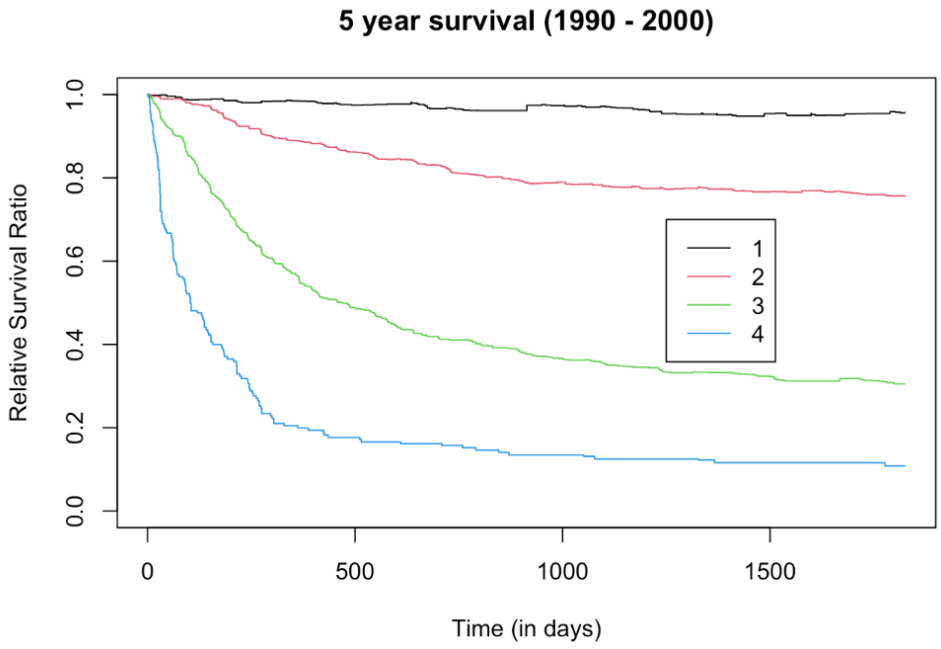


Figure 3.16 **T stage-specific 5-year relative survival of bladder cancer over whole period**

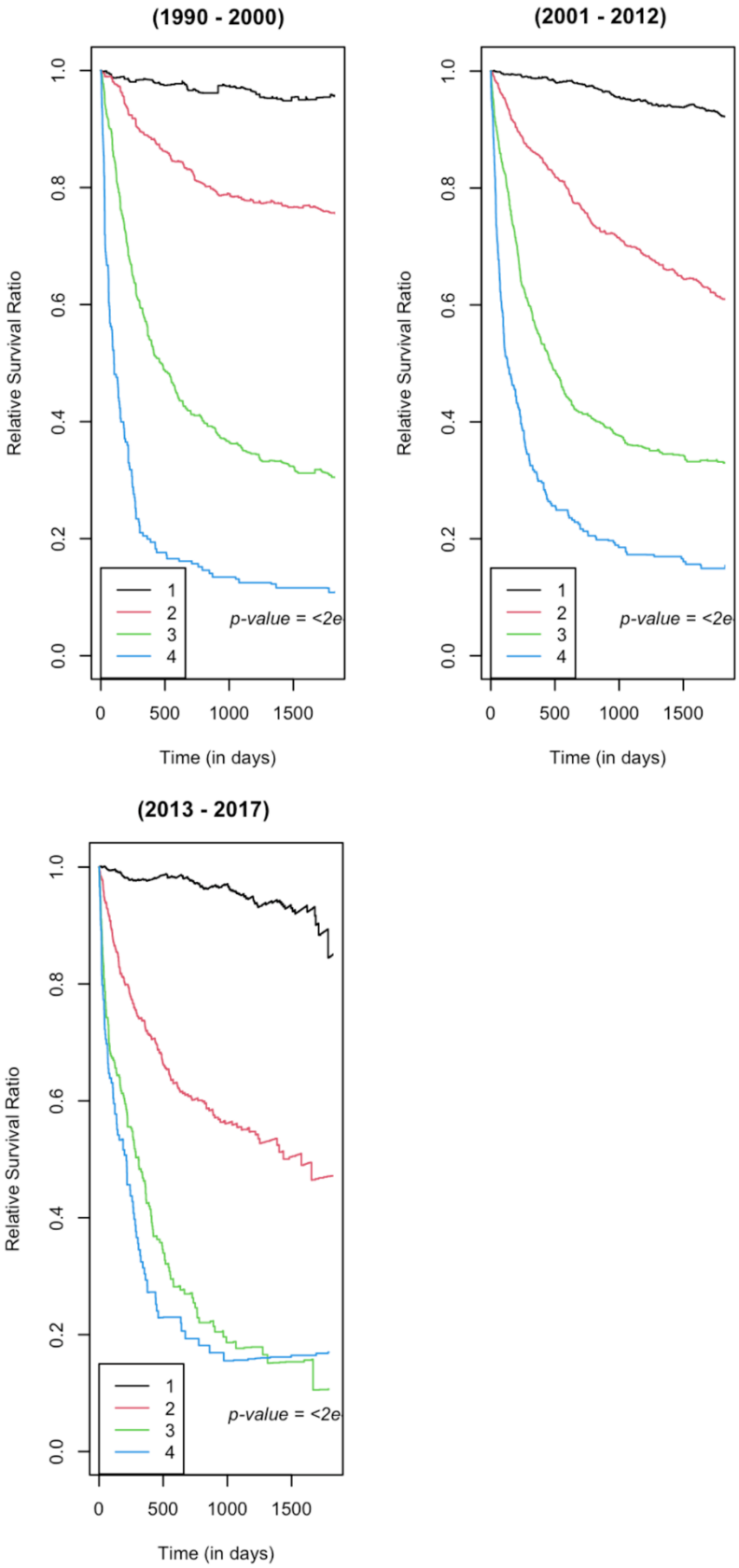


Figure 3.17 **T stage-specific 5-year relative survival of bladder cancer by three time periods**

3.4.2 Overall survival (OS) and cancer-specific survival (CSS)

Over the whole period, the median CSS was 8.45 years (95% CI, 7.562–9.348) (Figure 3.18).

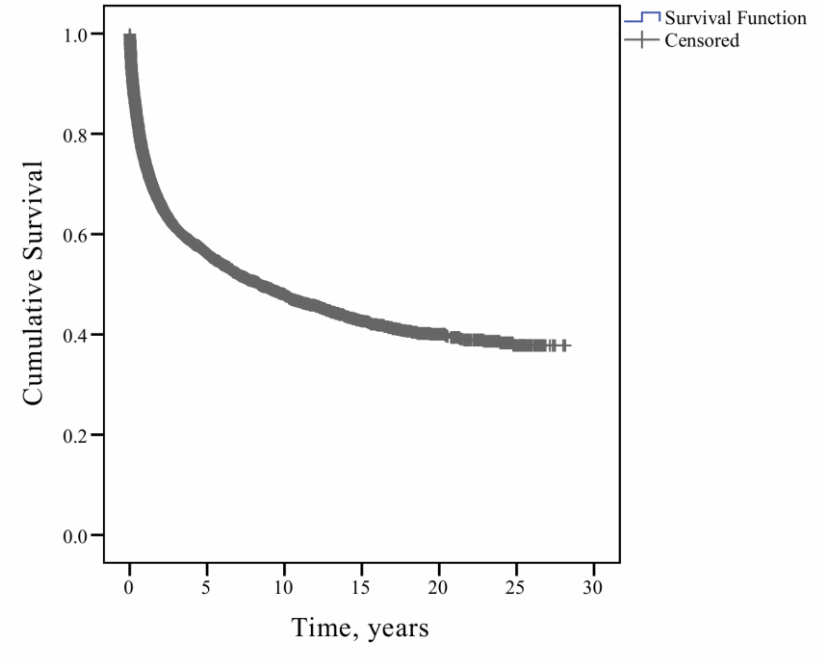


Figure 3.18 **Cancer-specific survival rate**

The 5- and 10-year OS rates were the lowest in the first analysis period following bladder cancer diagnosis: 38.5 % (95% CI 35.3–41.8) between 1996 and 1998 and 24.1% (95% CI 21.2–27.0). The 5- and 10-year OS rates improved during the assessment period, until the 5-year OS rate reached 48.6% (95% CI 45.6–51.6) between 2008 and 2010 and ten-year OS reached 31% (95% CI 28.2–34.0) between 2005–2007.

The lowest survival rates for cancer-specific 5 and 10 years were 47.2% (95% CI 43.4–51.0) and 41.3% (95.5% CI 37.5–45.1) for the period that was considered at the beginning of the period from 1990 to 1992. The survival rates for five and ten years of cancer have improved throughout the period studied, until the five-year CSS reached 62.0% (95% CI 58.9–65.0) between 2008 and 2010 and ten-year CSS reached 52.4% (95% CI 48.8–56.0) between 2005 and 2007.

More details on the 5- and 10-year OS and CSS rates calculated from the year of diagnosis are shown in Table 3.8.

Table 3.8

5- and 10-year survival rates

Survival rates		1990–1992	1993–1995	1996–1998	1999–2001	2002–2004	2005–2007	2008–2010
5-year	OS	41.4 (37.7–45.0)	41.4 (37.9–45.0)	38.5 (35.3–41.8)	40.0 (36.7–43.2)	41.4 (38.2–44.5)	47.3 (44.3–50.4)	48.6 (45.6–51.6)
	CSS	47.2 (43.4–51.0)	48.2 (44.5–51.9)	48.6 (45.1–52.1)	53.2 (49.8–56.7)	55.2 (51.8–58.6)	59.8 (56.7–62.9)	62.0 (58.9–65.0)
10-year	OS	31.3 (27.8–34.8)	27.0 (23.8–30.2)	24.1 (21.2–27.0)	26.4 (23.5–29.2)	31.1 (28.3–34.0)	31.6 (28.2–34.0)	–
	CSS	41.3 (37.5–45.1)	42.1 (38.3–45.9)	41.5 (37.9–45.1)	46.6 (43.1–50.2)	50.4 (47.1–53.8)	52.4 (48.8–56.0)	–

OS – overall survival rate; CSS – cancer-specific survival

When considering OS and CSS by sex, the 5-year survival improved across all groups (Table 3.9). At the end of the reference period, women demonstrated significantly higher survival rates compared with men, which is in stark contrast to the initial period ($p < 0.05$). (Table 3.9.)

Table 3.9

5-year survival rates in different gender groups

Survival rates		1990–1992	1993–1995	1996–1998	1999–2001	2002–2004	2005–2007	2008–2010
Men	OS	42.3 (38.2–46.5)	40.1* (36.0–44.2)	37.7 (33.9–41.4)	38.8* (35.0–42.6)	39.3 (35.7–43.0)	45.2* (41.7–48.7)	48.3 (44.9–51.7)
	CSS	48.5 (44.3.–52.8)	46.8* (42.5–51.0)	48.7 (44.7–52.8)	52.6* (48.5–56.6)	53.0* (49.1–57.0)	58.9 (55.2–62.5)	61.7 (58.2–65.2)-
Women	OS	37.8 (30.0–45.5)	46.4* (39.2–53.7)	41.0 (34.5–47.6)	43.3* (36.7–49.9)	47.4 (41.0–53.8)	53.8* (47.6–60.0)	49.8 (43.5–56.1)
–	CSS	42.2 (34.1–50.2)	52.5* (45.1–60.0)	48.6 (41.8–55.4)	55.1* (48.2–61.9)	61.5* (55.0–68.0)	62.5 (56.3–68.7)	62.8 (56.3–69.2)

OS – overall survival, CSS – cancer-specific survival

*$p < 0.05$

3.5 Patient groups

This study was conducted to identify miRNAs of interest in the urinary exomes of patients with bladder cancer. 12 patients were identified in bladder cancer groups (LG 6 men, median age 71.5 years vs HG 2 men, 4 women, median age 71.5 years) and 7 healthy control patients (6 men and 1 woman, median age 66 years). For a more detailed overview of the cohorts, see Table 3.10.

Table 3.10

Clinical-pathological parameters of the study cohorts

Variable	Study groups		
	Control (N = 7)	LG (N = 6)	HG (N = 6)
Age (years)			
Median	66	71.5	71.5
Range	53–81	50–78	61–86
Gender			
Men	6	6	2
Women	1	0	4
Pathological stage			
≤ pT1	–	6	0
≥ pT2	–	0	6
Grade			
LG	–	6	0
HG	–	0	6

N – number; LG – low grade; HG – high grade

3.6 Differentially expressed urinary exosomal miRNAs in bladder cancer patients

After comparing the overall profiles of exosomal miRNAs in the urine samples of patients with cancer, miRNAs presenting differently – with more than 2 fold regulation (FR) or less than −2 FR – were analysed compared to the control group. The number of miRNAs meeting these threshold values was 96 miRNAs in the LG group (93 up-regulated and 3 down-regulated) and 78 in the HG group (72 up-regulated and 6 down-regulated) (Tables 3.11 and 3.12; Figures 3.19 and 3.20). Of these differentially expressed exosomal miRNAs, 42 overlapped between the LG and HG groups, all of them up-regulated relative to the controls.

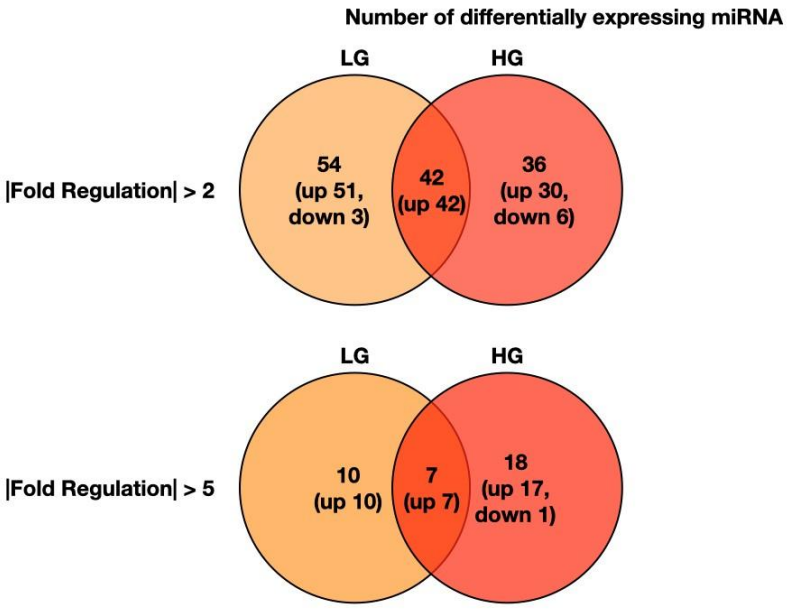


Figure 3.19 **Number of differentially expressing miRNA in low grade and high grade groups**

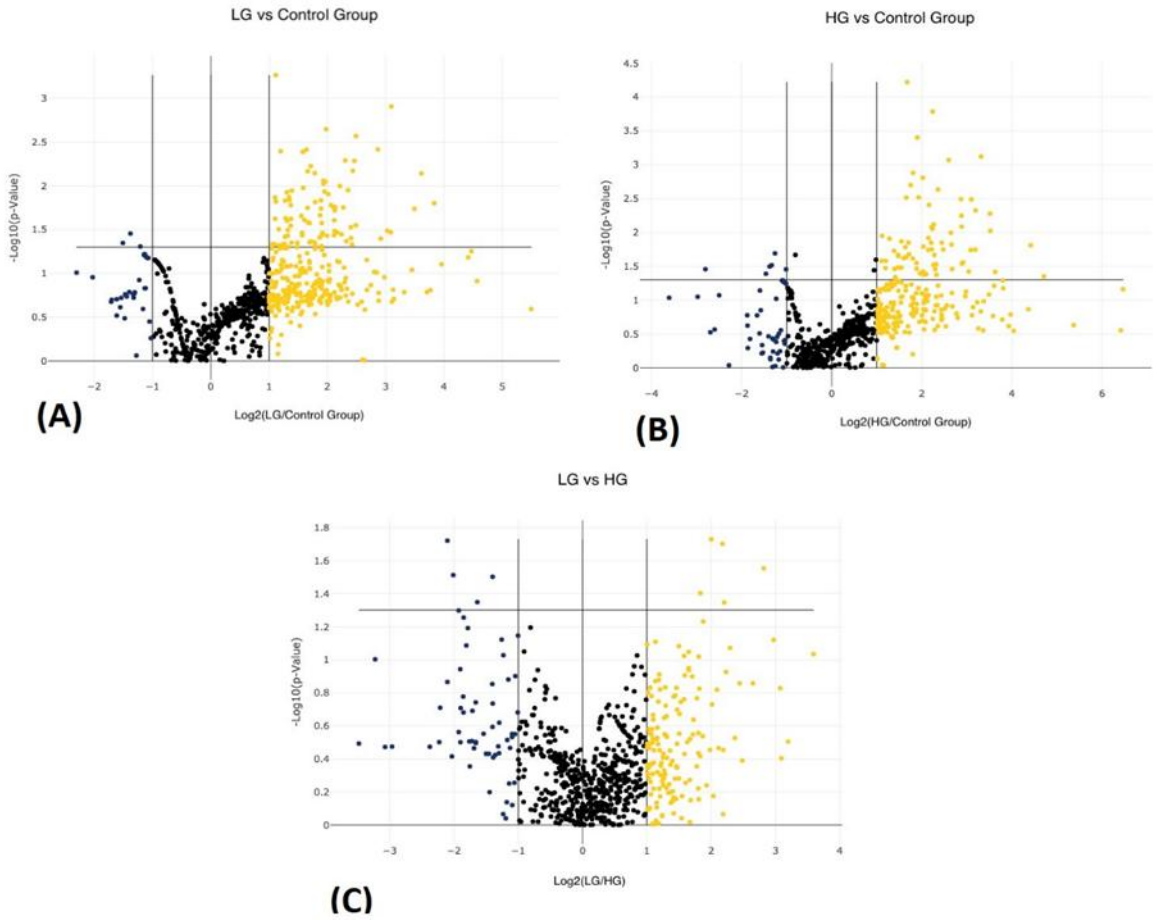


Figure 3.20 **Volcano plots of comparison (A) wild type(wt) low grade to control group, (B) wt high grade to control group, (C) wt low grade to high grade group**

