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NOVEL DIAGNOSTICS IN BLADDER CANCER: A SYSTEMATIC REVIEW

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ΑΜ: 194

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Περίληψη

Ο καρκίνος της ουροδόχου κύστης αποτελεί μία από τις πιο συχνές μορφές καρκίνου παγκοσμίως και χαρακτηρίζεται από σημαντική ετερογένεια ως προς την κλινική του πορεία και την ανταπόκριση στη θεραπεία. Η ανάγκη για βελτιωμένα διαγνωστικά και προγνωστικά εργαλεία είναι επιτακτική, ώστε να ενισχυθεί η ακρίβεια στη διάγνωση, να προβλεφθεί η εξέλιξη της νόσου και να βελτιστοποιηθεί η θεραπευτική προσέγγιση.

Η παρούσα διπλωματική εργασία είχε ως στόχο τη συστηματική ανασκόπηση και σύνθεση της υπάρχουσας βιβλιογραφίας σχετικά με τα νέα διαγνωστικά εργαλεία στον καρκίνο της ουροδόχου κύστης. Συγκεντρώθηκαν και αξιολογήθηκαν μετα-αναλύσεις και συστηματικές ανασκοπήσεις που διερευνούν καινοτόμες τεχνολογίες με βιοδείκτες, με στόχο την ανάδειξη των πιο υποσχόμενων προσεγγίσεων με βάση τον χαρακτηρισμό τους μέσα στην βιβλιογραφία ως υποσχόμενα, καινοτόμα ή με συναφή προσδιορισμούς. Επίσης ο χρονολογιακός περιορισμός της έρευνας πάνω στην δράση τους περιορίζεται από 2012 μέχρι το 2023 και σημαντικό κριτήριο να αποτελεί η ευκολία στην εξέτασή τους (αίμα και ούρα). Από τις επιλεγμένες μελέτες συγκεντρώθηκαν μόνο όσα διαγνωστικά πληρούσαν τα θεσπισμένα κριτήρια ώστε να χαρακτηριστούν καινοτόμα.

Τα αποτελέσματα της εργασίας ανέδειξαν τη σημασία της χρήσης μη επεμβατικών βιοδεικτών, όπως οι ανιχνεύσεις DNA και RNA στα ούρα, καθώς και η εφαρμογή τεχνικών υγρής βιοψίας για την παρακολούθηση της νόσου, προσφέροντας μια προσβασιμότερη, οικονομικότερη και μη επεμβατική προσέγγιση της νόσου, σε σχέση με τις συμβατικές μεθόδους. Η παρούσα διπλωματική εργασία καταλήγει στο συμπέρασμα ότι η ενσωμάτωση αυτών των καινοτόμων διαγνωστικών εργαλείων μπορεί να συμβάλει στη βελτίωση της κλινικής διαχείρισης του καρκίνου της ουροδόχου κύστης, υπογραμμίζοντας την ανάγκη για περαιτέρω έρευνα και κλινικές δοκιμές που να επικυρώσουν την αποτελεσματικότητά τους.

Abstract

Bladder cancer is one of the most common types of cancer worldwide characterized by significant heterogeneity in its clinical course and response to treatment. The need for improved diagnostic and prognostic tools is imperative to enhance diagnostic accuracy, predict disease progression, and optimize therapeutic approaches.

The present post-graduate dissertation aimed to systematically review and synthesize the existing literature on novel diagnostic tools for bladder cancer. Meta-analyses and systematic reviews were gathered and evaluated, focusing on innovative detection technologies based on biomarkers, in order to identify the most promising approaches, based on their characterization in the literature as promising, innovative, or similar designations. The chronological limitation of research on their activity being restricted to the period from 2012 to 2023, with an additional important criterion being the ease of their examination (blood and urine). From the selected studies, only diagnostic tools meeting the established criteria to be classified as novel were included.

The findings highlighted the importance of non-invasive biomarkers, such as DNA and RNA detection in urine, as well as the application of liquid biopsy techniques for disease monitoring, offering a more accessible, cost-effective, and non-invasive approach to the disease compared to conventional methods. The present study concludes that integrating these innovative diagnostic tools can contribute to improving the clinical management of bladder cancer, emphasizing the need for further research and clinical trials to validate their effectiveness.

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1. An Introduction to Bladder Cancer

Bladder cancer (BCa) is the second most prevalent urogenital cancer, following prostate cancer, and is the 9th most common cancer worldwide (Lenis et al., 2020). It primarily affects older adults, with men being more frequently diagnosed than women (Saginala et al., 2020). The disease presents a significant healthcare burden due to its high recurrence rate, the need for continuous monitoring, and the associated treatment costs (Leal et al., 2016). While significant progress has been made in understanding its pathogenesis, bladder cancer remains a challenging condition to diagnose and manage effectively. Early detection and accurate prognostic evaluation are crucial for improving patient outcomes, reducing mortality, and minimizing the risk of recurrence (Dyrskjøt et al., 2023).

Currently, the gold standard for bladder cancer diagnosis includes cystoscopy and urine cytology (Devlies et al., 2024). Cystoscopy, although highly effective, is invasive, expensive, and uncomfortable for patients, requiring frequent follow-ups. Urine cytology, on the other hand, is non-invasive but lacks sensitivity, particularly in detecting low-grade tumors (Yafi et al., 2015). To address these limitations, researchers have been actively investigating novel diagnostic and prognostic approaches that are more precise, less invasive, and capable of providing real-time insights into tumor behavior.

Emerging technologies, including biomarker-based assays, liquid biopsies, next-generation sequencing (NGS), radiomics, and artificial intelligence (AI)-driven imaging analysis, are revolutionizing bladder cancer diagnostics (Lopez-Beltran et al., 2024). Biomarkers derived from urine, blood, and tissue samples offer promising alternatives to traditional methods by providing molecular-level insights into tumor presence, progression, and therapeutic response (Maas et al., 2023)**.** Liquid biopsies, which analyze circulating tumor DNA (ctDNA) and extracellular vesicles, have the potential to detect cancer at an early stage and monitor treatment efficacy without requiring invasive procedures (Crocetto et al., 2022). Furthermore, advances in multi-omics approaches, integrating genomics, transcriptomics, proteomics, and metabolomics, are paving the way for personalized medicine in bladder cancer management (Crocetto et al., 2022). The abovementioned advancements hold the promise of significantly reducing overtreatment, minimizing unnecessary procedures, and improving overall survival rates.

This thesis explores the latest advancements in diagnostic biomarkers for bladder cancer, critically evaluating their clinical applicability, advantages, and challenges, trying to emerge non-invasive, more cost-effective procedures compared to existing methods helping to improve patient outcome. By synthesizing recent research findings and technological developments, this study aims to contribute to the ongoing efforts in refining bladder cancer detection, risk assessment, and personalized treatment approaches. The ultimate goal is to highlight innovative strategies that could transform the current field of bladder cancer management and improve patient care in the years to come.

2. Types of Bladder Cancer

Urothelial carcinoma, also known as transitional cell carcinoma, is the most prevalent form of bladder cancer and represents around 90% of all cases. This cancer originates from the urothelial cells that line the inner surface of the bladder wall. When these cells become cancerous, they can develop into a tumor, invading the deeper layers of the bladder wall and potentially spreading to nearby lymph nodes and other organs. Another type of bladder cancer is squamous cell carcinoma, accounting for around 4% of bladder cancers, which originates in the lining of the bladder because of irritation or inflammation. These types of cells may eventually become cancerous. Adenocarcinoma is another type of bladder cancer, accounting for around 2% of bladder cancer cases. Adenocarcinoma is usually invasive and consists of glandular-type cells. Small cell carcinoma, is a rare bladder cancer type, making up less than 1% of all bladder cancer cases, which tend to spread rapidly and grow quickly. Sarcoma is a type of cancer that develops in the body's supportive tissues, and very rarely may arise from the muscle or fat layers of the bladder (Zingg & Wallace, 2012).

3. The Development of Bladder Cancer

3.1 Bladder Cancer Development

Bladder cancer progresses through two distinct pathways: papillary and nonpapillary, each associated with different clinical manifestations of the disease, in a background of behavioral, industrial, and environmental risk factors and as a result of genetic predisposition. Around 80% of bladder tumors are superficial papillary lesions that develop from diffuse mucosal hyperplastic changes wide, known as low-grade intraurothelial neoplasia. These tumors may be multifocal and present a tendency for recurrence after surgical removal. They usually do not invade the bladder wall or metastasize, in contrast to nonpapillary type, that arise from in situ precursor conditions such as severe dysplasia or carcinoma in situ, collectively known as high-grade intraurothelial neoplasia. Patients with superficial papillary tumors often experience multiple recurrences, but only a small percentage progress to high-grade invasive bladder tumors. In contrast, most high-grade invasive bladder cancers occur in individuals with no prior history of superficial papillary lesions (Czerniak et al., 2016).

3.2 Bladder Cancer Stages

There are several different staging systems for cancer, but bladder cancer is usually staged using the Tumour, Node, Metastasis (TNM) staging system.

T stands for Tumor

TX: Primary tumor cannot be assessed

T0: No evidence of primary tumor

Ta: Noninvasive carcinoma

T1: Tumor invades lamina propria (subepithelial connective tissue)

T2: Tumor invades muscle

- T2a: Tumor invades superficial muscle
- T2b: Tumor invades deep muscle

T3: Tumor invades perivesical tissue

- T3a: Microscopically
- T3b: macroscopically (extravesical mass)

T4: Tumor invades any of the following: prostatic stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall

- T4a: Tumor invades prostate stroma, uterus, vagina
- T4b: Tumor invades pelvic wall, abdominal wall

N stands for Regional lymph nodes

NX: Regional lymph nodes cannot be assessed

N0: No regional lymph node metastasis

N1: Single regional lymph node metastasis in the true pelvis

N2: Multiple regional lymph node metastasis in the true pelvis

N3: Lymph node metastasis to the common iliac lymph node(s)

M stands for Distant Metastasis

M0: No distant metastasis

M1: Distant metastasis

- M1a: Distant metastasis limited to lymph nodes beyond the common iliacs
- M1b: Non–lymph node distant metastases

Another way of staging bladder cancer which is not often used due to its generality and lack of precise and personalized details about patient status, consists of 5 main stages, numbered from stage 0 to stage 4. Stage 0 is the earliest cancer and stage 4 is the most advanced.

Stage 0 - Noninvasive bladder cancer: Cancer cells are inside the tissue lining the inside of the bladder but have not invaded the bladder wall. Stage 0 is divided into two stages, 0a and 0is, based on the type of tumor. Stage 0a is also called noninvasive papillary carcinoma and Stage 0 is also called carcinoma in situ.

Stage I - Non-muscle-invasive bladder cancer: The cancer has spread into the connective tissue, but has not reached the muscle layers of the bladder.

Stage II - Muscle-invasive bladder cancer: At this point, cancer has spread through the connective tissue into the muscle layers of the bladder.

Stage III - Locally advanced bladder cancer: This stage is divided into stages IIIA and IIIB. In stage IIIA, cancer has grown all the way through the bladder muscles and bladder wall into the layer of fat surrounding the bladder and there is a possibility that it has spread to the reproductive but has not yet spread to lymph nodes, or cancer has spread to one lymph node in

the pelvis that is not near the major arteries in the pelvis, and its known as the common iliac arteries. When it comes to stage IIIB, cancer has spread to more than one lymph node in the pelvis, which is not near the common iliac arteries or it has spread to at least one lymph node, that is near the common iliac arteries.

Stage IV - Metastatic bladder cancer: Stage IV is also divided into two stages IVA and IVB. In stage IVA, cancer has spread to the abdominal wall or pelvic wall, or to lymph nodes that are above the major arteries in the pelvis. When it comes to stage IVB, cancer has spread to other parts of the body (lung, bone, liver) (NIH, 2024).

3.3 Clinical Features and Symptoms of Bladder Cancer

A range of symptoms describes bladder cancer, primarily associated with the urinary system. The variation of the clinical presentation depends on the stage and aggressiveness of bladder cancer.

3.3.1 Hematuria (Blood in Urine)

Hematuria means the presence of blood in the urine, and it is a significant symptom, that is often associated with bladder cancer. It is a condition that frequently presents to the emergency department (ED) and there are many possible causes, both benign and life-threatening. Any condition arising in the genitourinary tract anywhere from the glomerulus to the urethral meatus can lead to presence of RBCs in the urine. Macroscopic or gross hematuria is called the type of hematuria, where blood is visibly noticeable in the urine. In macroscopic hematuria urine could be bright red with or without visible clots, or cola-colored. On the other hand, microscopic hematuria can be detected only under the microscope and characterized by the presence of more than 3- 5 red blood cells (RBCs) per high power field (HPF) of spun urine sediment. The incidence of visible (macroscopic) hematuria in children is approximately 1.3 per 1,000, while the prevalence of microscopic hematuria varies between 0.15% and 2%. There is no specific therapy to treat or prevent hematuria, but treating the underlying reason of its existence causes resolution of hematuria (Vedula & Iyengar, 2020; Willis & Tewelde, 2019).

Bladder hematuria is most commonly caused by infections, trauma, or malignancies. Cystitis, can be either infectious or non-infectious, although it is typically due to infections. Hemorrhagic cystitis is a common cause of hematuria, particularly in patients who have undergone radiation therapy or have been exposed to certain chemicals or medications. Distinguishing it from infectious cystitis can be challenging, as gross hematuria is rare in infections but more common in noninfectious cases like radiation-induced cystitis, which also tends to cause significant pain. Radiation-induced hemorrhagic cystitis is most often linked to pelvic radiation and can develop at any point from days to years after treatment. Additionally, bladder trauma, especially from pelvic fractures, can lead to hematuria, necessitating imaging if gross hematuria is present. Painless gross hematuria is a typical presentation in 80-90% of bladder cancer cases, highlighting the importance of follow-up for patients with hematuria to rule out malignancy (Willis & Tewelde, 2019).

3.3.2 Irritative Lower Urinary Tract Symptoms (LUTS)

Lower urinary tract symptoms (LUTS) is a condition that includes various urination problems, such as, sensation of incomplete bladder emptying, excessive urine volume, and sudden, uncontrollable urges to urinate. These symptoms may involve frequent urination, including in the middle of the night, a weak or interrupted urine stream, and involuntary leakage, which can occur during activities like sneezing, coughing, or laughing. Other LUTS include straining to urinate, as well as unintentional dribbling when feeling no urge to urinate or while rushing to the bathroom.

Lower urinary tract symptoms (LUTS) can affect individuals of any gender, but they are more frequently observed in men, and people over age 50. In a follow up study (1996 – 2010) conducted by Jiachen Zhou, among 30.183 men, risk of bladder cancer was 64% higher (relative risk (RR): 1.64, 95% confidence interval (CI): 0.87, 3.08) compared with men who reported no LUTS (Cleveland Clinic, 2024; Zhou et al., 2015).

3.3.3 Symptoms of Advanced Bladder Cancer

If bladder cancer reaches an advanced stage, it means that the tumor has grown and penetrated the bladder lining and surrounding layers of tissue and muscle, and possibly has spread to other parts of the body. Symptoms can include pelvic pain, weight loss or loss of appetite, bone pain, urination problems and pain in the lower back (Zingg & Wallace, 2012).

4. Epidemiology of bladder cancer

4.1 Incidence and Prevalence of Bladder cancer

Bladder cancer is the second most common urogenital malignancy, after prostate cancer. It has moved up from the 10th to the 9th most commonly diagnosed cancer worldwide, with both incidence and mortality rates increasing (see Figure 1) (GLOBOCAN, 2024).

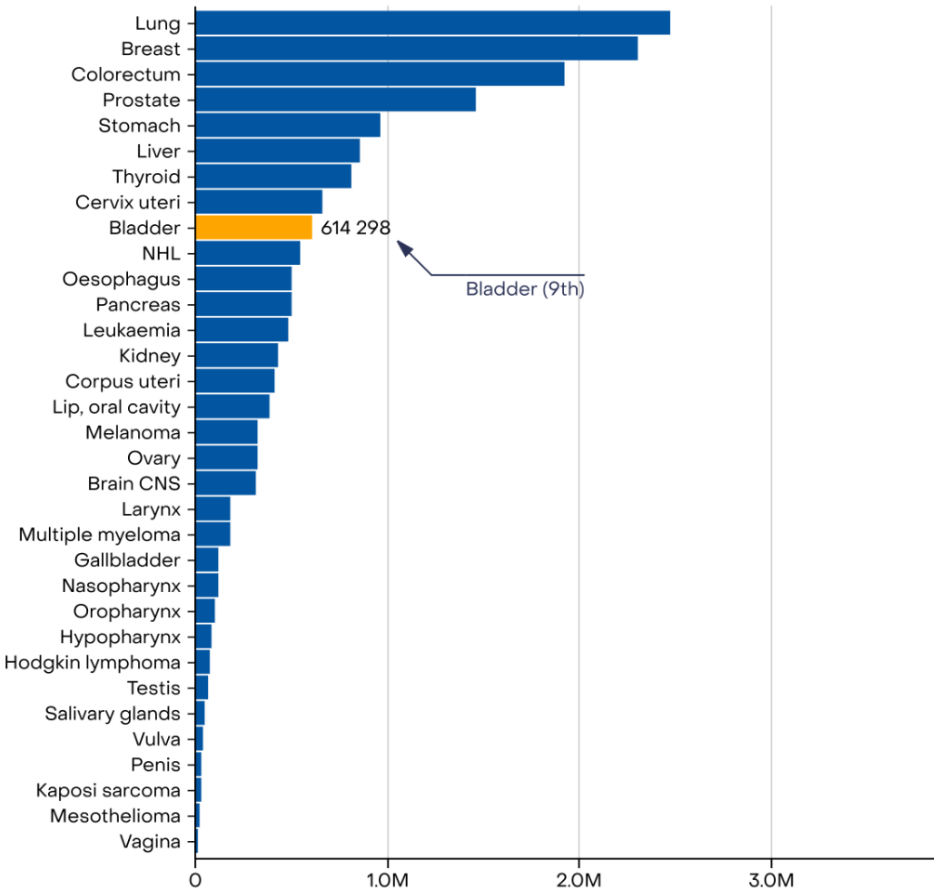

Figure 1: Bladder cancer has moved up to the 9th most diagnosed cancer worldwide Source: GLOBOCAN (2024)

Bladder cancer is the 6th most common cancer in men and the 17th most common cancer in women. There were more than 573,000 new cases of bladder cancer in 2020. Every year, around 600,000 individuals worldwide are diagnosed with bladder cancer, and more than 200,000 people die because of this disease. A higher incidence of bladder cancer occurs as a person ages and it is three to four times higher in men than in women (Figure 2). In the United States in 2023, an estimated 82,290 individuals (62,420 men and 19,870 women) would be diagnosed with bladder cancer from which 16,710 deaths (12,160 men and 4,550 women) from this disease will occur GLOBOCAN (2024).