Table 3.11

Differentially expressed urinary exosomal miRNAs and corresponding target genes in LG bladder cancer patients group

miRNA	Fold regulation	p-value	Target gene
Up-regulated			
hsa-miR-21-3p	14.25	0.016	*PTEN*
hsa-miR-324-5p	12.24	0.007	
hsa-miR-31-3p	11.27	0.018	
hsa-miR-30b-3p	8.57	0.001	
hsa-miR-188-5p	8.50	0.034	
hsa-miR-769-5p	8.15	0.033	
hsa-miR-132-3p	7.55	0.040	*EGFR*
hsa-miR-628-3p	7.30	0.004	
hsa-miR-126-3p	5.62	0.003	*AKT1*
hsa-miR-221-3p	5.62	0.028	*PTEN*
hsa-miR-455-3p	5.51	0.005	
hsa-miR-34a-3p	5.42	0.007	*AKT1, CTNNB1, MYC*
has-let-7f-2-3p	5.37	0.047	
hsa-miR-28-5p	5.30	0.039	*TP53*
hsa-miR-210-3p	5.29	0030	
hsa-miR-20a-3p	5.11	0.011	*PTEN, MYC*
hsa-miR-10b-3p	5.01	0.043	
hsa-miR-196b-3p	4.94	0.005	
hsa-miR-652-3p	4.84	0.018	

Table 3.11 continued

miRNA	Fold regulation	p-value	Target gene
Up-regulated			
hsa-miR-582-5p	4.73	0.036	
hsa-miR-15b-5p	4.64	0.010	
hsa-miR-27b-3p	4.49	0.034	
hsa-miR-29a-5p	4.39	0.018	
hsa-miR-193b-5p	4.36	0.019	
hsa-miR-301a-3p	4.36	0.033	*PTEN*
hsa-miR-450a-5p	4.35	0.018	
hsa-miR-29c-5p	4.34	0.025	
hsa-miR-135b-5p	4.33	0.037	
hsa-miR-21-5p	4.32	0.018	*PTEN, EGFR*
hsa-miR-152-3p	4.18	0.017	*PTEN*
hsa-miR-16-5p	4.05	0.012	*TP53*
hsa-miR-200a-5p	3.99	0.037	*PTEN, CTNNB1*
hsa-miR-320b	3.94	0.002	
hsa-miR-151a-3p	3.90	0.009	
hsa-miR-20b-3p	3.87	0.012	
hsa-miR-18a-5p	3.82	0.009	*PTEN*
hsa-miR-425-5p	3.82	0.009	*PTEN*
hsa-miR-148b-3p	3.78	0.034	
hsa-miR-31-5p	3.75	0.036	
hsa-miR-15a-5p	3.74	0.027	
hsa-miR-129-5p	3.73	0.049	
hsa-miR-27a-3p	3.71	0.015	
hsa-miR-376c-3p	3.71	0.018	
hsa-miR-101-3p	3.67	0.022	
hsa-miR-197-3p	3.65	0.039	
has-let-7e-3p	3.46	0.015	
hsa-miR-24-2-5p	3.46	0.049	
hsa-miR-181a-3p	3.45	0.044	
hsa-miR-664a-3p	3.44	0.007	
hsa-miR-106b-3p	3.30	0.043	*PTEN*
hsa-miR-23a-3p	3.28	0.006	
hsa-miR-331-3p	3.24	0.044	
hsa-miR-185-5p	3.23	0.027	*AKT1*
hsa-miR-99b-5p	3.18	0.026	
hsa-miR-28-3p	3.17	0.007	*TP53*
hsa-miR-501-3p	3.16	0.018	
hsa-miR-191-5p	3.12	0.004	
hsa-miR-502-3p	3.04	0.024	
hsa-miR-151a-5p	3.00	0.011	
hsa-miR-378a-3p	2.99	0.004	
hsa-miR-200a-3p	2.97	0.034	*PTEN, CTNNB1, EGFR*
hsa-miR-324-3p	2.97	0.037	
hsa-miR-532-3p	2.93	0.022	
hsa-miR-500a	2.80	0.045	
hsa-miR-222-3p	2.80	0.050	*PTEN*
hsa-miR-141-3p	2.62	0.011	*PTEN*
hsa-miR-24-3p	2.61	0.045	*TP53, MYC*
hsa-miR-19a-3p	2.60	0.024	*PTEN*
hsa-miR-22-3p	2.59	0.017	*AKT1*
hsa-miR-25-3p	2.59	0.028	*TP53*

Table 3.11 continued

miRNA	Fold regulation	p-value	Target gene
Up-regulated			
hsa-miR-200c-3p	2.55	0.014	*PTEN*
hsa-miR-200b-3p	2.54	0.013	
hsa-miR-125a-5p	2.51	0.050	*AKT1*
hsa-miR-320c	2.50	0032	
hsa-miR-423-5p	2.50	0.037	
hsa-miR-33a-5p	2.48	0.025	*MYC*
hsa-miR-429	2.47	0.012	*PTEN*
hsa-miR-320d	2.43	0.023	
hsa-miR-26b-5p	2.43	0.038	*PTEN*
hsa-miR-181a-5p	2.42	0.015	*PTEN*
hsa-miR-19b-3p	2.42	0.038	*TP53*
hsa-miR-320a	2.40	0.046	*PTEN*
hsa-miR-424-5p	2.38	0.046	
hsa-miR-10b-5p	2.38	0.047	*TP53*
hsa-miR-203a-3p	2.29	0.004	
hsa-miR-107	2.26	0.048	
hsa-miR-374b-5p	2.24	0.031	
hsa-miR-934	2.17	0.001	
hsa-miR-361-5p	2.17	0.0150	
hsa-miR-93-5p	2.15	0.014	*PTEN*
hsa-miR-29c-3p	2.15	0.024	*PTEN*
hsa-miR-103a-3p	2.14	0.047	*PTEN*
hsa-miR-628-5p	2.09	0.046	
Down-regulated			
hsa-miR-1247-5p	−2.31	0.049	
hsa-miR-492	−2.60	0.035	*PTEN*
hsa-miR-135b-3p	−2.84	0.045	

Table 3.12

Differentially expressed urinary exosomal miRNAs and corresponding target genes in high grade bladder cancer patients group

miRNA	Fold regulation	p-value	Target gene
Up-regulated			
hsa-miR-150-5p	26.15	0.044	*TP53, CTNNB1*
hsa-miR-126-3p	21.35	0.015	*AKT1*
hsa-miR-205-5p	12.37	0.038	*PTEN*
hsa-miR-324-5p	11.47	0.009	
hsa-miR-628-3p	11.42	0.005	
hsa-miR-28-5p	9.95	0.001	*TP53*
hsa-miR-18a-5p	9.14	0.005	*PTEN*
hsa-miR-21-3p	9.10	0.018	*PTEN*
hsa-miR-24-1-5p	8.57	0.003	
hsa-miR-342-3p	8.49	0.018	
hsa-miR-130b-3p	8.24	0.038	*PTEN*
hsa-miR-197-3p	7.54	0.028	
hsa-miR-376c-3p	7.41	0.029	
hsa-miR-182-5p	7.38	0.008	*PTEN*
hsa-miR-20a-3p	7.36	0.006	*PTEN*
hsa-miR-590-3p	7.30	0.003	
hsa-miR-21-5p	6.38	0.035	*PTEN, EGFR*
hsa-let-7f-2-3p	6.31	0.015	

Table 3.12 continued

miRNA	Fold regulation	p-value	Target gene
Up-regulated			
hsa-miR-200b-5p	6.26	0.010	
hsa-miR-374a-5p	6.05	0.001	
hsa-miR-7-1-3p	5.56	0.018	*EGFR*
hsa-miR-130b-5p	5.44	0.014	
hsa-miR-505-5p	5.14	0.002	
hsa-miR-183-5p	5.01	0.042	
hsa-miR-320b	4.97	0.029	
hsa-miR-105-5p	4.91	0.035	*AKT1*
hsa-miR-27b-3p	4.85	0.021	
hsa-miR-450a-5p	4.81	0.008	
hsa-miR-454-3p	4.79	0.030	
hsa-miR-28-3p	4.73	0.000	*TP53*
hsa-miR-146b-5p	4.71	0.009	
hsa-miR-425-5p	4.66	0.018	*PTEN*
hsa-miR-191-5p	4.65	0.008	
hsa-miR-301a-3p	4.62	0.014	*PTEN*
hsa-miR-151a-3p	4.47	0.004	
hsa-miR-320c	4.43	0.012	
hsa-miR-18a-3p	4.43	0.018	
hsa-miR-148b-3p	4.27	0.041	
hsa-miR-342-5p	4.12	0.042	
hsa-miR-664a-3p	4.10	0.016	
hsa-miR-455-3p	4.09	0.038	
hsa-miR-574-3p	4.06	0.002	*EGFR*
hsa-miR-618	3.89	0.040	
hsa-miR-365a-3p	3.81	0.003	
hsa-miR-200b-3p	3.74	0.000	
hsa-miR-181d-5p	3.69	0.034	
hsa-miR-193b-5p	3.68	0.028	
hsa-miR-200c-3p	3.50	0.001	*PTEN*
hsa-miR-23b-3p	3.48	0.024	*PTEN*
hsa-miR-374b-5p	3.37	0.002	
hsa-miR-92a-3p	3.29	0.021	*PTEN*
hsa-miR-320d	3.26	0.014	
hsa-miR-429	3.19	0.000	*PTEN, MYC*
hsa-miR-151a-5p	3.15	0.003	
hsa-miR-339-3p	3.15	0.023	
hsa-miR-26b-5p	3.12	0.023	*PTEN*
hsa-miR-99b-5p	3.11	0.016	
hsa-miR-489-3p	3.03	0.024	
hsa-miR-361-5p	2.92	0.014	
hsa-miR-320a	2.91	0.033	*PTEN*
hsa-miR-34a-3p	2.79	0.022	*AKT1, CTNNB, MYC*
hsa-miR-26a-5p	2.78	0.021	*PTEN*
hsa-miR-187-5p	2.77	0.033	
hsa-miR-218-5p	2.66	0.045	*EGFR*
hsa-miR-532-3p	2.66	0.047	
hsa-let-7d-3p	2.63	0.034	
hsa-let-7d-5p	2.61	0.019	
hsa-miR-125a-5p	2.43	0.012	*AKT1*
hsa-miR-106b-5p	2.30	0.026	*PTEN*

Table 3.12 continued

miRNA	Fold regulation	p-value	Target gene
Up-regulated			
hsa-let-7g-5p	2.29	0.033	
hsa-miR-423-3p	2.17	0.011	
hsa-miR-423-5p	2.15	0.026	
Down-regulated			
hsa-miR-548m	−2.02	0.035	
hsa-miR-1247-5p	−2.39	0.020	
hsa-miR-492	−2.52	0.030	*PTEN*
hsa-miR-302b-3p	−2.60	0.032	
hsa-miR-135b-3p	−2.75	0.041	
hsa-miR-1-3p	−6.99	0.035	

3.7 *TP53* and *PTEN* related exosomal miRNAs

Most exosomal miRNAs in the LG and HG group profiles are up-regulated (Tables 3.11 and 3.12), suggesting that many of these exosomal miRNAs may be involved in downregulating tumour suppressors. In the LG group, seven miRNAs (miR-28-5p, miR-16-5p, miR-28-3p, miR-24-3p, miR-25-3p, miR-19b-3p, and miR10b-5p) and in HG group three miRNAs (miR-150-5p, miR-28-5p, and miR28-3p) targeting *TP53* were found in the miRTargetLink database with strong validation. The changes in levels of these *TP53* target miRNAs from the control group to the LG and HG groups are shown in Figure 3.21. Two of these miRNAs, miR-28-5p and miR-28-3p, were significantly up-regulated in both groups and reached higher fold regulation in the HG than in the LG group, whereas miR-150-5p was significantly up-regulated only in the HG group and did not reach statistical significance in the LG group.

There are 22 exosomal miRNAs in the LG group and 17 exosomal miRNAs in the HG group (Tables 3.11 and 3.12) that directly target *PTEN* with strong validation. The levels of these *PTEN*-targeting exosomal miRNAs with greater than 5 FR in LG or HG are also shown in Figure 3.22.

When focusing on exosomal miRNAs with greater than 5FR, the HG group had more miRNAs related to *TP53* and *PTEN* (two *TP53* targeting miRNAs and seven *PTEN* targeting miRNAs) than the LG group (one *TP53* targeting and three *PTEN* targeting miRNAs) (Table 3.11 and 3.12). This observation indicates *that TP53* and *PTEN* are more strongly suppressed in the HG group than in the LG group.

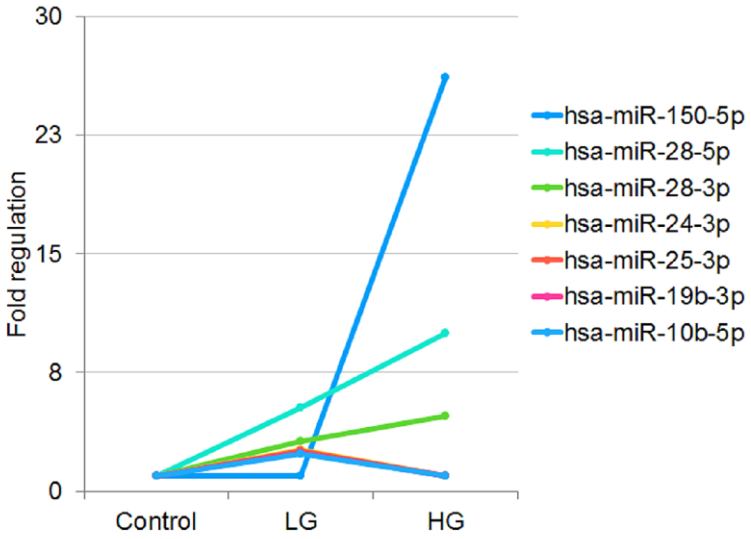


Figure 3.21 **Changes in levels of *TP53*-targeted exosomal miRNAs during bladder cancer progression**

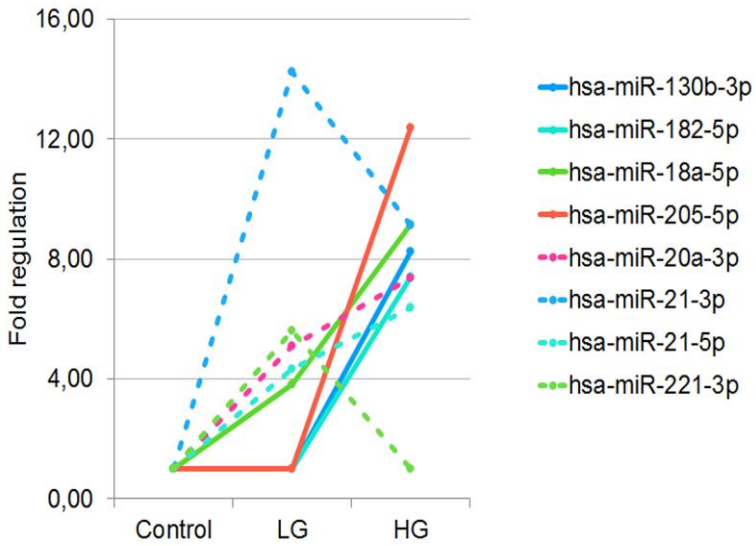


Figure 3.22. **Changes in levels of *PTEN*-targeted exosomal miRNAs during bladder cancer progression**

3.8 Pathway enrichment analysis of exosomal miRNAs

The main results of the pathway enrichment analysis of miRNAs with more than 2 FR in LG and HG groups are listed in Tables 3.11 and 3.12. The enriched localisation of miRNAs was exosome, microvesicle and circulating fractions, with high significance in both LG and HG groups. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Mammalian ncRNA-Disease Repository (MNDR) disease categories mainly found cancer-related subcategories. No statistically significant enriched pathways were identified using the list of down-regulated miRNAs, due to the small number of miRNAs in each group.

3.9 Analysis of target genes of up-regulated miRNAs and their interactions

A total of 1425 predicted target genes for 91 upregulated miRNAs in the LG group and 805 target genes for 71 upregulated miRNAs in the HG group were identified. Using these genes, regulatory network interactions between genes were created. To select the hub genes, the top 50 genes were compared using four CytoNCA and CytoHubba methods (Figure 3.23, Annexes 1 to 4). The common genes of CytoNCA and CytoHubba lists were extracted (Table 3.13). In total, 19 hub genes from the LG-regularised miRNA target group and 32 from the HG-regularised miRNA target group were extracted. The 17 target genes were overlapping in both groups. In both hub gene analyses with each of the four methods, *TP53* was ranked first, with the exception of MCC from CytoHubba (ranked 34th), and *PTEN* was ranked between 6 and 11 in LG (Annexes 1–2) and 7–10 in HG (Annexes 3–4).

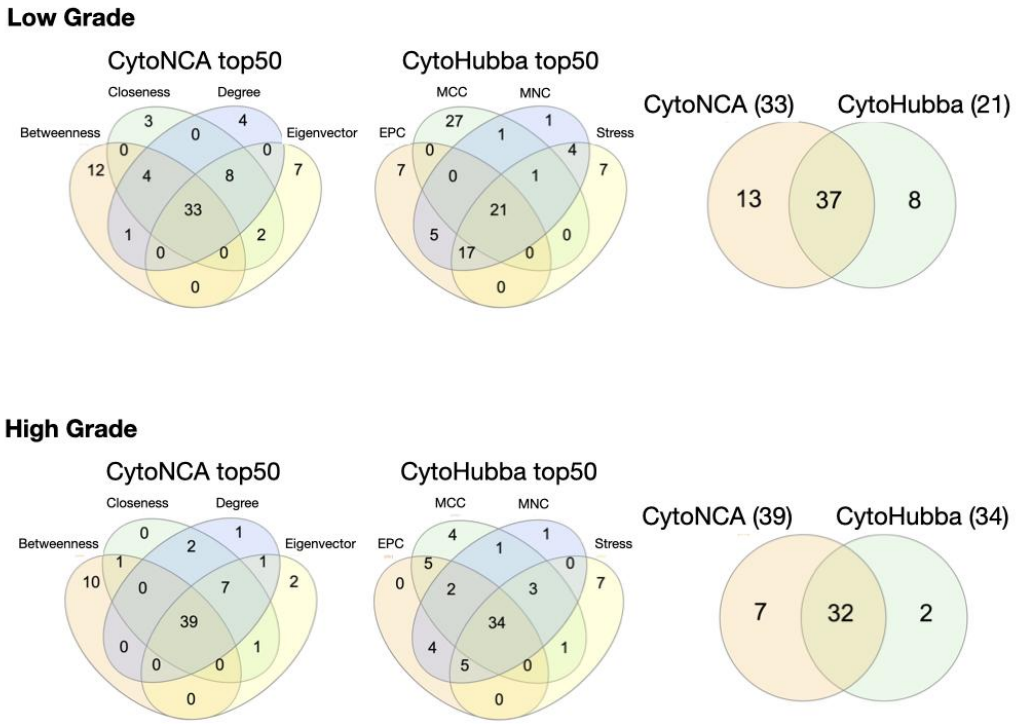


Figure 3.23 **Network of the target genes of up-regulated differentially expressed miRNAs in LG group and in HG group**

Table 3.13

Key target genes of urinal exosomal miRNAs in low grade and high grade groups

Low grade	High grade	
TP53	*TP53*	*NOTCH1*
CTNNB1	*AKT1*	*ERBB2*
GAPDH	*CTNNB1*	*RHOA*
AKT1	*MYC*	*IL1B*
HSP90AA1	*EGFR*	*SMAD3*
MYC	*JUN*	*MTOR*
EGFR	*SRC*	*KRAS*

Table 3.13 continued

Low grade	High grade	
PTEN	*IL6*	*PPARG*
SRC	*PTEN*	*MAPK1*
HSPA8	*CDH1*	*SMAD4*
ESR1	*HSP90AA1*	*FOXO3*
NOTCH1	*STAT3*	*CDKN2A*
JUN	*HRAS*	
MTOR	*ESR1*	
KRAS	*HIF1A*	
CDH1	*CCND1*	
HIF1A	*FN1*	
CDKN2A	*VEGFA*	
VEGFA	*CASP3*	

3.10 Enhancing bladder cancer diagnosis using machine learning on miRNA expression and clinical data

The machine learning analysis was performed on a cohort of 152 participants (89 bladder cancer patients and 63 healthy controls) who met all inclusion criteria and had complete clinical data. The clinical–pathological characteristics of this cohort are summarised in Table 3.14.