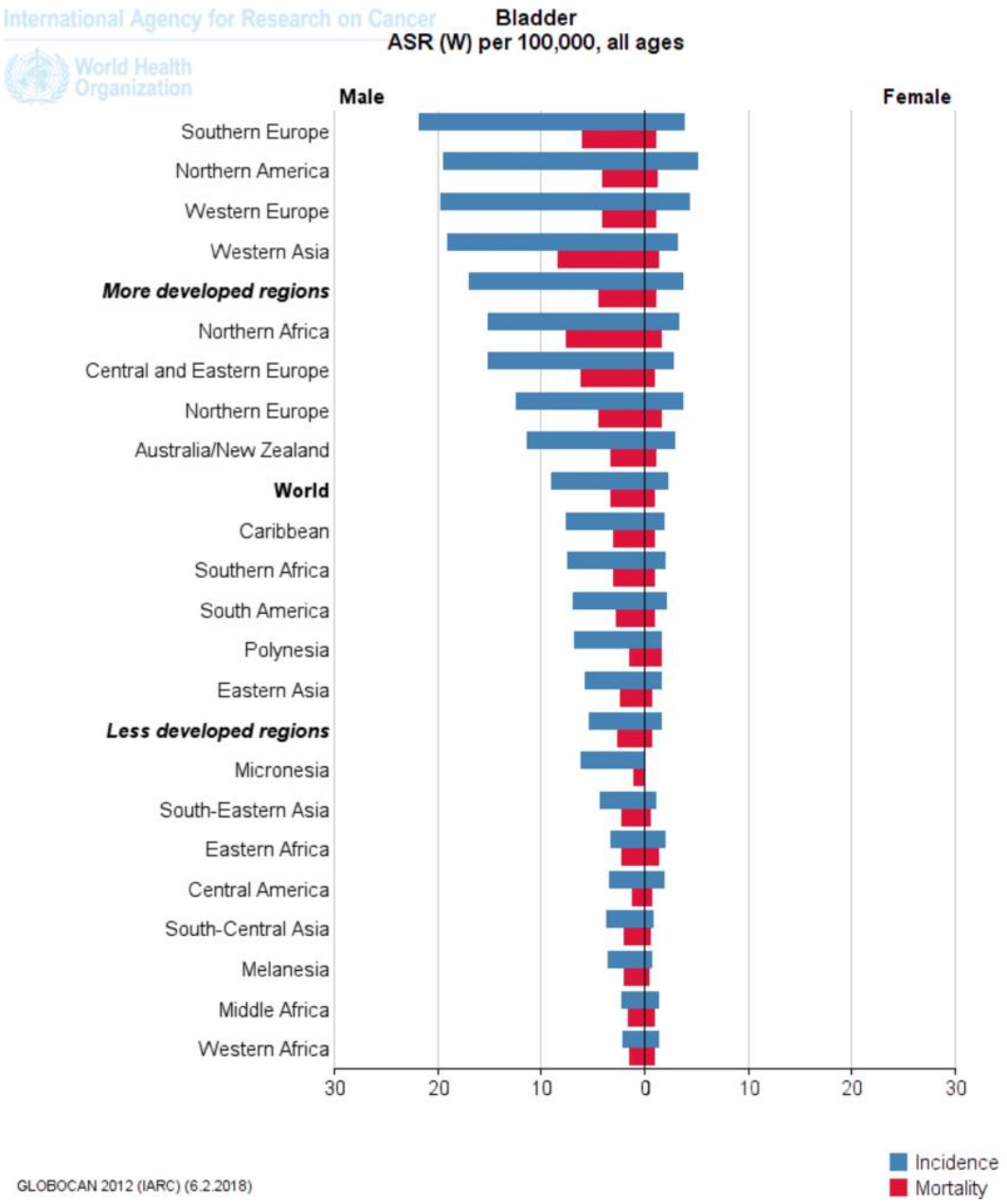

Figure 2*:* Age-standardized incidence (blue) and mortality (red) rates of BC in men (left) and women (right) in different world areas in 2024. Source: GLOBOCAN (2024)

4.2 Risk Factors

Bladder cancer has a complex etiopathogenesis that is influenced by a number of factors, including chemical carcinogens (smoking, occupational exposure to carcinogens), diet (artificial sweeteners, coffee consumption and meat consumption, total fluid intake), previous treatments (pelvic radiation, drug abuse, chronic treatments with analgesics and anti-inflammatory drugs, hormone therapy), and genetic factors (genetic polymorphisms, microRNAs) (Drake, 2007). Sometimes these risk factors can interact synergistically resulting in compounded effects, such as when tobacco smoking is combined with workplace exposure to aromatic amines. Aromatic amines (2-naphthylamine, 4-aminobiphenyl, and benzidine) and 4,4 -methylenebis (2-chloroaniline) in the dye and rubber industries as well as exposure to hair

dyes, house paints, fungicides, tobacco smoke, plastics, metals, and motor vehicle exhaust have all been reported as risk factors that contribute to the increase likelihood of occurrence of bladder cancer in around 10–15% of cases (Czerniak et al., 2016).

Genetic factors play also a significant role in bladder cancer risk, since having a family history of bladder cancer increases the risk of developing the disease, but it is rare for multiple family members to be affected by bladder cancer. Genome-wide association studies (GWAS) have provided insights into the genetic predisposition to bladder cancer, identifying multiple loci on different chromosomes that may influence susceptibility such as 22q13 (rs1014971 in a nongenic region), 19q12 (rs8102137 mapping to CCNE1), 2q37.1 (rs11892031 mapping to UGT1A cluster), 3q289 (mapping to TP63), 4p16.3 (mapping to TMEM129 and TACC3-FGFR3), 8q24.21 (centromeric to MYC), 8q24.3 (mapping to PSCA), 5p15.33 (near TERT-CLPTM1L), 19q12 region (of the CCNE1 gene that encodes cyclin E) and rs7257330 (Czerniak et al., 2016).

4.2.1 Genomic Characterization and Bladder Cancer

The Cancer Genome Atlas Research Network conducted a comprehensive genomic analysis of urothelial bladder cancer, revealing a high mutational burden. High-grade invasive bladder cancers have around 300 exonic mutations, 200 segmental copy number changes, and 20 positional rearrangements. Common alterations involve genes such as CDKN2A, E2F3/SOX4, CCND1, RB1, EGFR, PPARG, PVRL4, and YWHAZ. CDKN2A and TP53, mutations are present in nearly 50% of cases. A significant proportion also shows deletions in CDKN2A, mutations in RB1, and alterations in MLL2. Fusion genes, such as FGFR3-TACC3, are observed in a smaller subset of cases.

It is possible to notice three distinct genomic subtypes of bladder cancer, each with different patterns of genetic alterations, driving the disease in distinct ways:

Group 1: Characterized by **copy number changes** and **focal amplifications**, with a specific enrichment for mutations in the **MLL2** gene, which is involved in chromatin remodeling.

Group 2: Defined by **deletions of CDKN2A** and an enrichment of **FGFR3** mutations, a receptor tyrosine kinase gene associated with bladder cancer.

Group 3: Features predominant mutations in **TP53** and **RB1**, alongside **amplifications of E2F3 and CCNE1**, which are involved in cell cycle regulation and are often seen in more aggressive cancers (Czerniak et al., 2016).

4.2.2 Smoking and Bladder Cancer

Tobacco smoking is the leading cause of urinary bladder cancer in humans, with more than half of the cases in men and a significant portion in women linked to smoking. The risk of developing bladder cancer increases with the intensity and duration of smoking, showing a clear dose-response, linear relationship. In epidemiological studies conducted since the late 1950s, an association between smoking and bladder cancer has already been observed. Tobacco smoke contains harmful chemicals like polycyclic aromatic hydrocarbons and aromatic amines, such as β-naphthylamine, which are known to cause urothelial bladder cancer (UBC). Nicotine can activate nicotinic acetylcholine receptors that promote tumor progression. Cigarette smoke is a source of 4-aminobiphenyl (4-ABP), a well-established human bladder

carcinogen. Smoking various tobacco products, including cigarettes, pipes, cigars, and e-cigarettes, leads to the inhalation of carcinogens that are filtered by the kidneys and come into contact with the bladder. These carcinogens contribute to cancers of the bladder, ureter, and even renal-cell carcinoma (Alouini, 2024).

These carcinogenic compounds cause DNA damage. Studies in mice exposed to polycyclic aromatic hydrocarbons (PAHs) and aldehydes have shown DNA adducts formation in bladder tissue. Aldehydes, in particular, are potent carcinogens that cause DNA damage and impair its repair mechanisms. Smokers have elevated levels of methylated metabolites, such as PAHs and aromatic amines, which contribute to smoking-related bladder cancer. The activation of NNK and PAHs by cytochrome P450 enzymes leads to the formation of bulky DNA adducts. These damaged adducts can result in mutations and disrupt tumor suppressor genes. Bladder cancer tissues from smokers exhibit higher levels of NNK and BaP DNA adducts compared to non-smokers (Alouini, 2024).

A cohort study of 422.010 participants with 30-yr follow-up demonstrated around two- and three-times increased risks of BC with smoking (HR: 2.32, 95% CI: 1.98–2.73 in males and HR: 2.75, 95% CI: 2.07–3.64 in females) (Jacob et al., 2018).

The risk of bladder cancer decreases over time after quitting smoking. In a prospective cohort study of 143,279 postmenopausal women, ex-smokers had a 25% lower risk within the first 10 years of quitting (HR: 0.75, 95% CI: 0.56–0.99), and this risk continued to decrease over time. However, even 30 years after quitting, ex-smokers still had a higher risk of bladder cancer compared to those who never smoked (HR: 1.92, 95% CI: 1.43–2.58) (Li et al., 2019).

Another study of 646,526 participants demonstrated that non-smokers exposed to second-hand smoke throughout their lifetime had a 22% higher risk of developing bladder cancer compared to non-smokers who were not exposed (RR: 1.22, 95% CI: 1.06–1.40) (Yan et al., 2018).

A dose-response meta-analysis between cigarette smoking and risk of bladder cancer demonstrated a positive non-linear dose–response relationship between all smoking intensity, pack-years of smoking, smoking duration (years) and the risk of bladder cancer, but the plateau only occurred when smoking intensity reached 20 cigarettes/day (Zhao et al., 2022).

4.2.2.1 Smoking products other than cigarettes

The latest scientific data and issues related to alternative smoking methods are under scrutiny. Smoking products other than cigarettes, such as cigars and e-cigarettes, still pose significant health risks. E-cigarettes, though marketed as a less harmful alternative, contain nicotine and various harmful chemicals that can lead to lung damage and potential long-term risks (including bladder cancer) that are still being studied (NIH, 2024).

4.2.3 Dietary factors

It is biologically reasonable to consider that dietary factors could affect bladder cancer risk, given that both beneficial and harmful dietary substances are excreted through the urinary system, coming into direct contact with the bladder's epithelium. However, research examining the link between diet and bladder cancer has produced non-conclusive findings.

4.2.3.1 Intake of macronutrients and risk of bladder cancer

Multiple case-control studies demonstrate that diets rich in meat or fats are linked to an increased risk of bladder cancer (BC). However, these studies often lack detailed dietary data, making it challenging to analyze the actual impact of different types of fats or sources of protein on BC risk. Addressing these gaps, a more detailed study revealed that a 3% rise in energy intake from animal protein was associated with a 15% increase in BC risk, whereas a 2% increase in energy from plant protein intake was linked to a 23% reduction in BC risk (Allen NE, 2013). Although some case-control studies have linked higher red meat consumption with an increased risk of bladder cancer (BC), a meta-analysis involving 1,558,848 participants found no such association. However, it did suggest that a high intake of processed meats was associated with an elevated risk of BC, particularly in the United States (Li et al., 2014; Lin et al., 2012).

4.2.3.2 Consumption of fruits and vegetables and the risk of bladder cancer

Despite numerous studies examining the relationship between fruit and vegetable intake and bladder cancer (BC) risk, the findings have been inconsistent, particularly regarding the specific types of fruits and vegetables consumed. A prospective cohort study focusing on male smokers found no significant link between the consumption of fruits and vegetables, specific types of fruits (like berries), or groups of vegetables (such as cruciferous types) and the risk of bladder cancer. Additionally, the study observed that intake of various nutrients, including alpha-carotene, beta-carotene, lycopene, lutein/zeaxanthin, beta-cryptoxanthin, as well as vitamins A, E, C, and folate, did not influence BC risk (Michaud et al., 2002). Another prospective cohort study conducted among Swedish men and women found no significant association between the intake of total fruits and vegetables, including specific categories like citrus fruits, cruciferous vegetables, and leafy greens, and the risk of bladder cancer (Larsson et al., 2008). Although the results from the studies mentioned above, another comprehensive study combined of 11 case-control studies, comprising 5,637 BC cases and 10,504 controls provides compelling evidence that the consumption of fruits overall, citrus fruits, pome fruits and tropical fruits reduces the BC risk. The pooled analysis of the study finds that higher total fruit intake is associated with a reduction in BC risk. Also, examining the results of the total vegetable consumption, they noticed, that in men the highest intakes of total vegetables were associated with a decreased BC risk with no considerable heterogeneity (OR 0.80; 95% CI 0.71–0.88, I^2 =1.0%). They noted similar results for participants ≥ 60 years (OR 0.81; 95% CI 0.71–0.91, I^2 = 0.0%), while greater intakes of total vegetables among participants < 60 years were significantly associated with a decreased BC risk (OR 0.70; 95% CI 0.52-0.88) (Boot et al., 2024).