Table 3.14

Clinical–pathological parameters of the machine learning study cohort

Variable	Study Groups	
	Control (N = 63)	Cancer (N = 89)
Age (years)		
Median	66	74
Gender		
Male	39	70
Female	24	19
Pathological stage		
≤ pT1	–	80
≥ pT2	–	9
Grade		
LG	–	52
HG	–	37
Eritrocituria		
Yes	4	50
No	59	39
Smoking history		
Present/Past	30	47
Never	33	42

N – number; LG – low grade; HG – high grade.

Results demonstrated notable differences in miRNA expression levels between the cancer and control groups, with miRNAs such as mir-30b-3p, mir-28-3p, mir-339-5p, mir-20a-3p, mir-126-3p, mir-429, mir-28-5p and mir-324-5p showing substantial statistical significance (p-value < 0.05) (Figure 3.24).

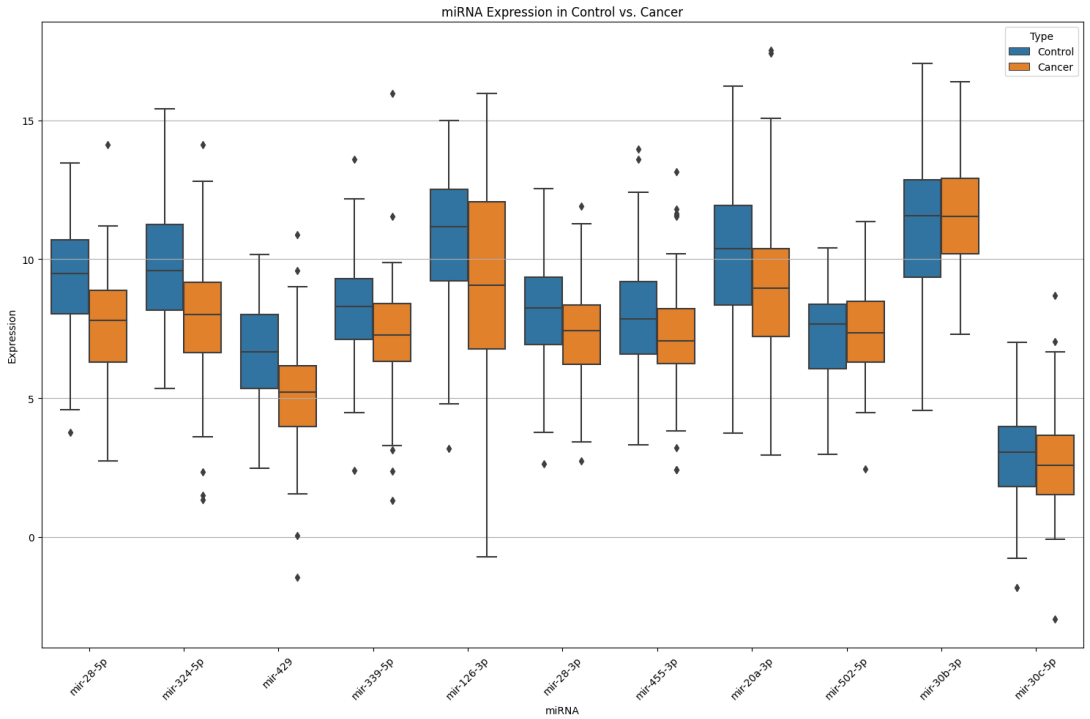


Figure 3.24. **Expression differences of 11 miRNAs between tumour and normal samples using box plots**

To visualise the separability of the cancer and control groups based solely on the selected miRNA panel, a Uniform Manifold Approximation and Projection (UMAP) dimensionality reduction was applied to the miRNA expression data. UMAP clustering revealed two distinct clusters corresponding to the cancer and control groups, supporting the diagnostic potential of the selected miRNAs prior to formal classification (Figure 3.25).

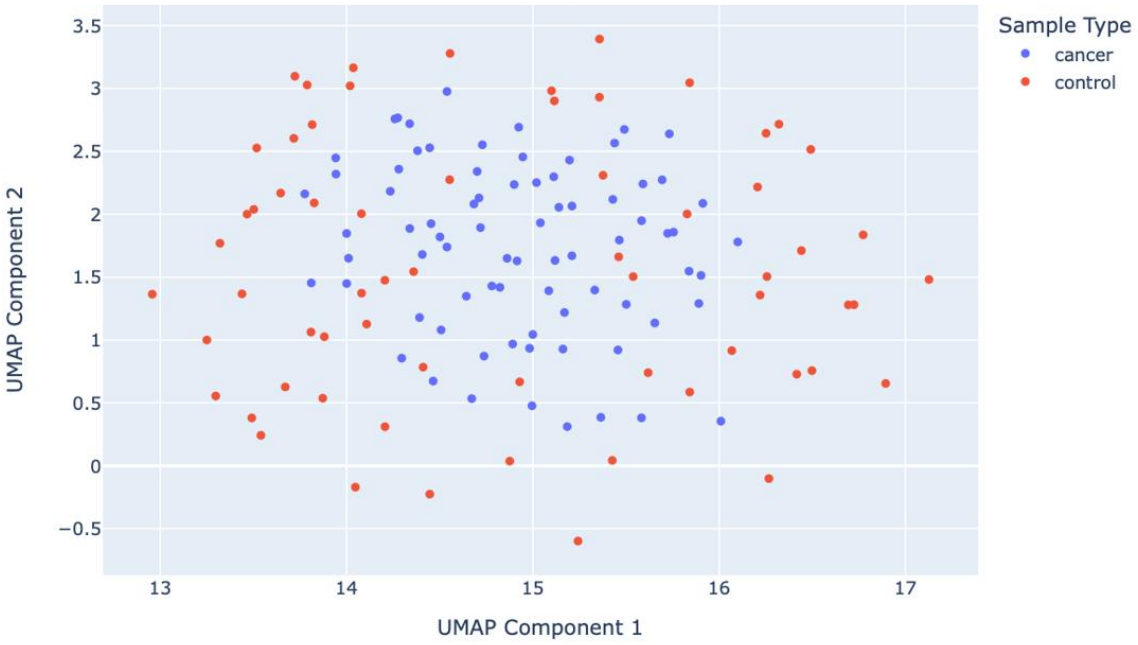


Figure 3.25 **UMAP cluster plot**

Initially, distinct models were constructed using solely miRNA data (Figure 3.26) and clinical analysis data (Figure 3.27) to evaluate their individual predictive capabilities. Based solely on miRNA data, the best-performing classifier (SVM) achieved an AUC of 0.75 (sensitivity 0.71, specificity 0.71, F1-score 0.67) see Figure 3.26.

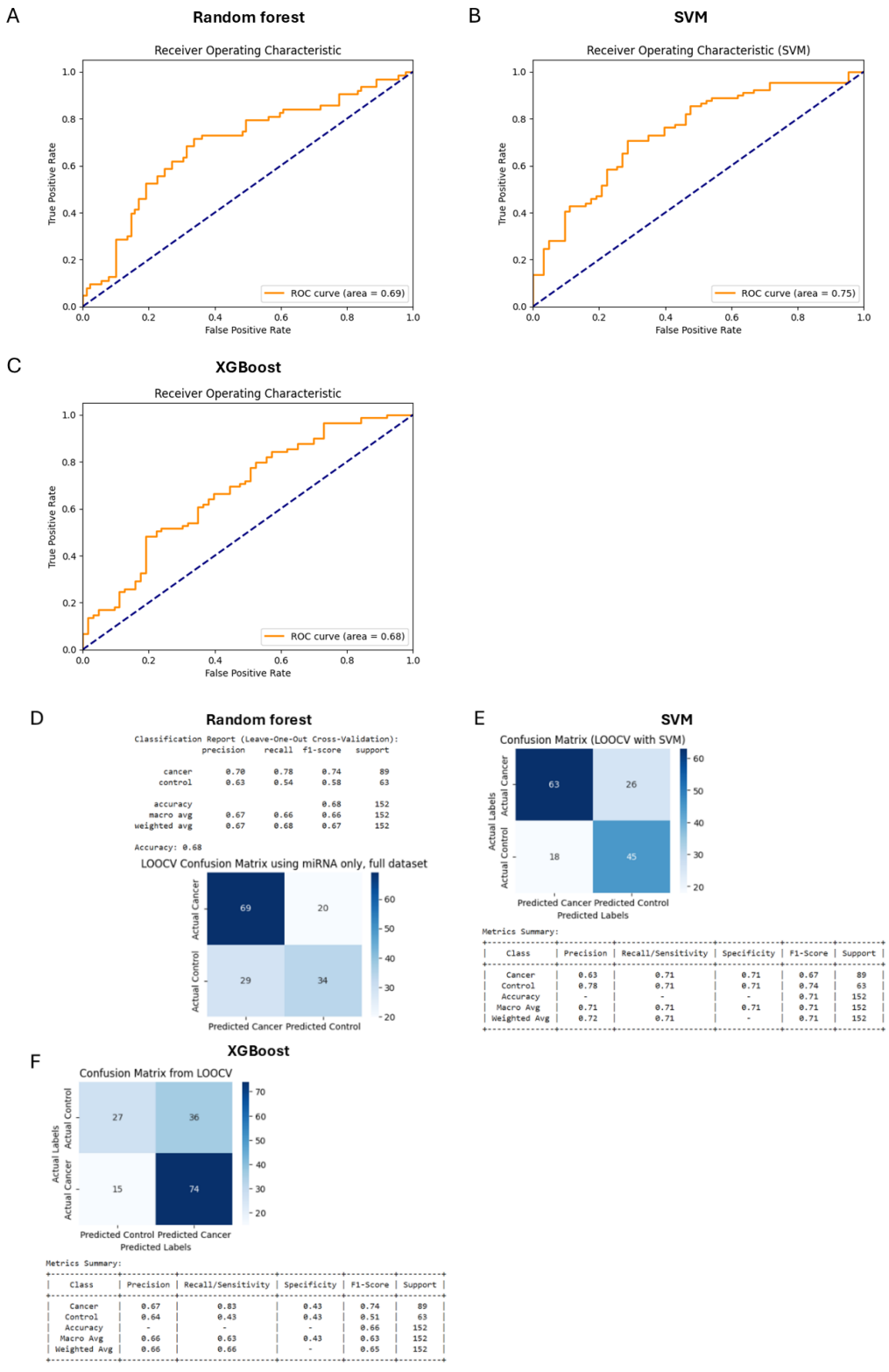


Figure 3.26 **Receiver-operating characteristic (ROC) curve analyses (A–C) and confusion matrices (D–F) for models trained on differentially expressed miRNA data using Random Forest (A, D), SVM (B, E), and XGBoost (C, F)**

When trained on clinical and demographic data only, the SVM model produced an AUC of 0.80 (sensitivity 0.90, specificity 0.58, F1-score 0.73); see Figure 3.27.

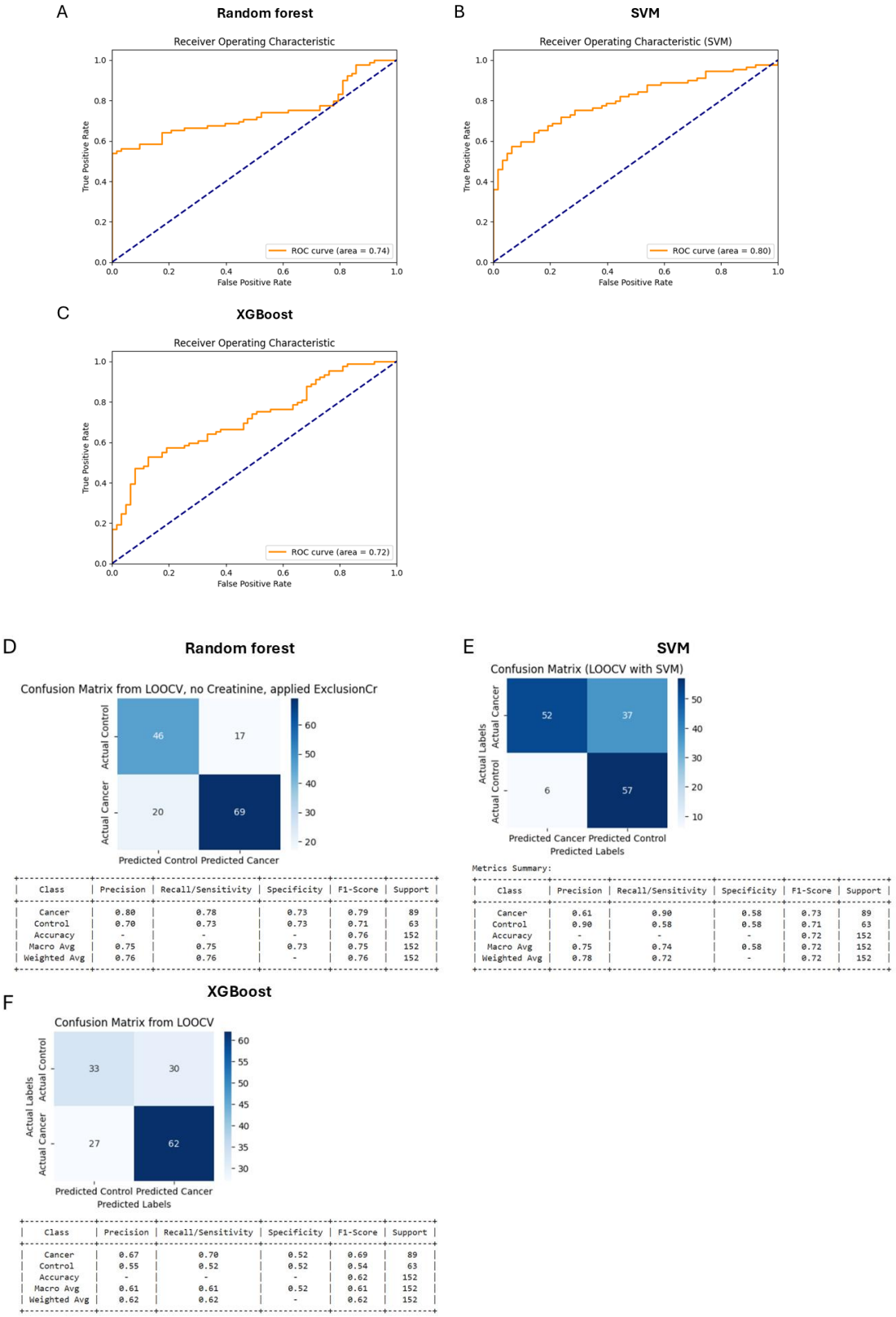


Figure 3.27 **Receiver-operating characteristic (ROC) curve analyses (A–C) and confusion matrices (D–F) for models trained on clinical and demographic data using Random Forest (A, D), SVM (B, E), and XGBoost (C, F)**

Feature importance was assessed independently for all three classifiers (Random Forest, SVM and XGBoost) trained on clinical and demographic data alone. Across the three models, erythrocyturia in urine tests and age were consistently ranked among the most discriminating clinical variables, followed by anthropometric parameters (weight, height) and patient sex. Lifestyle and history-based variables – smoking history (current or previous), family history of oncology and exposure to aromatic amines – contributed to a lesser extent, while symptom- and urinalysis-based variables (dysuria, chronic urinary tract infection, leukocyturia, positive nitrite test) ranked lowest in all three classifiers. The full ranking for each classifier is presented in Figure 3.28.

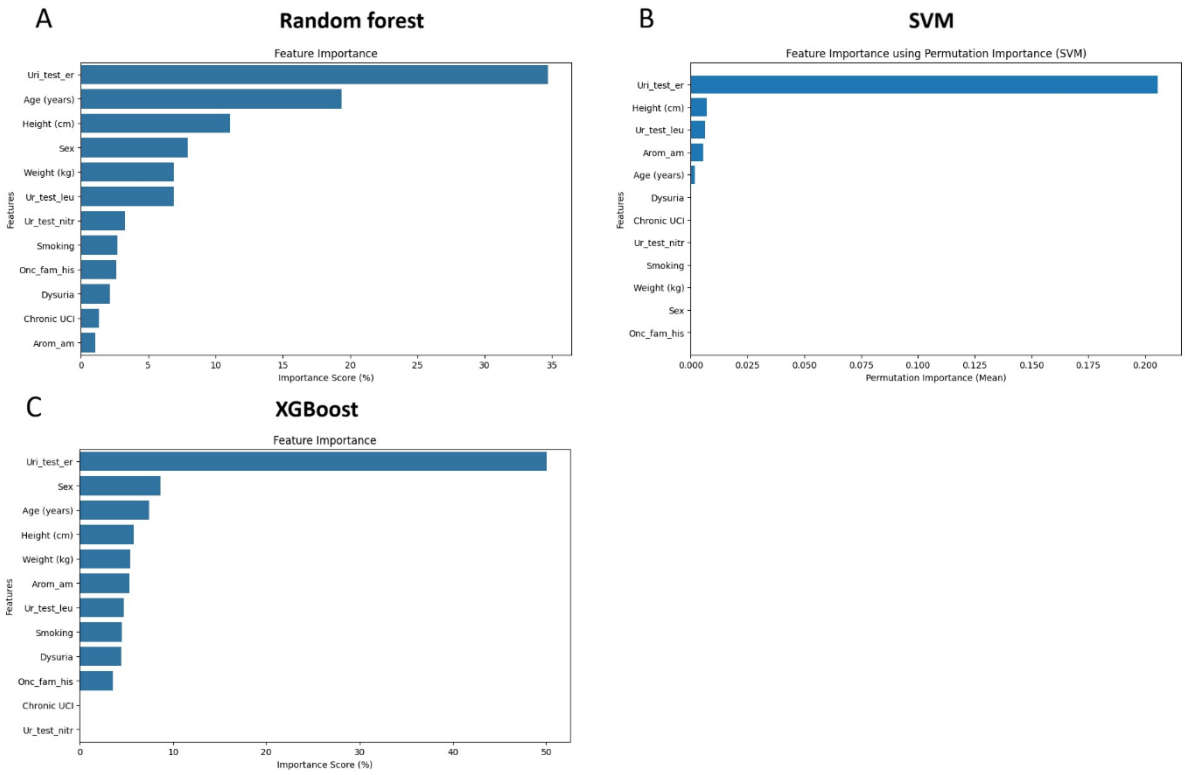


Figure 3.28. **Feature of importance based on clinical data analysis for Random Forest (A), SVM (B), and XGBoost (C)**

Uri_test_er – eritocituria in dipstick urine analysis; Ur_test-leu – leucocituria in dipstick urine analysis; Ur-test_nitr – positive nitrite test in dipstick urine analysis; Onc-farm_hist – oncological history in family; Chronic UCI – chronic urinary tract infection; Arom_am – exposure to aromatic amines.

However, the integrated model that combined both datasets (Figure 3.29) exhibited superior performance compared to the standalone models, underscoring the effectiveness of the integration approach. The best-performing classifier (SVM) on the combined miRNA + clinical dataset achieved an AUC of 0.85 (sensitivity 0.76, specificity 0.76, F1-score 0.79); see Figure 3.29. This amalgamated dataset accurately captures the multifaceted nature of bladder cancer, resulting in improved diagnostic precision.

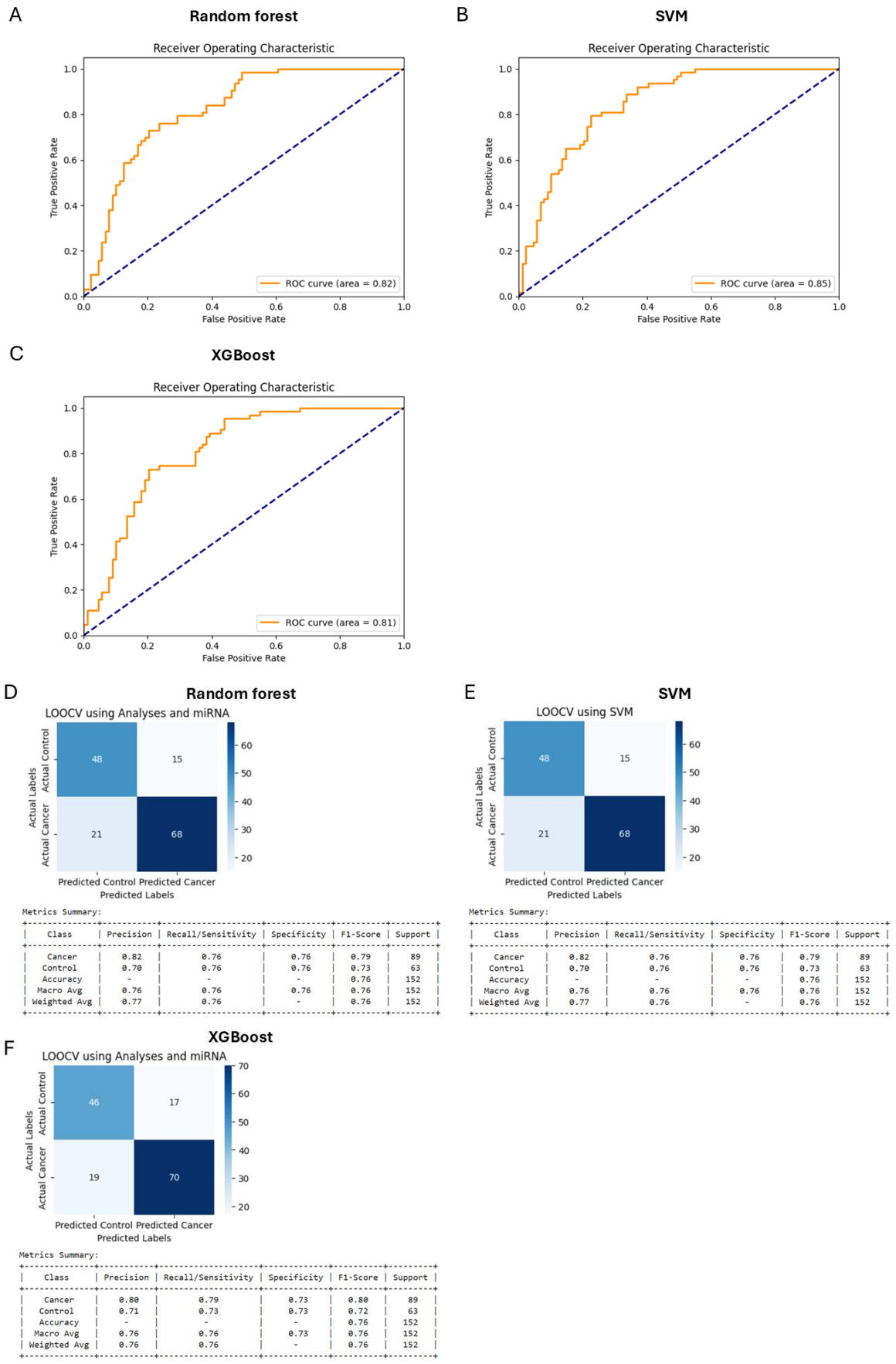


Figure 3.29 **Receiver-operating characteristic (ROC) curve analyses (A–C) and confusion matrices (D–F) for models trained on the combined miRNA and clinical dataset using Random Forest (A, D), SVM (B, E), and XGBoost (C, F)**

An assessment of feature importance revealed that the number of urinary erythrocytes and patient age were the most crucial discriminating factors across all three classifiers, representing key distinctions between patients with cancer and non-cancer control subjects. Among the miRNAs, hsa-miRNA-20a-3p, hsa-miRNA-324-5p, and hsa-miRNA-339-5p were consistently ranked among the top features by Random Forest, SVM, and XGBoost, while hsa-miRNA-28-5p, hsa-miRNA-429, and hsa-miRNA-455-3p contributed strongly to the tree-based models in particular (Figure 3.30).

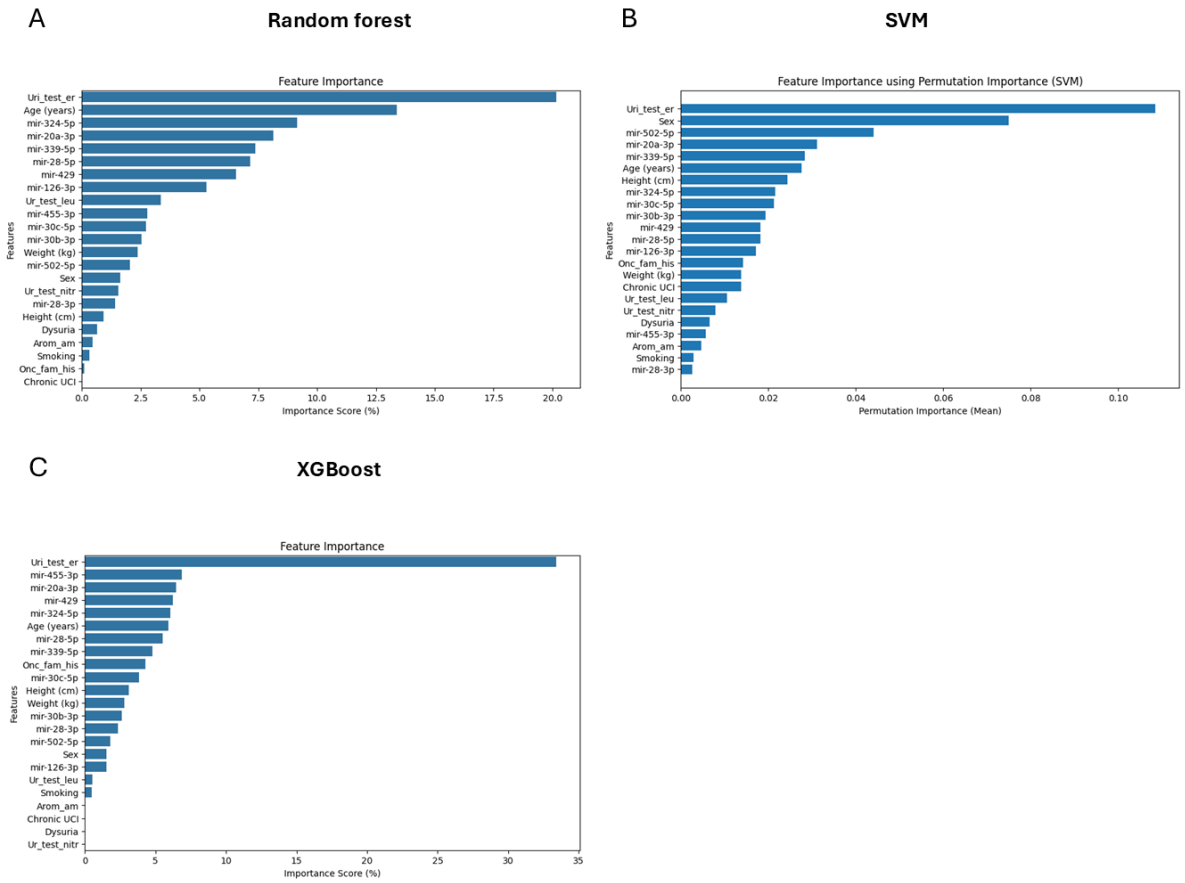


Figure 3.30 **Feature importance analysis on the combined miRNA and clinical dataset for Random Forest (A), SVM (B), and XGBoost (C)**

Uri_test_er – eritocituria in dipstick urine analysis; Ur_test-leu – leucocituria in dipstick urine analysis; Onc-farm_hist – oncological history in family; Chronic UCI – chronic urinary tract infec-tion; Ur-test_nitr – positive nitrite test in dipstick urine analysis; Arom_am – exposure to aromatic amines.

These findings suggest that combining miRNA expression levels with clinical data enhances diagnostic accuracy for bladder cancer. The diagnostic performance of the three classifiers (Random Forest, SVM, XGBoost) was evaluated for each of the three data configurations: miRNA expression data only (Figure 3.26), clinical and demographic data only (Figure 3.27), and the combined miRNA + clinical dataset (Figure 3.29). The integrated model consistently outperformed the standalone models, with the best performance achieved by SVM on the combined dataset (AUC 0.85).

4 Discussion

4.1 Epidemiology

Bladder cancer is one of the most common cancers in the world. There are substantial epidemiological data on bladder tumours in different countries, but such studies have not been carried out in Latvia before. Furthermore, in 1991, Latvia regained its independence and began to follow the guidelines of the European Urology Association.

The global burden of urological cancer has increased worldwide over the past decades. According to Dy et al., ASR incidence has declined by 24.7% in recent decades, but new bladder cancer diagnoses have increased 1.5 times. These changes are due to population growth (35.0%) and age structure redistribution (Dy et al., 2017). Consequently, when interpreting trends in disease incidence, it is important to consider not only actual changes in disease prevalence but also factors related to diagnostic activity, as increased detection rates may partially contribute to the observed rise in incidence without necessarily reflecting a proportional increase in the underlying disease risk. In 2018, Wong et al. analysed the epidemiological data of 39 countries on cancer incidence in five continents (CIFCs) and other national registries, and observed an increase in B.C. incidence in seven countries (six of which are European) in the last 10 years, affecting both men and women (Wong et al., 2018). Our study shows that the incidence rate of ASR BC increased from 6.8 in 1990 to 10.8 in 2017, with an APC of 2.6 % between 1990 and 2014, followed by stabilisation until 2017 (APC = −3.6; p = 0.4). However, in the study by Wong et al., there is no statistically significant increase in the incidence rate of men in Latvia (Wong et al., 2018), as previously described by Antoni et al. (2017). Our study showed trends similar to those observed by Antoni et al. (2017), which estimated APCs of 2.0 % for men and 2.7 % for women, with the result in our case being 2.1 % compared to 2.7 %. However, our analysis found that the 2012 age-referenced BC incidence rate was 20.2 for men and 4.8 for women (Figure 3.4). This is very different from the data used in the GLOBOCAN database, which describes the age-specific incidence of 15.9 for men and 2.9 for women in BC (Antoni et al., 2017).

Previous studies show that BC incidence is tightly associated with smoking habits (Freedman et al., 2011) and HDI (Antoni et al., 2017; Wong et al., 2018), these affirmations can explain the divergence in incidence rates in different populations. According to CDPC of Latvia, during the past two decades, cigarette smoking prevalence in Latvia has decreased from 46 % in 2008 (Mārtiņsone, 2017) to 32 % in 2017 (Mārtiņsone & Pelne, 2018) in a population aged ≥ 15, This factor interrelates with a cancer incidence decline. However, since 2008, cigarette smoking among women in Latvia has increased from 13–20% in 2016 (Mārtiņsone, 2017; Mārtiņsone & Pelne, 2018) reflecting an increase in cancer incidence among women,

which also corresponds to global tendencies (Antoni et al., 2017). It was reported that BC incidence was positively correlated with HDI (Antoni et al., 2017; Wong et al., 2018), and by the United Nations Development Programme data, Latvia ranks 41st with index 0,847 (world: 0.728) (United Nations Development Programme, n.d.). According to previous reports, such risk factors as smoking, obesity, and excessive alcohol and meat consumption (Cumberbatch et al., 2018) are more prevalent in developed countries (WHO, 2020). This explains the incidence correlation with HDI. Furthermore, the rise in incidence might be related to the better diagnostic accuracy of new available approaches and more widespread performance of urine cytology, cystoscopy, and CT scans.

Fluctuations were observed in the number of unknown cancer T stages, with a tendency to decrease over time. The percentage of missing values has reduced from 44.2 % in 1990 to 17.8 % in 2017. This could be explained by improvements in the cancer registry and data input. Although there are still missing data on tumour stage, and one of reason could be a lack of information on tumour size, regional lymph node involvement, and distant metastasis in cancer registries. Furthermore, the stage of disease often is unknown at the time of report to the registry.

The data from Antoni et al. data show that a considerable reduction in mortality rates was observed in most countries (Antoni et al., 2017). Although from 1990 till 2017 bladder cancer-specific mortality rates from BC in Latvia have significantly increased (APC=0.5; $p< 0.001$), with an ASR mortality rate of 3.9 in 1990 to 4.4 in 2017. These findings can be associated with insufficient cancer-preventing measures. Compared to other countries in terms of mortality rates, Latvia ranks 2nd after Poland in terms of male mortality among other European countries (International Agency for Research on Cancer, 2022). Perhaps such an arrangement in mortality rates can be explained by the different prevalence of tobacco smoking (WHO, 2016) and occupational exposure to chemicals in different populations. In our study, there was a trend towards an increase in cancer-specific mortality rates for age groups from 60–79 and > 80 years (APC = 0.7 and APC = 1.9; $p< 0.05$), although in the younger age group (from 40 to 59 years) mortality rates have decreased over time (APC = −1.1; $p< 0.05$). Furthermore, various studies have shown that the cancer mortality rate also has a tendency to increase with age. Therefore, in individuals aged ≥ 65 years, the mortality rate is 15 times greater than in individuals aged < 65 (Messing, 2008). There are assertions that in younger patients (aged < 40 years) urothelial BC tends to be low-grade and therefore commonly has a slow-growing pattern (Günlusoy et al., 2015; Fitzpatrick & Reda, 1986). Furthermore, according to Shariat et al. the urothelial BC mortality rate might be higher in the elderly due to administration of less aggressive treatment and avoidance of radical therapy (Shariat et al.,

2010; Noon et al., 2013). Interpretation of mortality trends should also take into account population ageing and healthcare-related factors, as treatment accessibility, comorbidities, and differences in clinical management may substantially affect patient outcomes. In comparison with other countries, lower average life expectancy rates in Latvia becomes another reason for the increased mortality rates (WHO, 2020).

Bladder cancer prevalence in Latvia has grown noticeably, from 25.2 per 100 000 in 1990 to 69.7 per 100 000 in 2017 (AAPC 3.8 %). The prevalence is rising faster than the incidence, meaning that more patients are living longer with the disease. This puts a growing pressure on follow-up and surveillance. At the same time, BC still causes many early deaths – in 2017 alone, 7450 years of potential life were lost to this disease.

In this study, an increase in RS rates is demonstrated. However, at the beginning of the study (during the period of 1990–2000) the RS rate was only 55.0%, which is relatively low compared to other European countries, but eventually the RS rate increased to 59.0 % from 2013 to 2017, which is still much lower than the European average rates. According to *Cancer Research UK*, the ASR five-year net survival in England and Wales was 60.9 % between 1990 and 1991 but fell to 53.7% between 2010 and 2011 (Cancer Research UK, 2014). A similar situation with a decline in the 5-year relative survival was observed in Germany (Brenner et al., 2005). Comparing male and female 5-year survival rates, women have better survival rates than men (Table 3.5 and Figure 3.14); however, this difference is not statistically significant. It could be explained by the fact that women in Latvia have a higher average life expectancy compared to men. In the female group, RS tends to increase, in contrast to data from the United Kingdom (Cancer Research UK, 2014), where survival rates have deteriorated for both sexes since 1990. In addition, women's survival rates in the United Kingdom are particularly low, standing at only 43.9 % in the period from 2013 to 2017. On the other hand, our survival rates are quite similar to the estimated relative survival rates reported in Lithuania: 48.6% in 1995–1999, 54.6% in 2000–2004, and reached 56.4% in 2005–2009 (Krilaviciute et al., 2014). In the present study, 5-year RS rates by tumour T stage gradually dropped from 1990 until 2017. The apparent decrease in survival rates reflects criteria changes in TNM classification introduced in 1997 for the diagnosis of invasive bladder cancer compared with earlier years. However, survival rates are tightly related to number of factors such as tumour grade, presence of carcinoma *in situ* (CIS), tumour size and tumour multiplicity. High-grade tumours are associated with a less favourable prognosis due to greater risk of progression and metastasis. In cases of CIS poorer outcome is associated with higher likelihood of tumour recurrence and risk of developing into invasive bladder cancer. Also, less favorable outcomes are associated with multiple and large tumours. In patients with muscle-invasive bladder cancer (MIBC), one of the most important

prognostic factors is the presence of nodal involvement. Tumour size of pathologic stage T2 correlates with survival rates as well (Cheng et al., 1999). On condition of localised MIBC the most effective treatment is radical cystectomy (RC) with bilateral pelvic lymph node dissection. In comparison alternative methods such as chemotherapy and radiation therapy indicate worse outcomes. In the report by Gore et al., RC showed superior results over chemotherapy or radiation in 2-year and 5-year overall survival (OS) (67.8 % vs. 47.5 % and 42.2 % vs. 20.7 %) and disease-specific survival (DSS) (81.1 % vs. 68.3 % and 66.6 % vs. 48.0 %) (Gore et al., 2010). Another factor which impacts oncological outcomes is the histological variant of bladder cancer. Processali et al. reported that pure squamous cell and signet ring cell carcinoma histological variants are indicative of a poor prognosis (Processali et al., 2020).