4.2.3.3 Intake of micronutrients (Vitamin A, Carotenoids, Retinoids, Selenium)

The therapeutic efficacy of retinoids, as well as vitamin A, retinol, and carotenoid supplements for preventing incident or recurrent bladder cancer remains unproven. When it comes to selenium, a substantial body of epidemiological research suggests an inverse relationship between selenium levels and bladder cancer risk. Multiple studies have reported a decreased risk associated with higher selenium concentrations. For instance, a case-control study found a 33% reduction in cancer risk among individuals with the highest selenium levels in toenail

clippings compared to those with the lowest levels (rate ratio (RR): 0.67, 95% CI: 0.46–0.97). Similarly, a Belgian cohort study showed a 70% lower risk of bladder cancer for participants in the highest tertile of serum selenium levels versus the lowest tertile (RR: 0.30, 95% CI: 0.17–0.52, with a significant p-value of ≤ 0.001) (Silberstein & Parsons, 2010).

4.2.3.4 Mediterranean diet and bladder cancer

The Mediterranean diet emphasizes reducing the intake of red meat and processed foods. Widely recognized as a healthier dietary option, it has received significant scientific support for offering a range of health benefits. The Mediterranean diet is typically defined by a high intake of fruits, vegetables, legumes, and cereals, along with moderate-to-high consumption of fish. It includes moderate alcohol intake, mainly in the form of wine, low-to-moderate dairy intake, and minimal consumption of meat and processed meat products. A study conducted by Bravi et al. (2018), on an Italian population reported that higher adherence to the Mediterranean diet was related to a lower risk of bladder cancer (OR=0.72; 95%CI: 0.54–0.98)

4.2.4 Occupation

It is estimated that occupational exposures may be responsible for up to 20% of all bladder cancer cases. The specific chemicals β-naphthylamine, 4-aminobiphenyl (ABP), and benzidine have been linked to bladder cancer, affecting workers in the textile dye and rubber tire industries. Due to strict regulations, these chemicals are now banned from workplaces and as a result they have a minimal impact on the current bladder cancer rates in Western countries. However, other potential bladder carcinogens, like orthotoluidine, remain in use today, particularly in the production of dyes, rubber chemicals, pharmaceuticals, and pesticides (Kirkali et al., 2005).

4.2.5 Medical History

The patient's medical history may reveal risk factors linked to bladder cancer. Chronic urinary tract infections (UTIs), especially in patients with spinal cord injuries, can lead to invasive squamous cell carcinoma due to nitrite and nitrosamine formation by bacteria or inflammation that promotes cell. Cyclophosphamide, an alkylating agent used in cancer treatment, increases the risk of urothelial carcinoma in a clear dose-dependent manner, potentially due to its toxic metabolites like acrolein. Radiotherapy, particularly in women treated for ovarian cancer, also raises bladder cancer risk, especially when combined with certain chemotherapies. Additionally, squamous cell carcinoma is associated with Schistosoma haematobium infection, as shown in studies correlating its prevalence with bladder cancer rates in endemic regions (Kirkali et al., 2005).

5.Diagnosis of Bladder Cancer

5.1 Importance of early diagnosis and challenges in early detection

Early diagnosis of bladder cancer is crucial for improving patient outcomes, as it significantly increases the chances of successful treatment, providing more treatment options and reduces the risk of disease progression. Detecting bladder cancer in its initial stages, especially when

tumors are non-muscle-invasive, provides the opportunity for less aggressive therapies and better prognosis. However, early diagnosis is challenging due to the often-intermittent nature of initial symptoms, like hematuria or irritative urinary symptoms, which can be mistaken for benign conditions. Additionally, limitations in diagnostic tools can further complicate timely detection, highlighting the need for increasing awareness and improved diagnostic methods.

5.2 Diagnostic Methods of Bladder Cancer

Every clinical diagnostic technique has advantages and disadvantages, therefore finding a bladder cancer detection and diagnosis approach that is marked by high sensitivity, high specificity, low cost, non-invasive nature, ease of use, and good reproducibility becomes of utmost importance. Both sensitivity and specificity are used to portray the results of diagnoses in the medical diagnosis field. Sensitivity is the percentage of persons with the disease who are correctly identified by the test. Specificity is the percentage of persons without the disease who are correctly excluded by the test. Patients might consequently benefit from additional reference information when sensitivity and specificity are used to compare several diagnostic techniques (Zhu et al., 2019).

5.2.1 Cystoscopic Examination

White light cystoscopy (WLC) is the standard diagnostic tool used in the initial diagnosis of BCa in a clinical setting with a flexible cystoscope. Its sensitivity for detecting papillary bladder tumors ranges between 62% and 84%, while specificity varies from 43% to 98%. However, WLC is less effective in identifying small papillary lesions and carcinoma in situ (CIS). Most abnormal findings seen during office cystoscopy require further resection with a rigid cystoscope in the operating room to assess histology and tumor invasion depth. Both flexible and rigid WLC are operator-dependent, with limited sensitivity for small and flat lesions and challenges in accurately defining margins of resection (Ahmadi et al., 2021).

5.2.2 Enhanced Cystoscopy Technologies

Plenty of enhanced imaging technologies have been introduced in recent years, and they are generally categorized into 3 categories: (1) macroscopic technologies such as blue light cystoscopy (BLC) and narrow band imaging (NBI), (2) microscopic imaging technologies such as optical coherence tomography and confocal laser endomicroscopy, and (3) molecular imaging in which fluorescently labeled binding agents such as antibodies, peptides, or small molecules are being captured using macroscopic enhanced imaging technologies (Ahmadi et al., 2021).

5.2.3 Urine Cytology, Urine-based Tumor and Blood-based Tumor Markers

Cytology remains the most common adjunct procedure to cystoscopy for detection of high-grade (HG) bladder cancer, including CIS and HG upper tract urothelial cancer, because of its exceptionally high specificity, but it has low sensitivity when it comes to detection of BCa, ranging from 12% for low-grade to 64% for HG tumors. US Food and Drug Administration (FDA) approved urine-based tumor markers available, such as RNA, proteins, tumor-related DNA methylation changes, or cellular markers, in order to overcome the drawbacks of cytology. Some of these markers are BRA stat, BTA TRAK, Cxbladder, uCyst, UroVysion,

NMP22 ELISA and NMP22 BladderCheck (Ahmadi et al., 2021). While urine-based tests (e.g., NMP22, UroVysion) are more commonly used, blood biomarkers are also gaining attention for their potential in early detection, prognosis, and treatment monitoring.

5.2.4 Imaging Techniques

Imaging techniques play an essential role in the diagnosis and staging of bladder cancer. Computed tomography (CT) urogram has completely replaced intravenous urography, and not only provides the opportunity of assessment of renal parenchyma and upper tract urothelium, but it can also evaluate other genitourinary conditions that may cause hematuria, such as urolithiasis and renal masses. Multiparametric MRI is emerging as a promising tool for bladder BCa staging due to its non-use of ionizing radiation, excellent soft tissue contrast, and ability to capture images in multiple planes. Although early studies show MRI can effectively differentiate between organ-confined and locally advanced BCa, it struggles with detecting nodal disease and has inconsistent interobserver reliability. To improve consistency, the VI-RADS protocol was introduced in 2018, using T2-weighted, diffusion-weighted, and dynamic contrast-enhanced imaging. This system scores tumors from 1 to 5, helping to distinguish between non-muscle-invasive and muscle-invasive BCa (Ahmadi et al., 2021).

5.2.5 Radiomics

Recent years have seen growing interest in using radiomics for bladder cancer evaluation and treatment. This approach involves extracting quantitative data from clinical imaging (like CT and MRI) and analyzing it with artificial intelligence techniques. Radiomics has been applied to diagnose, grade, classify subtypes, assess treatment response, and predict outcomes, as well as in multiomic analysis to link imaging features with molecular data. While currently limited to retrospective, single-center studies, ongoing research aims to refine this method for broader clinical use (Feretzakis et al., 2024).

5.2.6 Transurethral resection of bladder tumor (TURBT)

Transurethral resection of bladder tumor (TURBT) is the primary procedure used for diagnosing and staging BCa. It can be performed under local, spinal, or general anesthesia, depending on factors like the patient's preoperative risk and the tumor’s size and location. The procedure typically involves using a loop resectoscope to remove most tumors, especially during initial diagnosis (Cleveland Clinic, 2024).

6.Evidence Based Medicine in the Present Study about Diagnostic Research for Bladder Cancer

Bladder cancer continues to present significant challenges in early detection. Recent advancements in diagnostic tools are improving disease management. Innovations like liquid biopsy, which analyzes urine or blood samples, along with RNA and protein biomarkers, are

enabling more non-invasive and precise detection methods. Additionally, genomic sequencing and imaging technologies are helping to identify distinct molecular signatures and subtypes of bladder cancer, allowing for better predictions of disease progression and treatment responses. These novel approaches aim not only to detect bladder cancer at earlier, more treatable stages but also to personalize treatment strategies based on the tumor's molecular characteristics, ultimately improving patient outcomes and survival rates.

6.1 The Role of Diagnostic Studies in Evidence-Based Medicine

Evidence-based medicine (EBM) relies on high-quality research to guide clinical decision-making, particularly in the diagnosis and prognosis of bladder cancer. The development and validation of novel diagnostic tools require robust study designs that ensure accuracy, reliability, and clinical applicability. Various types of diagnostic studies have contributed to advancements in bladder cancer detection, risk stratification, and treatment optimization.

6.3 Key Diagnostic Studies in Bladder Cancer

Several landmark studies have contributed to the evolution of bladder cancer diagnostics such as urinary biomarker studies (NMP22, UroVysion FISH, cytokeratins and other urinary biomarkers), blood-based biomarkers (NLR), and different kinds of combination schemes, (methylation of TWIST1 and NID2 genes and mutation of FGFR3 gene and protein expression of matrix metalloproteinase 2).

6.4 Evaluating the Validity of Diagnostic Research

In order to integrate new diagnostic approaches into clinical practice, their validity and reliability must be carefully evaluated: A) Sensitivity and Specificity: A diagnostic test should be able to balance high sensitivity and high specificity in order to manage to minimize false results, B) Reproducibility and External Validation: Findings gathering from a single study must be validated in independent cohorts to ensure generalizability. Multi-center trials are particularly valuable for confirming results across diverse populations, C) Clinical Utility and Cost-Effectiveness: Beyond statistical performance, a diagnostic tool must demonstrate practical benefits, such as improving early detection, guiding treatment decisions, or reducing healthcare costs.

6.5 Integrating Novel Diagnostics into Evidence-Based Clinical Practice

The implementation of novel diagnostic tools in bladder cancer management requires a structured approach based on EBM principles. Guidelines from organizations such as the European Association of Urology (EAU) and the American Urological Association (AUA) incorporate findings from high-quality studies into clinical practice. However, challenges such as regulatory approval, accessibility, and physician adoption remain barriers to widespread clinical use.

As of now, there appears to be no published systematic review of systematic reviews specifically focusing on trends in bladder cancer diagnosis. However, several recent systematic reviews, gathering primary studies have explored various aspects of bladder cancer diagnosis.

Conducting a systematic review of systematic reviews on novel diagnostics in bladder cancer is essential, despite these existing reviews, as this research area is rapidly evolving. Given the significant health burden of bladder cancer worldwide, these innovations provide hope for improved survival rates and quality of life for patients. However, as advancements continue to unfold, it is critical to reconduct systematic reviews periodically to incorporate new findings and ensure that diagnostic methods evolve with the latest research, ultimately improving clinical outcomes.

Thus, it would be useful to conduct an overeview of systematic reviews that could synthesize and evaluate the latest advancements in bladder cancer diagnosis.

7 Materials and Methods

The objective of this overview of systematic reviews is to evaluate the most recent developments in bladder cancer diagnostic instruments, with a particular focus on biomarkers. This section outlines the methods to systematically assess the diagnostic accuracy and of new biomarkers in bladder cancer, identify gaps in the current research, and suggest areas for future investigation.

7.1 Study Design

This study is an overview of systematic reviews. The purpose was to identify relevant articles on bladder cancer diagnosis novel biomarkers published from 2012 to 2023. The search encompassed one database: PubMed.

7.2 Information sources and search strategy

To compile our list, with index novel biomarkers, we followed a structured process. A comprehensive search was conducted in PubMed using the algorithm: *"systematic review" AND "bladder cancer" AND ("diagnosis" OR "surveillance" OR "prognosis")* using a publication date limit (2012-2023). We screened the remaining citations and further considered those where, at the title or abstract level, biomarkers were assessed and the terms novel or emerging or promising were used. Whenever the abstract was not informative, the full text was scrutinized. From the final list of systematic reviews, we extracted the names of the biomarkers that were characterized as novel or emerging and created a pertinent list.

Since most of the selected studies did not focus exclusively on a single biomarker but rather examined a variety of biomarkers with different specimens, population sizes, and primary studies, it was necessary to systematically assess all biomarkers from this pertinent list. The aim was to retain only those that met all predefined criteria, including the requirement that the specimen should not be tissue-based, the primary studies should not be older than 2012, and the sample size of every biomarker as confirmed in their primary studies, which were demonstrated in systematic reviews, should be at least 50 participants.

7.3 Study selection

The study selection process was managed using the online systematic review software Rayyan (https://www.rayyan.ai/) and Abstrackr (https://www.brown.edu/public-health/cesh/resources/software). These tools facilitated the organization and screening of articles retrieved from the bibliographic databases.

7.4 Eligibility criteria

The inclusion and exclusion criteria for the systematic review are presented in this subsection.

7.4.1 Types of Studies

In this systematic review, we considered systematic reviews and meta-analyses that included cohort studies, case-control studies, cross-sectional studies, randomized controlled trials (RCTs) that evaluated novel diagnostic biomarkers for bladder cancer. The studies could span various biomarkers, aimed at improving diagnostic accuracy. We included studies where a diagnostic performance estimate was reported (i.e. sensitivity and specificity). We only included studies conducted with human participants assessing clinical outcomes, with a cumulative sample size of more than 50 participants, written in English, and those published from 2012 until 2023. Studies assessing human tissue or human cell lines were excluded. This comprehensive inclusion of studies allows for an in-depth evaluation of emerging methods and their potential to enhance the clinical management of bladder cancer.

7.4.2 Study Population

We included adult bladder cancer patients (≥ 18 years old), spanning various disease stages and subtypes, encompassing both non-muscle invasive (NMIBC) and muscle invasive bladder cancer (MIBC). Studies involving patients younger than 18 years old were excluded. Studies involving patients with carcinoma in situ (CIS) were also considered.

7.5 Data Collection and Analysis

Title and abstracts were screened for eligibility, based on predefined inclusion and exclusion criteria. Furthermore, full-text articles were assessed independently by the same reviewers, to confirm their suitability. In order to provide a thorough assessment, the data taken from each research included a number of important variables. Among these factors were:

- Author: The name of the primary author responsible for conducting the study.
- Year of Publication: The year in which the study was published, in order to provide insight into the timeliness and relevance of the research.
- Country of Study: The geographical location where the study was conducted, which could help to synthesize the findings within specific populations or healthcare systems.
- Study Design: The methodological approaches used in this research are randomized controlled trials (RCTs), cohort studies, case-control studies, cross-sectional studies. This information is critical for assessing the strength and validity of the findings.

- Sample Size: The number of participants included in each study, which affects the statistical power and also the generalizability of the results.
- Age of Participants: The age range or mean age of the study population, which could help to synthesize the findings within specific age group.
- Gender Distribution: The proportion of male and female participants in each study, as gender differences can possibly impact the outcomes.
- Number of primary studies: The total amount of primary studies focusing on a biomarker
- Diagnostic Biomarker or Panel of Biomarkers: The specific diagnostic biomarkers being evaluated.
- Outcomes on Diagnostic Studies: Sensitivity and Specificity were used as the key performance metrics that were used to assess the accuracy of the diagnostic test.

8.Results

8.1 Literature search

A total of 993 articles were retrieved from the primary literature search, which was part of a broader project concerning novel diagnostic and prognostic techniques in bladder cancer. A total of 7 duplicate reports were excluded. Accordingly, after screening the titles and abstracts, 688 articles were excluded because they were found to be non-human studies, genetic variation studies, letters, case reports, reviews, commentaries. The remaining articles were viewed in full text. This process led to the exclusion of additional articles that did not meet the inclusion criteria. After a careful review of the potential articles, 17 articles were included in this study and used for data extraction (Aveta et al., 2023; Cui & Zhou, 2021; Ding et al., 2015; Hentschel et al., 2021; Khetrapal et al., 2018; Kutwin et al., 2018; Lozano, 2020; Malinaric et al., 2022; Masuda et al., 2018; Papavasiliou et al., 2023; Sathianathen et al., 2018; Shi et al., 2017; Soputro et al., 2022; Su et al., 2021; Wang et al., 2020; Xiao et al., 2016). The entire literature search process is summarized in Figure 3.

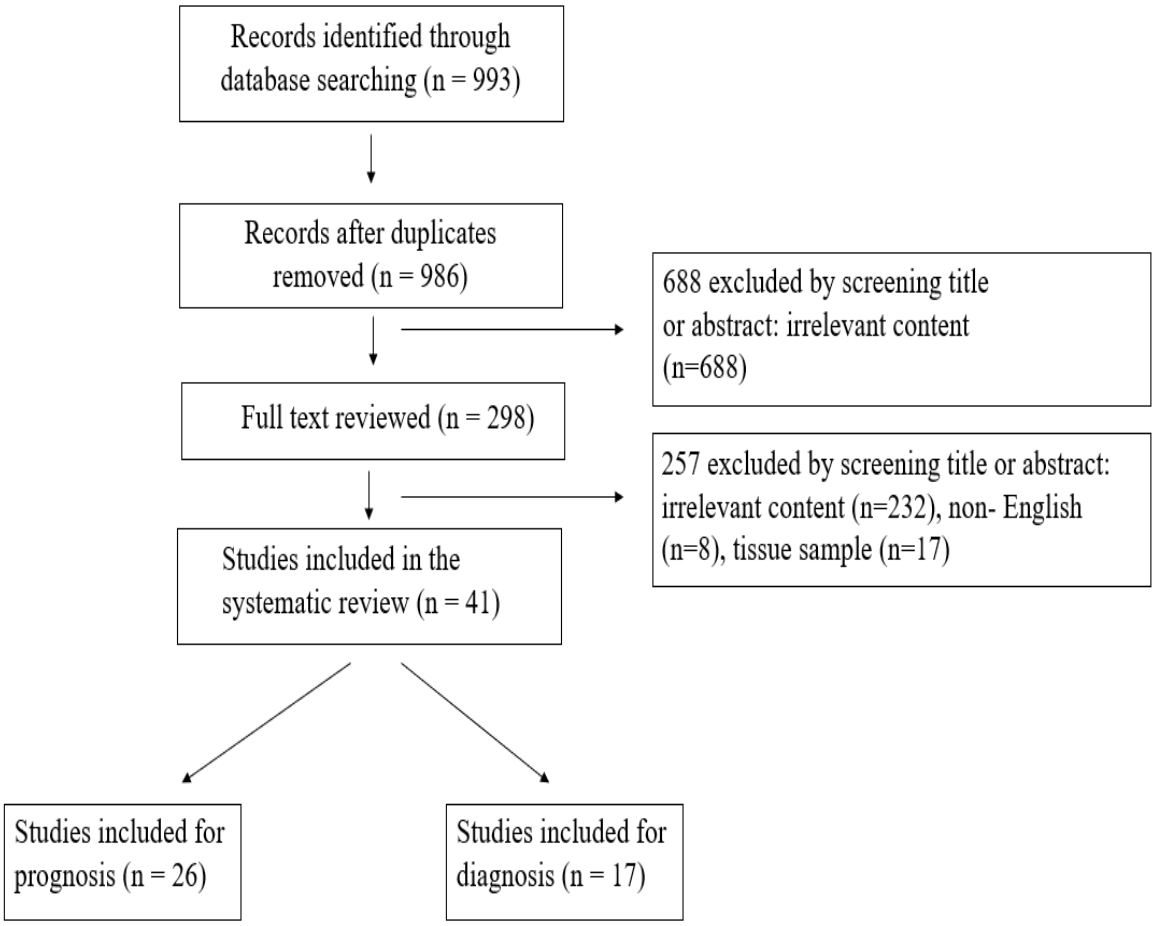

Figure 3: Flowchart for the study selection

The data extracted from the 17 selected studies aimed to identify diagnostic biomarkers (Table 1) for bladder cancer published from 2012 to 2023. All these studies were systematic reviews and/or meta-analysis publications, conducted across multiple countries, including China, Iran, Korea, UK, Canada, Spain, USA, Germany, Egypt, Australasia, Netherlands, France, Denmark, Japan, New Zealand, Sweden and Pakistan.