Taking into consideration the above-mentioned factors, the previously observed decrement of 5-year survival rates by T stage could be associated with these factors, which unfortunately were not available for analysis.

This Thesis has exposed epidemiological trends between 1990 and 2017. Since this specific issue has not been previously studied in Latvia, the information gathered in this study reflects the most accurate BC incidence, mortality, prevalence and survival rates in the country.

Due to the limited data available for this study, the analysis of survival was performed stratifyng the data by T stage. Although it would be much better to analyse it by individual stages, including N and M stages as well as tumour grade, this was not possible; however, it would considerably help to understand the changes in survival rates in more detail. Furthermore, the Latvian Cancer Register inherited the data collected before the restoration of the country's independence. It was only in 1992 that a personal identification number was introduced. Also, those who were historically registered with an error (with wrong/incomplete identification numbers) were removed from the database of the oncology registry during 2015 and 2016. This explains the reduced number of casualties in Latvia. Also, during the past 3 decades, the Latvian Cancer Registry has been reformed several times, as well as merged with other registers. Additionally, the 2009 financial crisis significantly influenced the statistics of newly registered patients. To be more precise, oncology practices were reformed, which was followed by a significant reduction in the number of newly discovered BC cases. The study also has its limitations due to multiple changes in disease coding throughout the years, which have also affected survival fluctuations. Further detailed studies are needed, including disease stage, grade, treatment data, income level and social exclusion, which may explain differences in incidence, prevalence, mortality and survival over time. Overall, these findings suggest that bladder cancer epidemiological trends in Latvia are influenced by a complex interaction of

demographic, behavioural, and healthcare-related factors rather than by isolated changes in incidence, mortality, or survival alone.

4.2 Urinary exosomal miRNA

Urinary exosomes contain miRNAs from cells in the urinary tract (Cheng et al., 2014). The profile of urinary exosomal miRNAs is considered to reflect the cellular conditions, and these miRNAs are known to modulate the other cells which received the exosomes (Melo et al., 2014). The aim of this study is to compare the urinary exosomal miRNAs in healthy control group and patients with LG and HG cancer patient groups to understand the cell–cell molecular information exchange and the cancer development.

Several miRNAs not previously described as altered in bladder cancer were identified in the present study. These novel miRNAs include miR-let-7f-2-3p, miR-28-5p, miR-196b-3p, miR-450a-5p, miR-320b, miR-151a-3p, miR-20b-3p, miR-425-5p, miR-376c-3p, let-7e-3p, miR-24-2-5p, miR-181a-3p, miR-664a-3p, miR-331-3p, miR-99b-5p, miR-28-3p, miR-501-3p, miR-500a, miR-628-5p, miR-7-1-3p, miR-105-5p, miR-342-5p, miR-455-3p, miR-365a-3p, miR-181d-5p, miR-423-3p, and miR-548m. Moreover, the profile of differentially expressing exosomal miRNAs in BC in this study widely covers BC-related miRNAs which have already been reported. Two patterns of up-regulation were observed relative to the control group: (1) HG > LG or HG alone, and (2) LG > HG or LG alone. In the following sections, the most informative individual miRNAs are first discussed by being grouped into these two patterns (Sections 4.2.1–4.2.2), followed by their convergence on the *TP53* and *PTEN* regulatory networks (Section 4.2.3), the cellular origin of the urinary exosomes (Section 4.2.4), and finally the translation of these molecular findings into a clinically applicable multimodal diagnostic model through machine learning (Section 4.2.5).

4.2.1 Up-regulated exosomal miRNAs in HG than LG or HG to control group alone

MiR-126-3p (LG 5.62 FR, HG 21.35 FR in this study) has been found in the plasma exosomes of HG BC patients (Sabo et al., 2020) and in the urine samples of BC patients (Hanke et al., 2010). The markedly higher fold-regulation in HG versus LG observed here suggests a grade-dependent increase that could reflect either greater tumour shedding of miR-126-3p-containing vesicles or altered biogenesis/secretion in high-grade tumours. Functionally, miR-126-3p has been implicated in angiogenesis and tumour progression in other cancers, and its enrichment in HG exosomes may contribute to a pro-angiogenic microenvironment or facilitate oncogenic signalling in recipient cells. While MiR-126-3p has been identified as a deregulated miRNA in other cancer tissues and plasma, its expression profile varies by cancer type and tissue origin. Therefore, it has been suggested that

miR-126-3p acts as both an oncogenic miRNA and a tumour-suppressor miRNA depending on tissue type and tumourigenesis timing. It has been suggested that the secretion of miR-126-3p into the exosome is accelerating tumour development, by leading angiogenesis stimulation and promoting oncotransformation of non-cancer cells (Sabo et al., 2020).

In the present study, up-regulation of miR-150-5p was detected in HG BC samples (26.15 FR). Due to data of multiple studies, circulating exosomal miR-150-5p has shown noteworthy diagnostic and prognostic value in colorectal cancer (Aherne et al., 2015; Hosseini et al., 2017). It was found that miR-150-5p promotes cancer cell proliferation by inhibiting the tumour suppressor gene *TP53* (Liu & Di Wang, 2019). Also, it has been suggested that the up-regulation of miR-150-5p mediates the progression of recurrent ovarian cancer (Tung et al., 2020).

Expression of miR-205 is crucial in formation of different epithelial tissues and its dysregulation is associated with development of a number of epithelial cancers. MiR-205 has both effects – oncogenic and tumour suppressive depending on impacted cell type (Ferrari & Gandellini, 2020). Several previous studies detected its up-regulation in BC tissues (Wiklund et al., 2011; Zabolotneva et al., 2013).

Substantial up-regulation of miR-21-5p (LG 4.32 and HG 6.38 FR in this study), miR-205-5p (HG 12.37 FR) and miR-141-3p (LG 2.62 FR) in urine samples has been detected in bothe bladder and prostate cancers (Ghorbanmehr et al., 2019).

MiR-21 is associated with cell proliferation, cancer metastasis and invasion, and is involved in the regulation of apoptosis (Hatley et al., 2010; Liu et al., 2013; Wu et al., 2017; Zhao et al., 2018). It has been widely explored, and it is declared that the up-regulation of this miRNA is involved in the oncogenic processes of multiple cancer types, and has been suggested as a potential circulating biomarker (Bautista-Sánchez et al., 2020). In the present study, mir-21-3p (LG 14.25 FR vs HG 9.10 FR) was more up-regulated in the LG group in contrast to miR-21-5-p. It has been reported that miR-21 overexpression is associated with a poorer prognosis in BC (Zhang et al., 2015). Moreover, miR-21-5p was implied as a potential biomarker for detecting urothelial cancer via urinary exosome examination (Matsuzaki et al., 2017); however, due to its implication in various malignant processes, it could not be used alone for specific condition verification.

Although miR-628-3p was up-regulated in both LG (FR 7.30) and HG (FR 11.42) groups in this study, it has been reported as down-regulated in various other cancers and suggested as a diagnostic cancer marker based on a meta-analysis (Li et al., 2018). In contrast, another study (Sabo et al., 2020) found that miR-628-3p was significantly elevated in the urine of muscle-invasive BC (MIBC) patients in comparison to miRNA isolated from plasma EVs,

in which it was down-regulated, while there was no statistically significant difference in miRNA expression from isolated MIBC tissues compared to the control. Irrespective of the previous meta-analysis results (Li et al., 2018), other studies have found that miR-628 is up-regulated in pancreatic and lung cancer (A. Li et al., 2013; Wang et al., 2016). Up-regulation of miR-628-3p, which is involved in the expression of tumour suppressors such as ATRX, SLC45A2 and TNRC6B, has been detected in the plasma of patients with lung adenocarcinoma (Wang et al., 2016).

Up-regulation of the miR-200 families in BC tissue has been reported and suggested as a marker of poor prognosis (Martínez-Fernández et al., 2015). In this study, the miR-200 family (miR-200a-3p, miR-200-5p, 200b-3p, miR-200-5p, miR-429, miR-200c and miR141) were found to be up-regulated exosomal miRNAs in the LG group, the HG group, or both.

MiR-182-5p (LG: FR = 2.8, p = 0.21; HG: FR = 7.38) has been reported as an oncogenic miRNA (oncomiR) detected in the urine of BC patients (Hanke et al., 2010). Krishnan et al. (2013) reported that the targets of miR-182-5p are genes involved in DNA repair systems (Krishnan et al., 2013). In contrast, miR-24-1-5p (LG 2.13 FR, HG 8.57 FR) has been spotted in two opposite results, up-regulated in BC tissue and down-regulated in BC cell lines (Yu et al., 2017) (Inoguchi et al., 2014). The miR-24 family is found in many cancers acting as both an oncogenic and an tumour-suppressive miRNA (Wang et al., 2020). Regarding miR-590-3p (LG 1.7 FR with p-value = 0.32, HG 7.30 FR), it has been reported as up-regulated circulating miRNA in BC patients and has been suggested as a possible biomarker of BC (Tölle et al., 2019).

In this study, up-regulation of miR-146b-5p was detected in the urine of of patients with HG bladder cancer (4.71 FR). Comparable results were reported by Baumgart et al., who analysed levels of miR-146b-5p in urinary extracellular vesicles and found notable overexpression in patients with MIBC compared to those with non-muscle-invasive BC (NMIBC) (Baumgart et al., 2019).

4.2.2 Up-regulated exosomal miRNAs in LG versus HG tumours, or in LG tumours relative to control group alone

MiR-221-3p (LG 5.62 FR, HG 1.42 FR with p-value = 0.30 in this study) and miR-210-3p (LG 5.29 FR, HG 1.13 FR with p-value = 0.123) have been reported as a non-invasive biomarkers in clear cell renal cell carcinoma (Petrozza et al., 2017). Since urine exosomes originate from the urinary tract, they also originate from the kidneys (Thongboonkerd, 2019).

Eissa et al. showed that miR-210 is overexpressed in urine sediment samples from bladder cancer patients compared to controls (Eissa et al., 2015). Another study found that

miR-210-3p expression levels are down-regulated in bladder tumour tissue. They demonstrated that miR-210-3p inhibits tumour growth and metastasis by acting on fibroblast growth factor receptor-like 1 (*FGFRL1)* (Yang et al., 2017).

Several studies have reported significant down-regulation of miR-31 in urothelial BC tissues (Wang et al., 2013; Xu et al., 2016). In this study, up-regulation of miR-31-3p (LG 11.27 FR) and miR-31-5p (LG 3.75 FR) was detected. Juracek et al. found that urinary miR-31-5p levels were significantly higher in bladder cancer patients compared to healthy controls and patients with renal clear cell carcinoma. Moreover, the mir-31-5p concentration decreased significantly in disease-free patients when urine was re-analysed three months after surgery compared to preoperative samples (Juracek et al., 2018) . Overexpression of miR-31 in bladder cancer tissues was found to suppress malignant cell proliferation by acting as a tumour-suppressor miRNA negatively regulating cell cycle, migration, and invasion, and directly targeting *ITGA5* (Xu et al., 2016).

Matsuzaki et al. measured miR-30b-3p (LG 8.57 FR in this study) in urinary EVs in patients with prostate cancer and observed its notable elevation in the study group (Matsuzaki et al., 2020) . This miRNA has also been previously spotted to play a role in neoplastic processes such as ovarian cancer, gastric cancer, HCC and lung adenocarcinoma (Gao et al., 2019; N. Li et al., 2022; Li et al., 2020; Liu et al., 2020).

MiR-324-5p (LG 12.24 FR, HG 11.47 FR in this study) has been reported as up-regulated miRNA in the urine samples of BC patients (Eissa et al., 2019; Tsai et al., 2020) and in colorectal cancer tissue (Chen et al., 2014). Regarding BC, miR-324-5p was found to be epigenetic regulator of *FOSB* mRNA and *RCAN1* mRNA (Eissa et al., 2019). It was reported that the up-regulation of miR-324-5p accelerated cell proliferation, colony formation ability, and invasion potential in BC (Tsai et al., 2020) . Moreover, it was discovered that urinary miR-324-5p levels are sig-nificantly higher in patients with BC compared to healthy controls and individuals with benign lesion (Eissa et al., 2019).

Excessive expression of miR-423-5p (LG 2.5 and HG 2.15 FR in this study) in BC cells as well as in urine samples was found in the study by Lin et al. and has been suggested as a potential non-invasive biomarker for bladder cancer detection (Lin & Tsai, 2021).

4.2.3 miRNAs targeting *TP53* and *PTEN*

KEGG pathway analysis was used to show the gene targets of the deregulated miRNAs and their role in biological processes. The results of their analyses further proved the reliability of the findings in this study, as several of the identified targets are associated with different malignancies. *TP53, AKT1, MYC, PTEN* have been previously described as target genes in

bladder cancer development in other studies (Canturk et al., 2014) , which is also reflected in the present data (Figure 3.23 and Table 3.13).

Previous studies have shown increased levels of mutations as *TP53, FGFR3, KDR, PIK3CA* and *ATM* in bladder cancer tissue (Braicu et al., 2019). It has been outlined that *TP53* mutations are associated with aggressive clinical features of BC, such as tumour grade, invasiveness, recurrence rate, and poor prognosis (Esrig et al., 1994; Mitra et al., 2006). A meta-analysis of seven studies reported that *TP53* mutation rate was significantly higher in MIBC compared with NMIBC (Liao et al., 2021). Moreover, immunohistochemical staining has revealed positive expression of *TP53* in high grade tumour tissues compared to low grade tissues (Braicu et al., 2019).

Comparing the LG and HG profiles, miRNAs which are targeting *TP53* and *PTEN* were found in HG with higher FR values (Tables 3.11 and 3.12). This observation suggests that HG BC is under the condition of higher accelerated cancer development than the LG BC. Based on the knowledge that exosomal miRNAs can modulate other cell conditions, (Alexander et al., 2015), miRNAs that target tumour suppressors could potentially modulate non-cancerous cells toward oncotransformation. Other previous studies have already shown that several altered miRNAs regulate the *TP53* network at different levels (Liu et al., 2017). Our hypothesis matches the observations that bladder cancer patients often have hyperplasia, dysplasia, and multiple synchronous cancers. Multiple synchronous and metachronous BC has been explained by intraepithelial migration of tumour cells and intraluminal seeding from a primary cancer (Hafner et al., 2002). According to Lindgren et al., synchronous cancers do not always show the same genomic rearrangements nor mutations in particular genes (Lindgren et al., 2008). This suggests a possible other mechanism capable of inducing new oncotransformation to the normal cells in the bladder which has BC. The urinary exosomal miRNA profiles in this study suggest the possible role of these miRNAs to repress *TP53* and *PTEN*, leading to the oncotransformation of normal cells that are exposed to urine containing exosomes from already developed BC cancer.

A regulatory relationship between multiple miRNAs and *PTEN* has been widely reported. MiR-21 is recognized as one of the most important miRNAs in BC tumourigenesis, which regulates proliferation and migration of cancer cells by its communication pathways with *PTEN* and *TP53*. Its overexpression has suppressive effect on *TP53* (Calderaro et al., 2014; Lei et al., 2015). Furthermore miR-18a, miR-20a, miR-21 and miR-221 in network analysis were found to target *PTEN.* And *PTEN* in its turn regulates miR-19a, miR-21 as well as miR-25, and in consequence of which miR-25 targets *TP53* (Y. Li et al., 2013). Moreover, up-regulated miR-205-5p has been shown to promote oncogenesis through p53 modulation (Braicu et al.,

2019), and a strong association between miR-205-5p and *PTEN* was identified in the HG group (FR = 12.37) (Table 3.12). However, previous attempts in *PTEN*-deficient mouse models have demonstrated mild phenotypes starting from 10 % for transitional cell carcinoma (Tsuruta et al., 2006), to no tumour development at all (Yoo et al., 2006). This only indicates that in bladder tumours there is more than one, but often multiple changes in the expression of various components, like the *AKT/PI3K/mTOR pathway* (Platt et al., 2009). In addition, combined p53 and *PTEN* alterations are common in invasive bladder cancer and are related to worse prognosis for patients (Puzio-Kuter et al., 2009). This implies the significance of synergistic effects of different tumour suppressors. Our regulatory gene interaction networks show multiple interactions of genes with the central hub gene *TP53* (Figure 3.23 and Table 3.13).

Members of the miR-130 family (miR-130b-3p and miR-301a) were found to be up-regulated among exosomal miRNAs. Notably, miR-130b-3p was up-regulated exclusively in the high-grade (HG) group (FR = 8.24). In previous studies, it has already been stated that these miRNAs are connected with *PTEN* gene, are responsible for cancer invasion and migration, rearrange the cytoskeleton, and promote stress fibre formation via *FAK* (Taheri et al., 2020). This presumes that this specific oncomiR is highly characteristic of aggressive bladder cancer.