Of the 17 included articles identifying novel diagnostic biomarkers for bladder cancer, n=8 were conducted in China, n=3 in Iran, n=2 in Korea, n=4 in UK, n=3 in Canada, n=4 in Spain, n=2 in USA, n=3 in Germany, n=4 in Egypt, n=2 in Australasia, n=3 in Netherlands, n=1 in France, n=2 in Denmark, n=1 in Japan, n=1 in New Zealand, n=1 in Sweden, n=1 in Pakistan, n=1 in India, and n=1 in Belgium. Across eligible studies about bladder cancer diagnosis, the median age was between 61.4 and 69.

Table 1: Characteristics of included studies reporting diagnostics in patients with bladder cancer

Study	Country	Biomarkers
Aveta, 2023	NM	miR-200, miR-21, miR-146a-5p, miR-141-3p, miR-205-5p, miR-20a, miR-21-5p, (miR-6124/miR-4511), (let-7c, miR-135a, miR-135b, miR-148a, miR-204, miR-345), miR-155, miR-214, (miR-18a, miR-25, miR-140-5p, miR-187, miR-142-3p, miR-204), miR-145, miR-200a
Hentschel, 2021	NM	TERT, (AKT1/ARID1A/BRAF/ CDKN1A/CDKN2A/EP300/ ERBB2/ERBB3/FBXW7/ FGFR3/KDM6A/KRAS/ MED12/PIK3CA/ PLEKHS1/RB1/STAG2/ TERT/TP53/TSC1), (ARID1A/CDKN2A/CREBBP/ ERBB2/ERBB3/FGFR1/ FGFR3/HRAS/KTM2D/ NF1/PIK3CA/STAG2/ TP53/TSC1), (CDKN2A/ERBB2/FGFR3/ HRAS/KRAS/MET/MLL/ PIK3CA/TP53/VHL), (FGFR3/HRAS/TERT), (FGFR3/TERT), (FGFR3/HRAS/PIK3CA/ RXRA/TERT/TP53)
Cui, 2021	China, Iran, Korea	miR-200a, miR-145, (miR-99a, miR-125b), miR-99a, miR-125b, miR-106b, miR-214, miR-155, (miR-34b and miR-10b), (miR-141, miR-34b, miR-10b and miR-103), (miR-6124/miR-4511), miR-21-5p, miR-141-3p, miR-205-5p, miR-192
Xiao, 2016	China, Japan, USA, Egypt, Germany, Spain, Canada, Korea, UK	(miR-135b, -15b, -1224-3p), miR-15a, miR-15b, miR-21, miR-23b, miR-24-1, miR-27b, miR-133b, miR-135b, miR-203, miR-211, miR-212, miR-328, miR-1224, miR-145, miR-200a, (miR-125b, miR-126), (miR-187, -18a, -25, -142-3p, -140-5p, -204), miR-26b-5p , miR-520e, miR-618, miR-1255b-5p, miR-106b, miR-99a, miR-125b, (miR-99a, miR-125b), (miR-152, -148b-3p, -3187-3p,-15b-5p, -27a-3p, -30a-5p), miR-214, miR-210, (miR- 18a, let 7a), miR-519a, (miR-497, -663b)
Malinaric, 2022	NM	Cytokeratins 8 and 18 (UBC rapid test), Cytokeratin 20, NMP22, SPARC, Orosomucoid-1, APE1/REF1, Soluble FAS, AURKA, Serum Irisin, AIB1, EIF5A2, (AIB1, EIF5A2, NPM22),

		LASP1, Unphosphorylated TF, Phosphorylated TF-pSer258, Phosphorylated TF-pSer253, (Multiplex immunoassay—A1AT, APOE, ANG, CA9, IL8, MMP9, MMP10, PAI1, SDC1, VEGFA), TERT promoter mutation, (TERT, FGFR3, KRAS), (TERT and PLEKHS1 promoters), (TERT, FGFR3, OTX1), (FEGFR3, Cyclin D3), (FGFR3, TP53, PIK3CA, ARID1A, STAG2, KTM2D), MCM5 overexpression, (Microsatellite instability (MIS) or Loss of heterozigosity (LOH) of D16S476, D9S171, FGA, ACTBP2), Urine-derived fc-DNA, Urine exosomes, Serum exosomes, (5 mRNA mutations panel: MDK, HOXA13, CDC2, IGFB5, CXCR2 (Cxbladder)), (5 mRNAs: CRH, IGF2, UPK1B, ANXA10, ABL1 (Xpert Bladder)), IQGAP3, N-Myc, (miRNA 130 family (-130a-3p, -130b-3p, -301a-3p)), miRNA 192, (miRNA 192, 2D ultrasound), (miR-31-5p, -93-5p), (miR-7-5p, -22-3p, -29a-3p, -126-5p, -200a-3p, -375, -423-5p), (miR -16, -21, -34a, -99a, -106b, -126, -129, -133a, -145, -200c, -205, -218, -221/222, -331), (miRNA urinary supernatant: -125b, -30b, -204, -99a, -532-3p), (miR-6087, -6724, -3960, -1343-5p, -1185-1-3p, -6831-5p, -4695-5p), (miR-140-5p, -142-5p, -199a-3p, -93, -652, -20a, -106b, -1305, -223, -18a, -191, -126, -26b, -26a, -145, -146a, -30a-3p, -96, -573, -221, -182,-142-3p, -19b, -224, -181a, -766, -146b-5p, -429, -200a, -200c, -20b, -324-3p, -19a, -106a, -143, -99b, -140-3p, -491-5p, -151-3p, -671-3, -222, -339-3p, -141, -200b, -7b, -21), (miR-652, -199a-3p, -140-5p, -93, -142-5p, -1305, -30a, -224, -96, -766), (DNA methylation test of 15 (unpublished) genes (Bladder EpiCheck)), (Methylation of tumor suppressor genes (p14ARF, p16INK4A, DAPK, RASSF1A, APC)), DNA hypermethylation of 150 loci panel (UroMark), (Hypermethylation of OTX1, ONECUT2, TWIST1, SEPTIN9, PCDH17, POU4F2, HS3ST2, SLIT2, FGFR3, CFTR, SALL3, GHSR, MAL, mutation of HRAS, TERT, FGFR3), Urinary lncRNA uc004cox.4, (3 lncRNAs panel: PCAT-1, UBC1 and SNHG16), (3 lncRNAs panel: MALAT1, PCAT1, SPRY4-IT1), (3 lncRNAs panel: MALAT1, MEG, SNHG16), (4 lncRNAs panel: LINC00355, UCA1-201, UCA1-203, MALAT1), (2 genes transcriptomic alterations: IGF2, MAGEA3), (Dimethyl amine, malonate, glutamine, lactate, histidine and valine metabolites panel), (Phosphatidylinositol, nucleic acids, collagen, aromatic amino acids, cholesterol fatty acids, glycogen, monosaccarides and carotenoids' changes (Rametrix)), (Bladder wash

		(>resectisol, urea, creatinine, uric acid, different types of cells, cylinders, crystals) analyzed by FTIR), (6 ions panel (>imidazoleacetic acid)), (116 peptides panel (>collagen fragments, APO-I peptides, basement-membrane specific heparan proteoglycan fragments)), Concentration matrices, (miRNAs: -19b1-5p, 21-5p, 136-3p, -139-5p, 210-3p combined with BLCA-4, NMP22, APE1/Ref1, CRK, VIM, and creatinine urinary concentrations), (miRNA-663, VIM hypermethylation), (Altered methylation of CFTR, SALL3, TWIST 1, NID2, TWIST1 in adjunct to Cytology), (Altered methylation of SALL3,ONECUT2, CCNA1,BCL2, EOMES, VIMcombined with altered mutations of TERT, FGFR3), (3 mRNAs: KLHDC7B, CASP14 and PRSS1; lncRNAs: MIR205HG and GAS5), (MMP-2, MMP-9 and TIMP-2 urinary and serum proteins' and genes' expression levels), (Cytology and CK20 immunostaining), (Cytology with CK20 and p53 immunostaining), (Cytology enriched with p53, ki67 coloration), (Cytology combined with AMARC coloration), (Cytology combined with p16/ki-67 dual-labeling), (p53, MCM5, MCM2, ki-67 coloration in adjunct to cytology)
Su, 2021	Iran, China	UCA1, PTENP1, MALAT1, PCAT-1, SPRY4-IT1, (MALAT1+PCAT-1+SPRY4-IT1), H19, UCA1-201, UCA1-203, (PCAT-1+UBC1+SNHG16), ANRIL, MIR205HG, GAS5, (MIR205HG + GAS5), ELNAT1
Khetrapal, 2018	NM	miR-210, (miR-497, -663b), miR-26b-5p, hsa-miR-144 5p, hsa-miR-374-5p, (miR-152, -148b-3p, -3187-3p,-15b-5p, -27a-3p, -30a-5p), (IGFBP7, SNX16, CSPG6, CTSD, CHD2, NELL2, TNFRSF7), S100A4 gene, S100A6 gene, S100A7 gene, S100A8 gene, S100A9 gene, S100A11 gene, ACTB-106, (Hypermethylation TIMP3, APC, RARB, TIG1, GSTP1, p14, p16, PTGS2 and RASSF1A)
Kutwin, 2018	NM	miR-106b, miR-125, miR-99a, miR-145, miR-155, miR-214, miR-125b, (miR-99a, miR-125b), (miR-26-a/93/191/940), (miR-135b, -15b, -1224-3p), miR-15a, miR-15b, miR-27b, miR-100, miR-135b, miR-203, miR-212, (hsa-miR-652, hsa-miR-199a-3p, hsa-miR-140-5p, hsa-miR-93, hsa-miR-142-5p, has-miR-1305, hsa-miR-30a, hsa-miR-224, hsa-miR-96, hsa-miR-766, hsa-miR-223, hsa-miR-99b, hsa-miR-140-3p, hsa-let-7b , hsa-miR-141 ,