4.2.4 Source of urine exosomes

Differentially expressed miRNAs were identified in urine exosomes; however, in this study, it is not possible to determine with certainty whether their source is cancer cells or non-cancer cells. Assuming that non-cancer cells maintain similar miRNA expression levels and secrete a comparable amount of exosomes, the differentially expressed miRNAs most likely originate from cancer cells. However, it should be considered that non-cancer cells within a cancer-bearing bladder may exist under different conditions compared with those in a healthy control bladder. It is possible that non-cancer cells are modified by receiving cancer-derived miRNAs, which may, in turn, trigger the production of tumour-suppressor miRNAs. Therefore, the urinary exosomal miRNA profiles of BC patients may represent a mixture of signals from both cancer and non-cancer cells. Some of the up-regulated exosomal miRNAs identified in the urine of BC patients in this study have been reported as onco-suppressor miRNAs in other contexts. This may be explained by differences in target gene repertoires across cancer types, the timing of onco-transition, or the stage of cancer development – or alternatively, by an onco-resistant response from non-cancer cells within the cancer-bearing bladder. Wu et al. found that cancer extracellular vesicles (EVs) in the cancerous urothelial field can contribute to de novo neoplasms (Wu et al., 2019). As exosomes are the smallest vesicles in the EVs group,

this suggests that exosomes containing different miRNAs may also act on the healthy urothelium and senesce the development of neo-plasia, which could explain development of multifocal synchronous tumours. Moreover, another study found that exosomes derived from bladder cancer cells inhibit tumour cell apoptosis in vitro (Yang et al., 2013). Taken together, this biological complexity – a mixed signal from cancer and non-cancer cells, the dual oncogenic and tumour-suppressive roles of many identified miRNAs, and overlap with other malignancies – makes it unlikely that any single miRNA will function as a reliable standalone biomarker. A multimodal approach combining routine clinical data with urinary exosomal miRNA expression, analysed through machine learning, therefore represents a more promising strategy for non-invasive bladder cancer diagnostics, as explored in the following section.

4.2.5 Possible usage as a non-invasive biomarker of BC

It should be noted that the sample size in this study was relatively small, and the association of specific miRNAs with specific genes was established using database-derived data but lacked confirmation from biological studies. Nevertheless, the results demonstrate that the methods used were effective, as many previously described miRNAs associated with BC were detected in the exosomal miRNA profile (Tables 3.9 and 3.10), along with their downstream influence on both tumour suppressors and oncogenes. The majority of urinary exosomal miRNAs in BC patients were up-regulated, with fold regulation values sufficient to distinguish between BC patients and the healthy control group, indicating that the identified miRNAs hold potential value as biomarkers for bladder cancer detection, particularly when incorporated into a multimodal approach alongside routine clinical data.

A further limiting factor is the age difference between the control and cancer groups, with the control group having a median age of 66 years compared with 71.5 years in both LG and HG groups. As recent studies have found a significant difference in the amount of freely circulating and extravesicular miRNAs in younger patients (VandenBussche et al., 2013), this may partly explain discrepancies between the present results and other studies among the newly identified differentially expressed miRNAs.

The main problem in selecting specific miRNAs for further use as biomarkers in bladder cancer is that their expression is frequently altered across a large number of other studies and conditions, making it difficult to verify their specificity. MiR-21 illustrates this well – it is substantially elevated in both LG and HG groups, but is also dysregulated in numerous other malignancies and diseases (Ghorbanmehr et al., 2019; Liu et al., 2013; Wu et al., 2017; Zhao et al., 2018), and could therefore serve as an additional component in a screening panel alongside more specific miRNAs to predict possible disease progression, rather than as

a standalone marker. This observation reinforces the rationale for a panel-based approach, which is further supported by published meta-analyses reporting that the combined diagnostic effectiveness of urinary exosomal miRNAs is relatively high, with a sensitivity of 0.69, a specificity of 0.81, and AUC of 0.83 (Long et al., 2024).

In the present study, three machine learning classifiers – Random Forest, SVM, and XGBoost – were evaluated across three data configurations. Applied to miRNA data alone, the best-performing classifier (SVM) achieved an AUC of 0.75; when clinical and demographic data were used alone, the SVM model reached an AUC of 0.80; and the integration of both data types yielded the optimal result – an AUC of 0.85 with the SVM classifier (sensitivity 0.76, specificity 0.76, F1 score 0.79), demonstrating that molecular and clinical information carry complementary diagnostic value. The SVM consistently outperformed the other two classifiers in every configuration, which may reflect its effectiveness with moderate-sized datasets. The convergent performance of all three classifiers on the combined dataset (AUC 0.81–0.85) supports the robustness of the multimodal approach.

Feature importance analysis identified the most discriminative variables across the three classifiers (Figure 3.30). The urinary erythrocyte count was the dominant feature in all three classifiers, while patient age and sex were the most influential demographic predictors, although their relative ranking varied between the models. Three miRNAs – hsa-miR-20a-3p, hsa-miR-324-5p, and hsa-miR-339-5p – appeared among the top-ranked features in Random Forest, SVM, and XGBoost, which suggests that their discriminative value does not depend on the choice of algorithm. Three additional miRNAs (hsa-miR-28-5p, hsa-miR-429, and hsa-miR-455-3p) were highly ranked in the tree-based models but contributed less in SVM, where hsa-miR-502-5p instead ranked among the top features. Interestingly, while smoking is commonly acknowledged as a risk factor (Antoni et al., 2017), it was not highlighted as a key characteristic in this analysis (Figure 3.30). This likely reflects the binary encoding of smoking status used in this study (current/former vs. never), since cumulative pack-years – a more precise measure of exposure intensity – were not analysed. Including pack-years in subsequent studies should help recover this signal. Other clinically significant features such as age and haematuria are consistent with those reported in the existing literature.

Several limitations should be acknowledged. Missing blood creatinine values were replaced with the group mean, which is a simple approach but does not capture individual variation in the data. More sophisticated imputation methods such as multiple imputation or model-based approaches exist, but require larger datasets than the one analysed here to estimate the underlying distribution reliably; applying them to a cohort of this size would risk overfitting. Deep learning architectures were not tested for the same reason – they require substantially

larger training sets than were available in this cohort, and overfitting would be likely on a sample size of this scale. The models were evaluated using internal leave-one-out cross-validation (LOOCV), repeated across 20 random seeds. This maximises the use of available data during training and gives a stable estimate of model performance, but does not replace external validation. Before this model can be considered for routine clinical use, it should be tested in an independent cohort, ideally from another centre. Patients with chronic comorbidities such as diabetes mellitus and autoimmune diseases were excluded from the study cohort, even though these conditions are common in elderly bladder cancer patients. Future studies should include these subclasses to confirm the generalisability of the findings in a broader, more representative patient population.

The miRNAs in this study were isolated from urinary exosomes, an approach that helps reduce the variability associated with whole-urine analyses. The agreement between three methodologically different classifiers – Random Forest, SVM, and XGBoost – on the most informative features adds further confidence that these features reflect a real biological signal, rather than something specific to one algorithm. Among the three classifiers, the SVM performed best, likely because it focuses on the boundary between classes and works well on smaller datasets, which makes it less prone to overfitting and better able to pick up the differences in miRNA expression levels between the cancer and control groups.

Taken together, the results suggest that urinary exosomal miRNA expression provides useful additional information alongside routine clinical and laboratory data for non-invasive bladder cancer diagnostics. The main limitation of this work at present is the lack of clinical validation in a real-world setting. The samples analysed here came from two hospitals; a prospective multicentre extension is currently underway, which will broaden the patient demographics and clinical settings included in future analyses. Beyond this, a double-blind validation study and a multicentre clinical trial with longitudinal follow-up will be needed to confirm how the model performs in everyday clinical practice and across different patient populations.

Conclusions

1 The increase in bladder cancer incidence and mortality rates has been observed during the past 28 years, with the exception of cancer-specific mortality in the 40–59 age group, which showed a decreasing trend.

2 Five- and ten- year relative survival rates in Latvia have improved over time but remain lower than the median survival rates reported in other European countries.

3 A number of differentially expressed miRNAs involved in the epigenetic regulation of the *TP53* and *PTEN* genes were identified as putative diagnostic biomarkers.

4 The application of machine learning, with three complementary classifiers (Random Forest, SVM, XGBoost) evaluated across miRNA-only, clinical-only, and combined data configurations demonstrated that the integration of clinical data and the identified miRNA biomarkers resulted in the best diagnostic performance, with the SVM classifier achieving an AUC of 0.85, sensitivity of 0.76, and specificity of 0.76.

5 The identified miRNA panel and the corresponding multimodal diagnostic approach require validation in larger cohorts and confirmation in biological studies before they can be considered for clinical use.

Proposals

1 Data collection within the Latvian Cancer Registry should continue to be systematically improved, with priority given to the complete recording of the TNM stage, tumour grade, treatment modalities, and comorbidities, so that future studies can provide a more detailed picture of survival and prognosis.

2 Although the clinical use of urinary miRNAs as cancer biomarkers is promising, several challenges remain. Many of them, although inherent to the field, could be overcome through closer collaboration within the scientific community – namely, by sharing datasets, using open-source platforms, applying standardised protocols for exosome isolation, RNA extraction and RT-qPCR, and making fuller use of emerging technologies.

3 Future studies should consider applying more advanced feature selection and dimensionality reduction methods to improve the interpretability and performance of the diagnostic model. Exploring the biological significance of the most influential features identified by the model could also provide insights into the molecular mechanisms underlying bladder cancer and may help to reveal new biomarkers for early diagnosis.

4 The urinary exosomal miRNA panel identified in this study – comprising miR-324-5p, miR-20a-3p, miR-429, miR-455-3p, miR-28-5p and miR-30b-3p – should be validated in an independent, prospectively recruited cohort, using the expression thresholds derived from the present work, to assess its reproducibility and clinical applicability.

5 The predicted interactions between the identified miRNAs and their target genes – particularly those targeting *TP53* and *PTEN* – should be confirmed using experimental approaches such as luciferase reporter assays, RNA immunoprecipitation or gene knockdown, in order to verify these regulatory relationships and gain a better understanding of the molecular mechanisms underlying bladder cancer.

6 The multimodal diagnostic dataset should be expanded through multicentre collaboration, in order to reach a sample size sufficient for robust external validation of the model. The machine learning analysis should also be extended to longitudinal follow-up data, to evaluate whether the urinary exosomal miRNA expression profile can predict disease recurrence, progression and response to treatment.

Publications and reports on topics of the Thesis

Publications:

1. Bitiņa-Barlote, Ē., Plonis, J., Cauce, V., Vjaters, E., Gardovskis, J., Miklaševičs, E., Nakazawa-Miklaševiča, M. 2021. Bladder cancer trends in Latvia during 1990-2017: incidence, mortality, and survival rates. *Central European Journal of Urology* 2021; 74(1): 14–23.. doi: 10.5173/ceju.2021.0266.R2
2. Bitiņa-Barlote, Ē., Plonis, J., Andrejeva, M., Vjaters, E., Gardovskis, J., Daneberga, Z., Miklaševičs, E., Nakazawa-Miklaševiča, M. Profiles of urinal exosomal miRNAs derived from bladder cancer. *Cent European J Urol*. 2024; doi: 10.5173/ ceju.2023.279.R1
3. Bitiņa-Barlote, Ē., Bļizņuks, D., Siliņa, S., Šatcs, M., Vjaters, E., Lietuvietis, V., Nakazawa-Miklaševiča, M., Plonis, J., Miklaševičs, E., Daneberga, Z., Gardovskis, J., Liquid Biopsy Based Bladder Cancer Diagnostic by Machine Learning. *Diagnostics.* 2025; 15(4), 492. https://doi.org/10.3390/diagnostics15040492

Reports and theses at international congresses and conferences:

1. Bitina-Barlote E., Plonis J., Vjaters E., Gardovskis J., Daneberga Z., Miklaševičs E., Nakazawa-Miklaševiča M. Urinary exosomal miRNA profiles and target genes in bladder cancer: Pilot study. *European Urology Open Science*, Volume 52, Supplement 1, June 2023, Page S22. https://doi.org/10.1016/S2666-1683(23)00294-X.
2. Bitiņa-Barlote Ē., Plonis J., Vasiļonoks J.K., Vjaters E., Gardovskis J., Daneberga Z., Miklaševičs E., Nakazawa-Miklaševiča M. Urine exosomal miRNA profiles implicmate the key role of TP53 in bladder cancer development. *European Urology Open Science*, Volume 40, Supplement 1, June 2022, Pages S31-S32. https://doi.org/10.1016/S2666-1683(22)00653-X
3. Bitiņa-Barlote Ē, Plonis J., Andrejeva M., Vjaters E., Gardovskis J., Miklaševičs E., Nakazawa-Miklaševiča M. Urinary exosomal miRNAs as potential biomarker for bladder cancer diagnosis: pilot study. Rīga Stradiņš University International Conference on Medical and Health Care Sciences. "Knowledge for Use in Practice", Abstracts, Riga. Latvia, 2021, p 114.
4. Bitiņa-Barlote Ē., Plonis J., Cauce V., Vjaters E., Gardovskis J., Miklaševičs E., Nakazawa-Miklaševiča M., Prevalence trends and years of life lost due to bladder cancer among the inhabitants of Latvia over past 28 years. Medicina (Kaunas); Volume 56, Supplement 1, 2020, p 128.
5. Bitina-Barlote E. Plonis J. Cauce V. Jukonis J. Vjaters E. Gardovskis J. Miklaševičs E. Bladder cancer incidence and mortality trends in Latvia: 1996-2017. *European Urology Supplements* Volume 18, Issue 3, 2019. p e2501-e2502. DOI: https://doi.org/10.1016/S1569-9056(19)32197-9
6. Bitina-Barlote E. Plonis J. Cauce V. Jukonis J. Vjaters E. Gardovskis J. Miklaševičs E. Bladder cancer 5-year survival trends in Latvia: 1996-2017. European Urology Supplements Volume 18, Issue 3, 2019, p e2498-e2499. DOI: https://doi.org/10.1016/S1569-9056(19)32195-5

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Annexes

Key target gene analysis of miRNAs in CytoNCA in low-grade bladder cancer patients group

CytoNCA							
Betweenness		**Closeness**		**Degree**		**Eigenvector**	
TP53	139671.39	*TP53*	0.02640	*TP53*	347	*TP53*	0.17362
CTNNB1	77327.39	*GAPDH*	0.02635	*AKT1*	289	*GAPDH*	0.16015
GAPDH	71795.57	*AKT1*	0.02634	*GAPDH*	277	*AKT1*	0.15996
AKT1	68962.64	*MYC*	0.02634	*MYC*	277	*MYC*	0.15962
HSP90AA1	66738.03	*CTNNB1*	0.02632	*CTNNB1*	266	*CTNNB1*	0.15120
MYC	64594.49	*HSP90AA1*	0.02632	*HSP90AA1*	242	*PTEN*	0.14304
EGFR	64423.48	*EGFR*	0.02631	*PTEN*	231	*EGFR*	0.13506
UBC	53594.86	*PTEN*	0.02630	*EGFR*	231	*HSP90AA1*	0.13330
UBA52	46076.90	*SRC*	0.02628	*JUN*	199	*JUN*	0.13301
CDC42	41823.67	*UBC*	0.02627	*UBC*	197	*HIF1A*	0.12128
PTEN	41206.15	*JUN*	0.02627	*SRC*	194	*KRAS*	0.12069
SRC	38859.36	*UBA52*	0.02627	*UBA52*	193	*SRC*	0.12068
HSPA8	37711.25	*HSPA8*	0.02625	*ESR1*	177	*ESR1*	0.12008
ESR1	34233.53	*ESR1*	0.02625	*KRAS*	177	*NOTCH1*	0.12001
BRCA1	30645.92	*MTOR*	0.02625	*NOTCH1*	170	*MTOR*	0.11088
NOTCH1	28079.76	*HIF1A*	0.02624	*HSPA8*	168	*VEGFA*	0.11065
TNF	27541.57	*NOTCH1*	0.02624	*HIF1A*	165	*CDH1*	0.10870
JUN	26984.02	*KRAS*	0.02623	*MTOR*	161	*CDKN2A*	0.10762
MTOR	25403.38	*TNF*	0.02623	*TNF*	160	*TNF*	0.10525
CDK1	24630.37	*CDC42*	0.02623	*CDH1*	155	*SIRT1*	0.10045
RAB5A	23991.12	*CDH1*	0.02622	*VEGFA*	150	*UBC*	0.09826
KRAS	23939.88	*CDKN2A*	0.02622	*CDC42*	149	*CREB1*	0.09559
CDH1	23554.02	*CDK1*	0.02622	*CDKN2A*	148	*PIK3CA*	0.09547
POLR2A	20306.50	*BRCA1*	0.02622	*BRCA1*	145	*SMAD4*	0.09393
RACK1	19890.60	*SIRT1*	0.02620	*SIRT1*	140	*FOXO3*	0.09306
SMARCA4	19504.41	*VEGFA*	0.02620	*PIK3CA*	139	*EZH2*	0.09269
HIF1A	19449.99	*RACK1*	0.02620	*CDK1*	137	*BRCA1*	0.09232
CDKN2A	18716.99	*SMAD4*	0.02619	*EZH2*	134	*UBA52*	0.09134
SIRT1	18499.02	*PIK3CA*	0.02619	*RACK1*	129	*SMAD3*	0.08997
CUL1	17619.86	*CREB1*	0.02619	*CREB1*	127	*GSK3B*	0.08909
ADAM12	17324.98	*APP*	0.02619	*SMAD4*	122	*SMAD2*	0.08823
APP	16838.32	*GSK3B*	0.02618	*SMARCA4*	122	*HSPA8*	0.08600
GRB2	16784.52	*EZH2*	0.02618	*FN1*	121	*FN1*	0.08288
FN1	16772.13	*GRB2*	0.02618	*FOXO3*	120	*CDC42*	0.08201
PAAT	16589.31	*SMAD3*	0.02618	*GRB2*	118	*CDK1*	0.08123
VCP	15717.12	*SMARCA4*	0.02618	*SMAD3*	118	*PIK3R1*	0.07941
RAB1A	15064.00	*SMAD2*	0.02618	*GSK3B*	118	*SMARCA4*	0.07718
COPS5	14224.77	*FN1*	0.02617	*POLR2A*	117	*NFKBIA*	0.07684
PCDHA10	13836.00	*FOXO3*	0.02617	*PIK3R1*	117	*FGF2*	0.07640
PIK3R1	13459.14	*PIK3R1*	0.02617	*SMAD2*	114	*RPS6KB1*	0.07617
SEC61A1	13308.79	*ACTG1*	0.02617	*RUVBL1*	108	*FOXO1*	0.07551
SMAD3	13284.94	*DICER1*	0.02616	*KAT2B*	105	*GRB2*	0.07440

CytoNCA							
Betweenness		**Closeness**		**Degree**		**Eigenvector**	
EZH2	13271.90	*VCP*	0.02616	*PABPC1*	102	*CCNA2*	0.07255
SRSF1	13234.46	*CCNA2*	0.02615	*DICER1*	102	*CDK6*	0.07156
PHB1	12975.83	*KAT2B*	0.02615	*RPS3*	100	*TGFB1*	0.07102
RUVBL1	12473.54	*POLR2A*	0.02615	*EFTUD2*	99	*DNMT1*	0.06930
VEGFA	12360.18	*FBXW7*	0.02615	*CCNA2*	98	*MCL1*	0.06890
PIK3CA	12281.99	*NFKBIA*	0.02614	*APP*	98	*KAT2B*	0.06883
HSP90B1	11969.34	*FGF2*	0.02614	*VCP*	95	*PTGS2*	0.06855
FMR1	11795.68	*HTT*	0.02614	*H2BC21*	95	*DICER1*	0.06821

Key target gene analysis of miRNAs in CytoHubba in low-grade bladder cancer patients group

CytoHubba							
EPC		**MCC**		**MNC**		**Stress**	
TP53	169.75	*RPL4*	1.30079E+27	*TP53*	346	*TP53*	2459550
GAPDH	169.39	*RPSA*	1.30079E+27	*AKT1*	289	*GAPDH*	1670310
MYC	167.50	*RPS8*	1.30079E+27	*GAPDH*	277	*CTNNB1*	1548678
AKT1	167.21	*RPS3A*	1.30079E+27	*MYC*	276	*AKT1*	1513244
CTNNB1	164.75	*RPL27*	1.30079E+27	*CTNNB1*	265	*MYC*	1483944
PTEN	160.78	*RACK1*	1.30079E+27	*HSP90AA1*	241	*HSP90AA1*	1465830
HSP90AA1	158.23	*RPS2*	1.30079E+27	*PTEN*	230	*UBC*	1293598
EGFR	156.94	*RPL6*	1.30079E+27	*EGFR*	228	*EGFR*	1265514
JUN	152.81	*RPS3*	1.30079E+27	*JUN*	197	*UBA52*	1155906
HIF1A	148.84	*RPL9*	1.30079E+27	*UBC*	195	*PTEN*	953772
ESR1	147.75	*EIF3I*	1.30016E+27	*UBA52*	193	*HSPA8*	866314
NOTCH1	147.52	*EEF1A1*	1.29952E+27	*SRC*	191	*SRC*	822966
SRC	145.35	*EIF3B*	1.29830E+27	*ESR1*	175	*CDC42*	774068
KRAS	144.20	*RPL14*	1.29805E+27	*KRAS*	175	*ESR1*	706210
MTOR	142.91	*PA2G4*	1.29701E+27	*NOTCH1*	170	*JUN*	665702
UBC	142.63	*CCT3*	1.29457E+27	*HSPA8*	167	*NOTCH1*	631136
UBA52	141.74	*RUVBL1*	1.29268E+27	*HIF1A*	165	*BRCA1*	626702
CDH1	140.53	*PSMD2*	1.29268E+27	*MTOR*	161	*TNF*	600260
CDKN2A	140.12	*CCT8*	1.27880E+27	*TNF*	159	*MTOR*	591232
VEGFA	139.27	*CCT6A*	1.27707E+27	*CDH1*	153	*CDK1*	590886
TNF	138.57	*RPL7A*	1.26842E+27	*VEGFA*	150	*CDH1*	540048
SIRT1	137.28	*RPL35A*	1.26829E+27	*CDC42*	149	*KRAS*	538298
HSPA8	135.16	*TCP1*	1.26164E+27	*CDKN2A*	147	*RACK1*	491350
PIK3CA	134.88	*HSPA8*	1.25727E+27	*BRCA1*	142	*HIF1A*	489466
EZH2	134.34	*RAN*	8.73154E+26	*PIK3CA*	139	*SMARCA4*	444384
CREB1	130.53	*PSMD11*	8.72352E+26	*SIRT1*	138	*RAB5A*	442400
BRCA1	130.10	*RPL12*	8.64560E+26	*CDK1*	137	*SIRT1*	441248
SMAD3	127.12	*PSMD4*	4.36227E+26	*EZH2*	134	*POLR2A*	438488
GSK3B	126.31	*RPS5*	4.27637E+26	*RACK1*	128	*CDKN2A*	436768
FOXO3	125.73	*RPS24*	4.26909E+26	*CREB1*	126	*GRB2*	392492
FN1	125.15	*HSP90AA1*	5.52272E+25	*SMAD4*	122	*FN1*	380422
SMAD4	124.21	*AIMP1*	3.41505E+25	*SMARCA4*	120	*APP*	367420
CDK1	123.73	*GAPDH*	3.06873E+25	*FN1*	120	*CUL1*	356552
PIK3R1	121.13	*TP53*	3.04799E+25	*FOXO3*	119	*EZH2*	342406
SMAD2	120.99	*CTNNB1*	3.04799E+25	*SMAD3*	117	*VEGFA*	334088
CDC42	118.30	*MYC*	3.04799E+25	*GSK3B*	117	*VCP*	330860
SMARCA4	118.12	*PTEN*	3.04798E+25	*POLR2A*	116	*SRSF1*	325274
RACK1	117.18	*ESR1*	3.04770E+25	*GRB2*	116	*SMAD3*	323756
GRB2	116.48	*KRAS*	3.04692E+25	*PIK3R1*	115	*PIK3CA*	321294
CCNA2	114.38	*SRC*	3.04324E+25	*SMAD2*	113	*DICER1*	316540
RPS6KB1	114.13	*VEGFA*	3.04146E+25	*RUVBL1*	108	*PIK3R1*	314746

CytoHubba							
EPC		**MCC**		**MNC**		**Stress**	
FOXO1	113.55	*NOTCH1*	3.03517E+25	*KAT2B*	104	*COPS5*	310048
CDK6	113.55	*HIF1A*	3.01924E+25	*PABPC1*	102	*RUVBL1*	298730
SP1	113.27	*JUN*	2.91634E+25	*DICER1*	102	*ADAM12*	295146
FGF2	113.19	*CDKN2A*	2.91570E+25	*RPS3*	98	*SMAD4*	293032
NFKBIA	112.98	*CDH1*	2.80904E+25	*CCNA2*	98	*CREB1*	276004
KAT2B	111.86	*MTOR*	2.77055E+25	*EFTUD2*	97	*GSK3B*	270180
DICER1	111.64	*SMAD4*	2.71931E+25	*APP*	95	*PAAT*	266302
MCL1	111.59	*AKT1*	2.71467E+25	*H2BC21*	95	*H2BC21*	265714
DNMT1	109.47	*EGFR*	2.69060E+25	*NFKBIA*	95	*PABPC1*	261368

EPC, Edge Percolated Component; MCC, Maximal Clique Centrality; MNC, Maximum Neighborhood Component

Key target gene analysis of miRNAs in CytoNCA in high-grade bladder cancer patients group

CytoNCA							
Betweenness		**Closeness**		**Degree**		**Eigenvector**	
TP53	43318.11	*TP53*	0.08692	*TP53*	400	*TP53*	0.14084
AKT1	34899.65	*AKT1*	0.08683	*AKT1*	390	*AKT1*	0.13901
CTNNB1	33186.80	*CTNNB1*	0.08656	*CTNNB1*	363	*CTNNB1*	0.13490
MYC	24278.85	*MYC*	0.08639	*MYC*	348	*MYC*	0.13356
EGFR	17742.88	*EGFR*	0.08604	*EGFR*	311	*STAT3*	0.12497
JUN	15688.20	*JUN*	0.08592	*JUN*	297	*JUN*	0.12306
SRC	14826.75	*PTEN*	0.08586	*PTEN*	294	*EGFR*	0.12272
IL6	12890.36	*STAT3*	0.08582	*STAT3*	290	*PTEN*	0.12237
EP300	12564.87	*SRC*	0.08564	*SRC*	272	*HRAS*	0.11447
PTEN	12175.20	*IL6*	0.08557	*HRAS*	264	*VEGFA*	0.11438
CDH1	11612.66	*HRAS*	0.08557	*IL6*	258	*SRC*	0.11318
HSP90AA1	10878.28	*VEGFA*	0.08550	*VEGFA*	256	*CCND1*	0.11273
STAT3	10463.25	*CCND1*	0.08549	*CCND1*	256	*HIF1A*	0.11113
HRAS	9998.29	*NOTCH1*	0.08537	*NOTCH1*	245	*NOTCH1*	0.11058
ESR1	9833.07	*CASP3*	0.08534	*HIF1A*	241	*IL6*	0.10920
HIF1A	9642.17	*HIF1A*	0.08534	*CASP3*	237	*CASP3*	0.10908
CCND1	8931.99	*ESR1*	0.08529	*KRAS*	236	*KRAS*	0.10757
FN1	8405.20	*CDH1*	0.08526	*CDH1*	236	*ESR1*	0.10533
VEGFA	8071.93	*KRAS*	0.08525	*ESR1*	235	*CDH1*	0.10354
CASP3	7862.63	*ERBB2*	0.08516	*EP300*	226	*ERBB2*	0.10037
NOTCH1	7647.77	*EP300*	0.08513	*FN1*	225	*FN1*	0.09700
ERBB2	7125.52	*FN1*	0.08511	*ERBB2*	224	*IL1B*	0.09356
RHOA	6872.49	*HSP90AA1*	0.08510	*HSP90AA1*	219	*CDKN2A*	0.09339
IL1B	6710.04	*IL1B*	0.08496	*IL1B*	211	*HSP90AA1*	0.09333
HDAC2	6475.09	*CDKN2A*	0.08486	*CDKN2A*	196	*MTOR*	0.09170
SMAD3	6356.20	*MTOR*	0.08478	*MTOR*	190	*IGF1*	0.09033
SMAD2	6248.53	*SMAD3*	0.08475	*SMAD3*	183	*EP300*	0.08876
MTOR	5964.37	*SMAD4*	0.08467	*SMAD4*	176	*SMAD4*	0.08460
SOX2	5825.79	*IGF1*	0.08463	*IGF1*	173	*SMAD3*	0.08439
KRAS	5246.49	*CREB1*	0.08463	*SMAD2*	172	*PIK3CA*	0.08390
PPARG	5108.88	*PPARG*	0.08463	*PPARG*	172	*CREB1*	0.08213
EZH2	4958.64	*SMAD2*	0.08460	*EZH2*	172	*PPARG*	0.08166
CREB1	4872.41	*MAPK1*	0.08460	*MAPK1*	172	*SMAD2*	0.08122
MAPK1	4640.42	*SIRT1*	0.08460	*RHOA*	171	*MAPK1*	0.08043
AR	4350.69	*RHOA*	0.08459	*CREB1*	171	*SIRT1*	0.07987

CytoNCA							
Betweenness		**Closeness**		**Degree**		**Eigenvector**	
SMAD4	4252.57	*PIK3CA*	0.08458	*PIK3CA*	170	*FOXO3*	0.07965
CDH2	4097.98	*FOXO3*	0.08454	*SIRT1*	169	*STAT1*	0.07936
BDNF	4096.94	*EZH2*	0.08452	*FOXO3*	166	*RHOA*	0.07857
FOXO3	4087.12	*SOX2*	0.08451	*SOX2*	163	*GSK3B*	0.07788
GRB2	4063.63	*MAPK14*	0.08444	*STAT1*	160	*MAPK14*	0.07698
PTPN11	4011.15	*MAPK8*	0.08439	*MAPK14*	155	*MAPK8*	0.07665
CDKN2A	3870.89	*STAT1*	0.08439	*GSK3B*	152	*IGF1R*	0.07567
CDK2	3835.93	*GSK3B*	0.08437	*MAPK8*	152	*BCL2L1*	0.07505
STAT1	3782.77	*KDR*	0.08431	*PIK3R1*	150	*KIT*	0.07490
MAPK8	3758.49	*PIK3R1*	0.08430	*ATM*	149	*CDKN1A*	0.07454
LRRK2	3439.97	*CDKN1A*	0.08429	*KIT*	149	*EZH2*	0.07435
HNF4A	3412.08	*ATM*	0.08429	*NFKBIA*	148	*JAK2*	0.07383
IGF1	3400.95	*KIT*	0.08429	*BCL2L1*	147	*SOX2*	0.07369
KDR	3383.64	*IGF1R*	0.08429	*TLR4*	146	*PIK3R1*	0.07275
DICER1	3267.78	*NFKBIA*	0.08428	*CDKN1A*	143	*PTGS2*	0.07271

Key target gene analysis of miRNAs in CytoHubba in high-grade bladder cancer patients group

CytoHubba							
EPC		**MCC**		**MNC**		**Stress**	
TP53	36.96	*TP53*	4.64876E+37	*TP53*	400	*TP53*	818632
CTNNB1	35.28	*CTNNB1*	4.64876E+37	*AKT1*	390	*AKT1*	718546
AKT1	34.75	*STAT3*	4.64876E+37	*CTNNB1*	363	*CTNNB1*	677670
EGFR	34.49	*MYC*	4.64876E+37	*MYC*	348	*MYC*	575480
JUN	33.75	*HRAS*	4.64876E+37	*EGFR*	310	*EGFR*	449474
HIF1A	33.42	*VEGFA*	4.64872E+37	*JUN*	297	*JUN*	402476
PTEN	33.29	*PTEN*	4.64871E+37	*PTEN*	294	*SRC*	369210
MYC	33.28	*HIF1A*	4.64870E+37	*STAT3*	290	*IL6*	362580
STAT3	32.55	*CCND1*	4.64867E+37	*SRC*	271	*PTEN*	344868
HRAS	31.71	*CASP3*	4.64862E+37	*HRAS*	264	*STAT3*	323652
IL6	31.31	*SRC*	4.64825E+37	*IL6*	258	*EP300*	304670
CASP3	31.15	*JUN*	4.64515E+37	*CCND1*	256	*CDH1*	292526
CCND1	31.03	*KRAS*	4.64328E+37	*VEGFA*	256	*HRAS*	291064
SRC	30.96	*MTOR*	4.61065E+37	*NOTCH1*	245	*HSP90AA1*	286176
NOTCH1	30.25	*AKT1*	4.60476E+37	*HIF1A*	240	*ESR1*	276576
KRAS	30.06	*IGF1R*	4.58689E+37	*CASP3*	237	*CCND1*	275162
VEGFA	28.77	*CDKN2A*	4.52123E+37	*CDH1*	236	*VEGFA*	260862
ESR1	28.60	*CDH1*	4.51446E+37	*KRAS*	236	*FN1*	254858
CDH1	28.45	*IL6*	4.46168E+37	*ESR1*	235	*HIF1A*	253298
FN1	27.72	*EGFR*	4.39080E+37	*EP300*	226	*NOTCH1*	242982
ERBB2	27.67	*NOTCH1*	4.35771E+37	*FN1*	225	*IL1B*	227416
MTOR	27.51	*IGF1*	4.16343E+37	*ERBB2*	223	*CASP3*	220624
IL1B	27.12	*HSP90AA1*	3.95631E+37	*HSP90AA1*	219	*ERBB2*	207914
HSP90AA1	26.35	*MAPK8*	3.78193E+37	*IL1B*	211	*SMAD3*	207866
CDKN2A	26.27	*CDKN1A*	3.54946E+37	*CDKN2A*	196	*RHOA*	204496
CREB1	25.68	*ERBB2*	3.49330E+37	*MTOR*	189	*SMAD2*	195824
IGF1	25.44	*FN1*	3.48111E+37	*SMAD3*	183	*KRAS*	195774
SMAD3	25.40	*SMAD2*	3.24648E+37	*SMAD4*	176	*SOX2*	177378
EP300	25.11	*SMAD4*	3.17361E+37	*MAPK1*	172	*MTOR*	169344
FOXO3	24.55	*ESR1*	3.16477E+37	*EZH2*	172	*EZH2*	169208
PIK3CA	24.35	*SMAD3*	3.13466E+37	*PPARG*	172	*HDAC2*	162266
SIRT1	24.19	*IL1B*	3.03260E+37	*IGF1*	172	*PPARG*	160972
RHOA	24.15	*MMP2*	2.67257E+37	*CREB1*	171	*SMAD4*	149714
IGF1R	24.11	*RHOA*	2.57949E+37	*RHOA*	171	*MAPK1*	147868
STAT1	23.91	*PPARG*	1.48338E+37	*PIK3CA*	170	*CREB1*	145682

CytoHubba							
EPC		**MCC**		**MNC**		**Stress**	
MAPK1	23.84	*CDKN1B*	1.17501E+37	*SMAD2*	170	*CDKN2A*	142236
KIT	23.76	*FOXO3*	1.15523E+37	*SIRT1*	169	*CDK2*	135550
PTGS2	23.54	*BCL2L1*	1.10569E+37	*FOXO3*	166	*FOXO3*	127036
BCL2L1	23.22	*SIRT1*	1.10102E+37	*SOX2*	163	*STAT1*	126868
SMAD4	23.21	*CASP8*	1.07225E+37	*STAT1*	160	*AR*	124004
TLR4	23.19	*AR*	9.87754E+36	*MAPK14*	155	*BDNF*	117584
EZH2	22.99	*PTGS2*	6.65197E+36	*GSK3B*	152	*SIRT1*	114494
MAPK14	22.66	*MAPK1*	6.64063E+36	*MAPK8*	151	*MAPK8*	113312
KDR	22.65	*ATM*	6.31194E+36	*PIK3R1*	150	*GRB2*	113286
GSK3B	22.63	*HGF*	5.61595E+36	*KIT*	149	*CDH2*	106958
NFKBIA	22.59	*BCL2L11*	4.97005E+36	*NFKBIA*	148	*PIK3CA*	106326
ATM	22.49	*TGFB1*	4.91611E+36	*ATM*	148	*MAPK14*	105788
HGF	22.49	*STAT1*	3.31280E+36	*BCL2L1*	147	*PTPN11*	105016
PPARG	22.26	*KDR*	3.25620E+36	*TLR4*	146	*ITGB1*	104348
MMP2	22.14	*SOX2*	*3.20016E+36*	*CDKN1A*	143	*ATM*	102894

EPC, Edge Percolated Component; MCC, Maximal Clique Centrality; MNC, Maximum Neighborhood Component

Approval of the Central Medical Ethics committee of Latvia

Centrālā medicīnas ētikas komiteja

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Rīgā

12.02.2019. Nr.1/19-02-12

Rīgas Stradiņa universitātes
Onkoloģijas institūtam

Atzinums par pētījumu
„Neinvazīvi diagnostiski un prediktīvi biomarķieri urīnpūšļa audzēja agrīnai diagnostikai un prognozēšanai"

Centrālā medicīnas ētikas komiteja 2019.gada 31.janvārī ir izskatījusi Rīgas Stradiņa Universitātes Onkoloģijas institūta iesniegto pētījumu *„Neinvazīvi diagnostiski un prediktīvi biomarķieri urīnpūšļa audzēja agrīnai diagnostikai un prognozēšanai".*

Pamatojoties uz Centrālās medicīnas ētikas komitejas 2019.gada 31.janvāra sēdes protokola Nr.2019-1 punktu Nr.2 un iesniegtajiem labojumiem, tiek izsniegts atzinums, ka Rīgas Stradiņa Universitātes Onkoloģijas institūta iesniegtais pētījums *„Neinvazīvi diagnostiski un prediktīvi biomarķieri urīnpūšļa audzēja agrīnai diagnostikai un prognozēšanai"* nav pretrunā ar bioētikas normām.