		hsa-miR-191, hsa-miR-146b-5p, hsa-miR-491-5p, hsa-miR-339-3p, hsa-miR-200c, hsa-miR-106b, hsa-miR-143, hsa-miR-429, hsa-miR-222, hsa-miR-200a), (miR-137/124-2/124-3/9-3), (miR-187, -18a, -25, -142-3p, -140-5p, -204), miR-520e, miR-618, miR-1255b-5p
Masuda, 2018	NM	(ANG, APOE, A1AT, CA9, IL8, MMP9, MMP10, PAI1, SDC1, and VEGF)
Sathianathen, 2018	Australasia, Netherlands	CxBladder, AssureMDx
Soputro, 2022	NM	CxBladder, AssureMDx
Ding, 2015	UK, Canada, China, Spain, Egypt	(miR-135b, -15b, -1224-3p), miR-200a, (miR-187, -18a, -25, -142-3p, -140-5p, -204), miR-520e, mir-618, miR-1255b-5p, miR-106b, (miR-210, -10b, -29c)
Wang, 2020	China, Iran, Israel	MALAT1, H19
Papavasiliou, 2023	Denmark, Germany, Australasia, The Netherlands, UK, China, Egypt, Pakistan, Sweden, Spain, New Zealand	(ADXBLADDER/Mcm5), (Base model (age and grade of haematuria) + UroVysion + Cytology), (Base model (age and grade of haematuria) + UroVysion + uCyt+), (Base model (age and grade of haematuria) + UroVysion), (Base model (age, gender, smoker, race, haematuria) + UroVysion + uCyt+ (+) Cytology), BCL2-gene methylation, (CCNA1 + ONECUT2 + BCL2 + EOMES + SALL3 + VIM (methylation genes)), CCNA1-gene methylation, (CxBladder Triage (CxBT) + Imaging), (Extended Model consisting of: Existing model (univariate analysis incl. age, mutation, methylation) + type of haematuria + gender), (FGFR3 + Cytology), (FGFR3, TERT, HRAS and OTX1, ONECUT2, TWIST1, mutation and methylation), (HOXA9 + PCDH17 + POU4F2 + ONECUT2, methylation), HOXA9- gene methylation, (HURP + Cytology), HURP-expression, (MADR Assay (Methylation of TWIST1 and NID2 genes, mutation of FGFR3 gene and protein expression of matrix metalloproteinase 2)), (Mcm5 + NMP-22, expression), (Mutation of TERT and FGFR3 genes plus methylation of CCNA1 and ONECUT2 genes), (Mutation of TERT and FGFR3 genes plus methylation of CCNA1 genes), (Mutation of TERT and FGFR3 genes plus methylation of CCNA1, ONECUT2 and BCL2 genes), (Mutation of TERT and

		FGFR3 genes plus methylation of CCNA1, ONECUT2, BCL2 and EOMES genes), (NMP-22 + Cytology), NMP-52- pr expression, ONECUT2-gene methylation, (Optimal (age + FGFR3, TERT, HRAS, ONECUT2 probes 1 + 4, OTX1 probe 2, TWIST)), (Optimal model consisting of: Existing model (univariate analysis incl. age, mutation, methylation) + type of haematuria), (RAB-B2 + Cytology), (RAB-B2 + HYAL-1 + Cytology), (RAB-B2 + HYAL-1), RAB-B2-gene methylation, SNCG- pr expression, (TERT, FGFR3, SALL3, ONECUT2, CCNA1, BCL2, EOMES, and VIM, mutation n methylation), (uCyt+ (+) NMP-22, expression), (UroVysion + Cytology), (UroVysion + NMP-22 + Cytology), (UroVysion + NMP-22), (UroVysion + uCyt+ (+) Cytology), (UroVysion + uCyt+ (+) NMP-22 + Cytology), (UroVysion + uCyt+ (+) NMP-22), (UroVysion + uCyt+)
Shi, 2017	China, UK, USA, Canada, Spain, Germany	miR-106b, (miR-99a, miR-125b), (miR-152, miR-148b-3p, miR-3187-3p, miR-15b-5p, miR-27a-3p, miR-30a-5p), miR-520e, miR-618, miR-1255b-5p, (miR-25, miR-18a, miR-187 miR-204, miR-142-3p, miR-140-5p), (miR-15a, miR-15b, miR-21, miR-23b, miR-24-1, miR-27b, miR-100, miR-133b, miR-135b, miR-183, miR-203, miR-211, miR-212, miR-328, miR-1224-3p)
Jing Quan, 2018	China, India, Belgium, Egypt	UCA1, MEG3, SNHG16, MALAT1
Lozano, 2020	NM	Xpert Bladder Cancer Monitor (ABL1, CRH, IGF2, ANXA10, UPK1B), (genes methylation EOMES, HOXA9, POU4F2, TWIST1, VIM, ZNF154), (genes methylation APC_a , TERT_a , TER _b ,EDNRB), (genes mutations TERT and FGFR3), (genes methylation TWIST1, NID2), (hyper and hypomethylated genes SOX1, IRAK3, L1-MET), (FGFR3 mutation, TERT mutation and OTX1 methylation), (FGFR3 mutation +DNA methylation HS3ST2, SLIT2 and SEPTIN9), (genes methylation CFTR, SALL3, TWIST1), (15 DNA methylation genes (Epicheck)), (10 genes mutations plus detection of aneuploidy (UroSEEK)), (TERT promoter and FGFR3 mutations (Uromonitor)), 6 miRNA signature(miR16, miR200c, miR205, miR21, miR221 and miR34a), 5 genes mRNA expression (Cx Bladder Monitor)

Notes: Each combination of biomarkers within the brackets consists of a distinct diagnostic scheme.

8.2 Novel Diagnostic Biomarkers that are Extracted from the Enrolled Studies

In Appendix A, all selected novel diagnostics are described. Their total amounts to 220, comprising proteins, genes, lncRNAs, miRNAs, mRNAs, different kinds of metabolites, cell-free circulating DNA, improving cytology methods, and even combinations of these. The sampling methods for these diagnostics, aimed at their analysis, were based on the collection of urine and blood (serum or plasma), or combinations thereof. The most prevalent method was urine collection (128 diagnostics), then follows the combination of blood and urine (75 cases, of which 3 were urine and serum), the sampling via blood (17 cases, of which 12 were serum and 2 were plasma). The total number of primary studies for each biomarker ranged from 1 to 11 studies.

Among these diagnostics, based on their availability in primary studies, the majority of diagnostics in the selected systematic reviews are identified in a single primary study. A total of eight diagnostics stand out for having a greater number of primary studies: CxBladder with 5 primary studies, Bladder EpiCheck with 5 primary studies, Cytokeratins 8 and 18 (UBC rapid test) also with 5 primary studies, Hypermethylation of OTX1, ONECUT2, TWIST1, SEPTIN9, PCDH17, POU4F2, HS3ST2, SLIT2, FGFR3, CFTR, SALL3, GHSR, MAL, mutation of HRAS, TERT, FGFR3 with 6 primary studies, ADXBLADDER, Mcm5 with 7 primary studies, Xpert Bladder with 10 primary studies, and the TERT mutation with 11 primary studies

Continuing, among the novel diagnostics, an miRNA panel, miR-6087, -6724, -3960, -1343-5p, -1185-1-3p, -6831-5p, -4695-5p, stands out due to its sensitivity and specificity, achieving rates of 95%-98% and 87%-91%, respectively (Malinaric et al., 2022). Additionally, the AssureMDx assay demonstrates a sensitivity of 95%-96.7% and a specificity of 82.1%-85% (Sathianathen et al., 2018; Soputro et al., 2022). The MMP-2, MMP-9, TIMP-2 also exhibits a sensitivity of 100% and a specificity of 100% (Malinaric et al., 2022). Furthermore, high sensitivity and specificity, ranging from 91%-95% and 96%-100%, respectively, are also observed in the genomic mutations on TERT and PLEKHS1 promoters (Malinaric et al., 2022). CxBladder also presents interesting data, with sensitivity and specificity rates of 81.3%-97.7% and 61%-85.1%, respectively (Malinaric et al., 2022; Sathianathen et al., 2018; Soputro et al., 2022). Moreover, UroMark stands out due to its sensitivity 96% and specificity 97%. Additionally, the combination panel of metabolites including dimethyl amine, malonate, glutamine, lactate, histidine, and valine exhibits sensitivity of 80.8%-98.1% and specificity of 66.7%-80.3% as also the Bladder wash (>resectisol, urea, creatinine, uric acid, different types of cells, cylinders, crystals) analyzed by FTIR with sensitivity 81.8%–100% and specificity 52.9%–80.9% (Malinaric et al., 2022). High sensitivity, albeit with lower specificity compared to the aforementioned diagnostics, is observed in miR-210 and the miRNA panels miR-497,-663b, miR-135b,-15b,-1224-3p.(Khetrapal,P.,2018) The combination of hypermethylation of OTX1, ONECUT2, TWIST1, SEPTIN9, PCDH17, POU4F2, HS3ST2, SLIT2, FGFR3, CFTR, SALL3, GHSR, MAL, and mutation of HRAS, TERT, FGFR3, also presents interesting potential, due to its sensitivity 93%–98% and specificity 40%–86% (Malinaric et al., 2022).

Another criterion by which certain diagnostics stand out is their prominence in systematic reviews. AssureMDx, MALAT1, and H19 are identified in two selected systematic reviews. Additionally, two separate lncRNA panels, PCAT-1, UBC1, SNHG16 and MALAT1, PCAT1, SPRY4-IT1, also appear in two systematic reviews. The combination of ANG, APOE, A1AT, CA9, IL8, MMP9, MMP10, PAI1, SDC1, VEGF, also appears in two systematic reviews. In the category of availability across two different systematic reviews, there are miRNAs or miRNA

panels, which includes the following: miR-135b, miR-141-3p, miR-15a, miR-15b, miR-203, miR-205-5p, miR-21, miR-210, miR-212, miR-21-5p, miR-26b-5p, miR-27b, miR-6124/miR-4511 and miR-497, -663b. CxBladder appears in three of the eighteen eligible systematic reviews related to diagnostics, along with the following miRNAs or miRNA panels: miR-125b, miR-135b, -15b, -1224-3p, miR-152, -148b-3p, -3187-3p,-15b-5p, -27a-3p, -30a-5p, miR-155, miR-187, -18a, -25, -142-3p, -140-5p, -204, miR-99a. In four systematic reviews, the following miRNAs or miRNA panels: miR-145, miR-618, miR-520e, miR-200a, miR-214, miR-99a, -125b and miR-1255b-5p. Only one miRNA is identified in five out of the eighteen total eligible studies, and that is miR-106b. It should be noted that although a diagnostic may appear in more than one systematic review, in some cases, the primary studies pertaining to it are the same across the mentioned systematic reviews.