Centrālās medicīnas ētikas
komitejas loceklis E.Strautiņš

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Annex 6

Supplement of Approval of the Central Medical Ethics committee of Latvia

Centrālā medicīnas ētikas komiteja

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Rīgā

17.03.2023 Nr. 01-29.1.2/1352
Uz 15.02.2023 Nr. _____

Rīgas Stradiņa universitātes
Onkoloģijas institūtam
erika.bitina@gmail.com
Zanda.Daneberga@rsu.lv

Par atļaujas saņemšanu pētījumam "Neinvazīvi diagnostiski un prediktīvi biomarķieri urīnpūšļa audzēja agrīnai diagnostikai un prognozēšanai"

Centrālā medicīnas ētikas komiteja izskatīja Rīgas Stradiņa universitātes Onkoloģijas institūta 2019.gada 1.janvāra precizēto pētījuma pieteikumu "*Neinvazīvi diagnostiski un prediktīvi biomarķieri urīnpūšļa audzēja agrīnai diagnostikai un prognozēšanai"* (reģistrēts Veselības ministrijā 2023.gada 15.februārī Nr.1687) ar papildinājumiem (reģistrēts Veselības ministrijā 2023.gada 16.martā ar Nr.2798).

Atbilstoši Centrālā medicīnas ētikas komitejas 2023.gada 9.marta sēdes protokola Nr.2023-2 I.daļas "*PRECIZĒTIE IESNIEGUMI*" 2.punktam, tiek sniegts atzinums, ka pētījums nav pretrunā bioētikas normām.

Centrālās medicīnas ētikas
komitejas priekšsēdētājs (paraksts*) V. Sīlis

Kristīne Kalniņa 67876116
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Explanation of the study for participants

PĒTĪJUMA „Neinvazīvi diagnostiski un prediktīvi biomarķieri urīnpūšļa audzēja agrīnai diagnostikai un prognozēšanai ”

SKAIDROJUMS

Pētījuma īstenotājs: Rīgas Stradiņa universitāte, Onkoloģijas institūts.

Lūdzu rūpīgi izlasiet esošo informāciju un, ja vēlaties, apspriediet to ar draugiem un radiniekiem. Lūdziet ārstam paskaidrot nesaprotamos vārdus vai sniegt papildus informāciju.

Pētījuma mērķis ir, izmantojot bioķīmiskās un molekulāri bioloģiskās metodes, izpētīt un atrast urīnā urīnpūšļa audzējam specifiskus miRNS biomarķierus, kas būtu jauns neinvazīvs, specifisks un ekonomiski izdevīgs diagnostikas veids, kas ļautu agrīni atklāt urīnpūšļa audzēju. Nākotnē tas mazinātu ielaista vēža gadījuma skaitu, kas ļautu jau agrīni sniegt pacientiem nepieciešamo ārstēšanu, tādējādi uzlabot kopējo pacientu dzīvildzi.

Pētījums ilgs 7 gadus un norisināsies laika posmā no 2019.gada februāra līdz 2026. gada janvārim. Pētījuma laikā tiks meklēti ar urīnpūšļa audzēju saistītie miRNS, kas ir ribonukleinskābes, kuras ietekmē audzēja attīstību. MiRNS var darboties kā audzēja veicinātājas, tā arī kavētājas.

Izmeklējuma laikā, kombinējot bioķīmiskās un molekulāri bioloģiskās metodes, tiks pārbaudītas audzēju šūnas un urīna paraugi. Tas ļaus atklāt specifiskās miRNS, kuras raksturīgas slimniekiem ar urīnpūšļa audzēju, bet ne veseliem indivīdiem.

Pētījumā tiks pētīti audu un/vai urīna paraugi, kas tiks iegūti diagnostikas vai ārstēšanas nolūkos veikto manipulāciju rezultātā. No iegūtā bioloģiskā materiāla izdalīs šūnas, no kurām tālāk izdalīs nukleīnskābes (RNS, DNS), kuras izmantos molekulāri bioloģiskām analīzēm. Nodotie bioloģiskā materiāla paraugi tik uzglabāti laboratorijā 5 gadus pēc pētījuma beigām.

Pētījuma laikā netiks noņemti nekādi papildus audu paraugi, kā vien tie, kuri medicīnisku iemeslu dēļ tiks atdalīti operācijas laikā.

Jūsu ieguldījums veicinās pētniecību, kas var rezultēties ar jaunu diagnostikas metožu ieviešanu. Lai arī šie pētījuma rezultāti, kas tiks iegūti un no tiem izdarītajiem secinājumiem nebūs tieša ietekme uz Jūsu slimības ārstēšanu, tomēr Jūsu iesaiste projektā būs nozīmīga citiem pacientiem tālākā nākotnē.

Kas ar mani notiks, ja piekritīšu piedalīties pētījumā?

Piekrītot piedalīties pētījumā:

- operācijas laikā (kas tiks veikta ārstnieciskos nolūkos) no jau iegūtā operācijas materiāla vai slimību diagnosticējošas manipulācijas (cistoskopijas) laikā tiks paņemti audu paraugi un/vai urīna paraugs.
- no Jūsu medicīniskās dokumentācijas tiks iegūta informācija par slimības klīniski patoloģiskajām īpašībām, saņemto ķirurģisko ārstēšanu, saņemto ķīmijterapiju, testu rezultātiem un ģimenes vēsturi.

- pacientu grupā urīna paraugi tiks ņemti pirms ķirurģiskās manipulācijas un pēc tam katrā novērošanas vizītē līdz recidīvam vai slimības progresēšanai.
- kontroles grupā urīna paraugs tiks ņemts tikai vienu reizi.
- terapijas kontroles grupā grupas pacientiem urīna paraugi tiks ņemti pirms operācijas, pirms terapijas uzsākšanas, pirms katras ķīmijterapijas reizes ievadīšanas (kopā 6 reizes) un pēc tam trešajā un sestajā mēnesī, un vienu gadu pēc pirmās ķīmijterapijas reizes katrā novērošanas vizītē līdz recidīvam vai progresēšanai.
- iegūtie klīniskie dati un bioloģiskais materiāls tiks kodēti, un uzglabāti atsevišķi no personas datiem, nodrošinot konfidencialitāti datus apstrādājot.

Iespējamie riski

Psiho-emocionāls risks - diskomforts un baiļu sajūta saistībā ar vēža diagnozi (pētījuma dalībniekiem ar saslimšanu), pētījumā iegūto datu drošību un konfidencialitāti.

Finansiāla kompensācija par fizisko un psiholoģisko kaitējumu nav paredzēta.

Kontaktinformācija:
Ja jums ir jebkādi jautājumi par šo pētījumu vai vēlaties pārtraukt savu dalību tajā, lūdzu, sazinieties ar RSU pētnieku dr. Juris Plonis, e-pasts Juris.Plonis@stradini.lv

Approval of the Rīga Stradiņš University

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Rīgas Stradiņa universitātes
Rektoram prof. Aigaram Pētersonam

atzinums.

Atbalstu RSU Onkoloģijas institūta Molekulārās ģenētikas laboratorijas iesaistīšanos pētījumā ***„Neinvazīvi diagnostiski un prediktīvi biomarķieri urīnpūšļa audzēja agrīnai diagnostikai un prognozēšanai"***. Pētījums tiks organizēts sadarbībā ar Paula Stradiņa Klīnisko universitātes slimnīcu.

Rektors A. Pētersons

Sagatavoja: J. Plonis
29159476

UROLOGS
JURIS PLONIS
1-21073-10917

Approval of the Pauls Stradiņš Clinical University Hospital

1.pielikums **Kārtība, kādā tiek veikta akadēmiskā pētniecība VSIA „Paula Stradiņa klīniskā universitātes slimnīca"**	ID P-Zin-1.2/01 Versija 02 Spēkā no 30.08.2016.

VSIA „Paula Stradiņa klīniskā universitātes slimnīca"
Zinātniskā institūta direktoram

IESNIEGUMS AKADĒMISKĀ PĒTĪJUMA ATĻAUJAS SAŅEMŠANAI

20 18. gada 15. novembris

IESNIEGUMA IESNIEDZĒJS / PĒTĪJUMA AUTORS VAI AUTORU KOLEKTĪVA VADĪTĀJS

Vārds, uzvārds	Erika Bitiņa-Barlote		
Tālr.	20092515	E-pasts	ERIKA.BITINA@GMAIL.COM
*Izglītības iestādes nosaukums**	Latvijas Universitāte		
Studiju programma*	Rezidentūra.		

PĒTĪJUMA ZINĀTNISKAIS VADĪTĀJS*

Vārds, uzvārds	UROLOGS JURIS PLONIS 1-281070-10917	Asoc. prof. Zanda Daneberga
Izglītības iestādes nosaukums, amats	RSU Uroloģijas institūts	RSU Onkoloģijas institūts
Zinātniskā vadītāja paraksts, datums	15.11.2018	15.11.2018

PĒTĪJUMA NOSAUKUMS

Neinvazīvi diagnostiski un prediktīvi biomarķieri urīnpūšļa audzēja agrīnai diagnostikai un prognozēšanai

PĒTĪJUMA NORISES VIETA

Klīnika/centrs/nodaļa	RSKUS uroloģijas centrs
Klīnikas/centra/nodaļas vadītāja saskaņojums par pētījuma veikšanu	
Slimnīcas darbinieks- galvotājs** paraksts, datums	UROLOGS JURIS PLONIS 1-281070-10917 15.11.2018

NORISES LAIKS

Pētījuma norises laiks, norādot sākuma un beigu datumu	01.01.2019. 01.01.2024.

IZMANTOJAMĀS PACIENTU SLIMĪBAS VĒSTURES

Pētījumā paredzēts izmantot ≤ *100* ☐ ≥ *100**** ☐

Slimības vēsturu diagnožu kodi (SSK-10) vai Slimības vēsturu numuri ja zināmi

C67

* Šo sadaļu aizpilda studenti, maģistranti un doktoranti

** ja iesnieguma iesniedzējs pats vai zinātniskā darba vadītājs nav Slimnīcas darbinieks, nepieciešams galvotājs (Slimnīcas darbinieks, ārstniecības persona, kura ir atbildīga par pētniecības norisi un juridiskajām sekām).

*** *ja paredzēts izmantot vairāk kā 100 Slimības vēstures, iesniegumam nepieciešams pievienot rakstisku pamatojumu brīvā formā.*

15.11.2018
/datums/

Urologijas ārsts-rezidents Erika Bitiņa-Barlote 10370054524
/iesniedzēja paraksts, atšifrējums/

Slimnīcas atļauju:

20.11.2018.
/datums/

VSIA "Paula Stradiņa klīniskā universitātes slimnīca" Zinātniskā institūta Zinātnes nodaļas vadītājs Pēteris Ērglis
/Zin. institūta kompet. darbinieka paraksts un atšifrējums/

Zin-1-006 versija 02

Template of informed consent for study participant

Pārzinis: Rīgas Stradiņa universitāte

Datu operators: Rīgas Stradiņa universitātes Onkoloģijas institūta
Molekulārās ģenētikas laboratorija
Pilsoņu iela 13, 13. korpuss, Rīga, LV-1012, Latvija

PACIENTA PIEKRIŠANA DALĪBAI RSU pētniecības projekta "Neinvazīvi diagnostiski un prediktīvi biomarķieri urīnpūšļa audzēja agrīnai diagnostikai un prognozēšanai." PĒTĪJUMĀ

Pētījuma mērķis – Atrast specifisku, neinvazīvu, ekonomiski izdevīgu biomarķierus, kuri var tikt izmantoti agrīnai urīnpūšļa vēža noteikšanai un paredzēt izdzīvošanu, nemuskuļinvazīva urīnpūšļa audzēja iespējamo recidīva risku.
Personas datu apstrādes mērķis – Analizēt pētījuma laikā iegūtos molekulāri bioloģiskos datus saistībā ar slimības klīnisko aprakstu.

1. Esmu saņēmis un iepazinies ar rakstisku informāciju par projekta **"Neinvazīvi diagnostiski un prediktīvi biomarķieri urīnpūšļa audzēja agrīnai diagnostikai un prognozēšanai."** mērķi, saturu un iespējamiem riskiem. Uz visiem maniem jautājumiem esmu saņēmis izsmeļošas atbildes. Man bija pietiekami daudz laika, lai pārdomātu savu lēmumu piekrist nodot bioloģisko materiālu zinātniskai izpētei.
2. Esmu saņēmis un iepazinies ar rakstisku informāciju par manu personas datu apstrādes tiesisko pamatu, mērķi, apjomu, glabāšanas ilgumu un iznīcināšanu, kā arī iespējamiem personas datu saņēmējiem.
3. Es piekrītu bez atlīdzības nodot urīna un/vai audu paraugus (bioloģisko materiālu) nukleīnskābju un/vai proteīnu izdalīšanai molekulāri – ģenētiskiem pētījumiem. Es saprotu, ka urīna vai audu paraugu nodošana nerada risku manai veselībai, kas man ir izskaidrots.
4. Urīna un/vai audu paraugi, ko nododu izpētei, ir nepieciešami, lai izdalītu nukleīnskābes (DNS; RNS) un/vai proteīnus, ar ko veikt molekulāri bioloģiskās analīzes.
5. Esmu informēts, ka jebkura mani identificējoša informācija būs konfidenciāla, un ka visi mani nodotie audu paraugi tiks kodēti.
6. Esmu informēts, ka jebkurā brīdī bez paskaidrojumiem varu pārtraukt piedalīšanos pētījumā un tas neietekmēs manu turpmāko ārstēšanos. Kā arī, esmu informēts, ka šādā gadījumā mani nodotie urīna un/vai audu paraugi, izdalītās nukleīnskābes un/vai proteīni, veselības stāvokļa apraksts un jebkura mani identificējoša informācija tiks iznīcināta.
7. Esmu informēts, ka man ir tiesības jebkurā brīdī labot, papildināt, vai pārtraukt manu personas datu apstrādi vai jautāt par savu personas datu iznīcināšanu, kā arī man ir tiesības iegūt visu informāciju, kas ir savākta par maniem personas datiem.
8. Esmu informēts, ka mani personas dati var tikt nodoti ārpus ES vai EEZ valstīm tikai ar manu atsevišķi parakstītu piekrišanu.

9. Veselības stāvokļa apraksti:

	Es ATĻAUJU sava veselības stāvokļa apraksta papildināšanu, atjaunošanu vai pārbaudi;
	Es AIZLIEDZU sava veselības stāvokļa apraksta papildināšanu, atjaunošanu vai pārbaudi.
	Es ATĻAUJU savu veselības stāvokļa aprakstu un tam atbilstošo attēlu (radioloģisko izmeklējumu attēlu, fotogrāfiju u.c.) anonīmu publicēšanu;
	Es AIZLIEDZU savu veselības stāvokļa aprakstu un tam atbilstošo attēlu (radioloģisko izmeklējumu attēlu, fotogrāfiju u.c.) anonīmu publicēšanu.

10. Ja mana bioloģiskā materiāla izpētē tiks atklāta informācija par kādu man līdz šim nezināmu apdraudējumu (vajadzīgo atzīmēt):

	Es PIEKRĪTU, ka man tiek paziņota šī informācija elektroniski uz manu privāto e-pastu _____ vai uz deklarēto dzīvesvietas adresi _____;
	Es PIEKRĪTU, ka man tiek paziņota šāda informācija tikai tādā gadījumā, ja risks veselībai ir novēršams;
	Es NEVĒLOS saņemt nekādu papildus informāciju.

11. Bioloģiskā materiāla uzglabāšana:

	Es PIEKRĪTU, ka, mans bioloģiskais materiāls turpmāk glabāsies RSU Onkoloģijas institūta Molekulārās ģenētikas laboratorijā un var tikt izmantots citos zinātniskos pētījumos, kuri ieguvuši LR Veselības ministrijas Centrālās medicīnas ētikas komitejas atzinumu par atbilstību bioētikas normām, bez ierobežojuma;
	Es AIZLIEDZU mana bioloģiskā materiāla turpmāku uzglabāšanu RSU Onkoloģijas institūta Molekulārās ģenētikas laboratorijā un tā izmantošanu citos zinātniskos pētījumos.

Bioloģiskā materiāla donors:

_____

Vārds un uzvārds (drukātiem burtiem)

Personas kods: | | | | | | | - | | | | | |

Paraksts:_____ Datums: __ __ / __ __ / 20__ (DD/MM/GGGG)

Ārsts/klīnicists: _____

Vārds un uzvārds, amats (drukātiem burtiem vai spiedogs):

_____Datums: __ __ / __ __ / 20__ (DD/MM/GGGG)

Apstiprinu, ka esmu informējis pacientu par šo pētījumu (paraksts)

Pacientam piešķirtais kods: _____

2

Research questionnaire

Pacienta piešķirtais kods

_____

Pielikums nr.5

Pētījuma anketa

1. Dzimums — S/V
2. Vecums — _____
3. Svars — _____
4. Augums — _____
5. Smēķē: — jā/ nē
   a. Ja atbilde ir jā — Pašreizējais/ kādreizējais smēķētajs
      i. Paku gadi _____
6. Saskarsme ar aromātiskiem amīniem — jā/nē
7. Hroniskas UCI — jā/nē
8. Hematūrija: — ir/nav
9. Dizūrija — jā/nē
10. Audzēju skaits — 1/2-7/ ≥ 8
11. Audzēja izmērs — <3cm/ >3cm
12. TNM kategorija — _____
13. CIS — jā/nē
14. Grade — 1/2/3 vai LG/HG
15. Iepriekš recidīvs — primārs/ ≤1recidīvs gadā/ ≥1recidīvs gadā
16. EORT kalkulatora noteiktais
    1 gadā progresijas/recidīva risks — _____/_____%
    5 gadu progresijas/recidīva risks — _____/_____%
17. EAU NMIBC riska kalkulators — _____
18. Operācijas veids — TUR-U/ parciāla cistektomija/ cistektomija
19. Urīna citoloģija — _____
    _____
    _____
20. Asins analīzes — _____
    _____
    _____

21. Onkoloģiska saslimšana ģimenē — jā/nē

Ja 20. Punktā jā, tad kāda

_____