Based on the sample size associated with each biomarker, some biomarkers stand out for having the largest populations. The largest sample size is for the TERT mutation, with a total 3,861 participants, followed by Xpert Bladder with 3019 participants. There is the combinations of ADXBLADDER and Mcm5 with 2,980 participants. Next are the FGFR3 and TERT mutation with 1,854. Following this is the combination of Mcm5 + NMP-22 with 1,677 participants. The combination of ANG, APOE, A1AT, CA9, IL8, MMP9, MMP10, PAI1, SDC1, VEGF with 1295. The Bladder EpiCheck with 1274 participants. Also, the NMP22 protein expression with a sample size of 1318. With a sample size of 1,038, we detect the mutation of FGFR3, TERT, and HRAS genes, along with the methylation of OTX1, ONECUT2, and TWIST1 genes. Next is CxBladder with a sample size of 970, miR-212 with 882, and miR-210 with 850. The Urovision method combined with uCyt, Cytology, NMP-22, or their combinations schemes that are presented in appendix - Table3 totals 808, along with the four Base Models referenced in the Table3. Lastly, ERBB2 has a sample size of 793.

From the above table results, we observe how widespread is the use of the gene scheme, which includes the mutation of FGFR3 and TERT, in combination with the methylation of other genes, as shown in the following patterns:

1. FGFR3 mutation, TERT mutation, and OTX1 methylation
2. Mutation of TERT and FGFR3 genes plus methylation of CCNA1, ONECUT2, and BCL2 genes
3. Mutation of TERT and FGFR3 genes plus methylation of CCNA1, ONECUT2, BCL2, and EOMES genes
4. Mutation of TERT and FGFR3 genes plus methylation of CCNA1 and ONECUT2 genes
5. Mutation of TERT and FGFR3 genes plus methylation of CCNA1 genes
6. Altered methylation of SALL3, ONECUT2, CCNA1, BCL2, EOMES, and VIM combined with altered mutations of TERT and FGFR3

8.3 Diagnostics with Higher Diagnostic Performance

We conducted a systematic second-pass evaluation of aforementioned diagnostics from Table 3 for bladder cancer based on criteria of high diagnostic accuracy (sensitivity and specificity, both ≥ 85%) and clinical utility across studies, aiming to recognize the top 15 effective diagnostics across the multiple categories, including biomarkers, and combined methods. The results are organized in Table 4.

Table 2: Top 14 diagnostics

Novel Diagnostic Biomarker	Biomarker Description	Sensitivity	Specificity
A1AT, APOE, ANG, CA9, IL8, MMP9, MMP10, PAI1, SDC1, VEGFA	Multiplex immunoassay, proteins	87–93%	93%
AssureMDx	urine-based test	95%-96.7%	85%-82.1%
CDKN2A, ERBB2, FGFR3, HRAS, KRAS, MET, MLL, PIK3CA, TP53, VHL	mutation markers	88.0%	96.6%
9LINC00355, UCA1-201, UCA1-203, MALAT1	lncRNAs panel	92%	91.7%
miR-152, -148b-3p, -3187-3p,-15b-5p, -27a-3p, -30a-5p	miRNA	90.0%	90.0%
miR-6087, -6724, -3960, -1343-5p, -1185-1-3p, -6831-5p, -4695-5p	miRNAs panel	95–98%	87–91%
miR-99a, miR-125b	miRNA	86.7%-90%	81.1%-90%
miRNA 192, 2D ultrasound	miRNA	93.2%	96.7%
miRNA-130a-3p, -130b-3p, -301a-3p)	miRNA 130 family	87.8%	93.3%
miRNA-663, VIM	miRNA, VIM hypermethylation	92.6%	90%
S100A4	Gene	90.0%	92.0%
S100A8	Gene	85.0%	92.0%
Soluble FAS	Protein	88.03%	89.19%
TERT, PLEKHS1 promoters	genomic mutation	91–95%	96–100%
TF-pSer258	Phosphorylated protein	88.2%	93.3%

8.4 Future Prospects

Examining the results of this study concerning the field of novel diagnostic biomarkers in bladder cancer, which are part of a broader project on novel diagnostic and prognostic biomarkers in bladder cancer, the presence of a dual role for certain biomarkers was observed, contributing both to diagnosis and prognosis of the disease, such as miR-21, miR-20a, miR-200, miR-200a, miR-145, MEG3, as well as and (let-7c-5p, miR-30a-5p, miR-486-5p).

8.5 Limitations in Conducting the Study

In the context of this overview of systematic reviews on novel diagnostics in bladder cancer, there were some limitations encountered during the study's execution. First, the search was conducting only in PubMed, no other search database was used. Second, the heterogeneity of included studies induces challenges in standardizing the methodologies and outcomes, given that different studies employed varying diagnostic markers, techniques, and assessment criteria. Also, another important limitation is the quality of evidence, which in some studies was poor, with small sample sizes. Another limitation is based on the rapidly evolving nature of the field, which means that some emerging technologies or biomarkers might not have been included due to publication timing. Lastly, differences in population demographics and clinical settings among the eligible studies may introduce confounding factors making it harder to draw clear and specific conclusions. The recognition of these limitations is crucial for accurately interpreting the results and for identifying future research directions to enhance the reliability and applicability of novel diagnostic tools in bladder cancer.

9. Conclusions

Bladder cancer is the 9th most common cancer worldwide, and early detection is important to improve survival of BC patients. Although random biopsy guided by cystoscopy is currently the most reliable means of screening for bladder cancer, its invasiveness, inconvenience, and risk of sampling errors have significantly limited its widespread clinical adoption. Urine cytology is a simpler and more specific, though its diagnostic sensitivity is quite low. Recently, there has been growing interest in utilizing blood-based and urine-based biomarkers as potential diagnostics for bladder cancer due to their feasibility. Although those novel biomarkers are urgently needed, and many studies have indicated their potential diagnostic value, there were inconsistencies between studies, about the diagnostic accuracy. For example, genomic mutation on scheme *TERT*, *FGFR3*, and *KRAS* in the Sieverink et al. (2020) study about detecting NMIBC recurrence in BC patients, displayed a sensitivity of 93.1% and a specificity of 85.4%, while in the Batista et al. (2020) study about detecting NMIBC recurrence in BC patients, it displayed a sensitivity of 73.5% and a specificity of 93.2%.

Urological cancers make up a considerable share of all solid tumors, with a high likelihood of local recurrence or metastasis. For instance, nearly 75% of high-risk bladder cancer cases will experience recurrence, progression, or result in death within a decade of the initial diagnosis (Chamie et al., 2013). Thus, apart from their contribution to cancer diagnosis, novel biomarkers can also play a significant role in prognosis providing promising and inexpensive methods that can be used in clinical management and foresee survival outcome in bladder cancer.

Focusing on the eligible biomarkers of the above study, it is noticed that TERT mutation, CxBladder, and AssureMDX present significant interest in the field of bladder cancer diagnosis

as a great potential for improving bladder cancer management, offering more accurate, non-invasive, and personalized diagnostics. Additionally, some biomarkers found in both prognosis and diagnosis could suggest their potential future dual use, serving both as diagnostic and prognostic tools. Overall, the review underscores the growing body of evidence supporting the integration of these biomarkers into clinical practice, though standardization in methodology and further research is necessary to confirm their prognostic and diagnostic accuracy.

References

Ahmadi, H., Duddalwar, V., & Daneshmand, S. (2021). Diagnosis and staging of bladder cancer. *Hematology/Oncology Clinics*, *35*(3), 531-541.

Alouini, S. (2024). Risk factors associated with urothelial bladder cancer. *International Journal of Environmental Research and Public Health*, *21*(7), 954.

Aveta, A., Cilio, S., Contieri, R., Spena, G., Napolitano, L., Manfredi, C., Franco, A., Crocerossa, F., Cerrato, C., & Ferro, M. (2023). Urinary MicroRNAs as biomarkers of urological cancers: a systematic review. *International Journal of Molecular Sciences*, *24*(13), 10846.

Batista, R., Vinagre, N., Meireles, S., Vinagre, J., Prazeres, H., Leão, R., Máximo, V., & Soares, P. (2020). Biomarkers for bladder cancer diagnosis and surveillance: a comprehensive review. *Diagnostics*, *10*(1), 39.

Boot, I. W., Wesselius, A., Jochems, S. H., Yu, E. Y., Bosetti, C., Taborelli, M., Porru, S., Carta, A., Golka, K., & Jiang, X. (2024). Fruits and vegetables intake and bladder cancer risk: a pooled analysis from 11 case–control studies in the BLadder cancer Epidemiology and Nutritional Determinants (BLEND) consortium. *European Journal of Nutrition*, *63*(7), 2477-2498.

Bravi, F., Spei, M.-E., Polesel, J., Di Maso, M., Montella, M., Ferraroni, M., Serraino, D., Libra, M., Negri, E., & La Vecchia, C. (2018). Mediterranean diet and bladder cancer risk in Italy. *Nutrients*, *10*(8), 1061.

Chamie, K., Litwin, M. S., Bassett, J. C., Daskivich, T. J., Lai, J., Hanley, J. M., Konety, B. R., Saigal, C. S., & Project, U. D. i. A. (2013). Recurrence of high-risk bladder cancer: a population-based analysis. *Cancer*, *119*(17), 3219-3227.

Cleveland Clinic. (2024). *Lower urinary tract symptoms*. https://my.clevelandclinic.org/health/symptoms/24248-lower-urinary-tract-symptoms

Crocetto, F., Barone, B., Ferro, M., Busetto, G. M., La Civita, E., Buonerba, C., Di Lorenzo, G., Terracciano, D., & Schalken, J. A. (2022). Liquid biopsy in bladder cancer: State of the art and future perspectives. *Critical Reviews in Oncology/Hematology*, *170*, 103577.

Cui, Y., & Zhou, T. (2021). Diagnostic value of urinary microRNAs for bladder cancer in Asia. *J Coll Physicians Surg Pak*, *31*(10), 1207-1213.

Czerniak, B., Dinney, C., & McConkey, D. (2016). Origins of bladder cancer. *Annual Review of Pathology: Mechanisms of Disease*, *11*(1), 149-174.

Devlies, W., de Jong, J. J., Hofmann, F., Bruins, H. M., Zuiverloon, T. C., Smith, E. J., Yuan, Y., van Rhijn, B. W., Mostafid, H., & Santesso, N. (2024). The diagnostic accuracy of cystoscopy for detecting bladder cancer in adults presenting with haematuria: a systematic review from the European Association of Urology Guidelines Office. *European Urology Focus*, *10*(1), 115-122.

Ding, M., Yi, L., Wang, H., Lv, Y., Liang, J., Wang, J., & Li, C. (2015). Diagnostic value of urinary microRNAs as non-invasive biomarkers for bladder cancer: A meta-analysis. *International Journal of Clinical and Experimental Medicine*, *8*(9), 15432.

Drake, M. J. (2007). The integrative physiology of the bladder. *The Annals of the Royal College of Surgeons of England*, *89*(6), 580-585.

Dyrskjøt, L., Hansel, D. E., Efstathiou, J. A., Knowles, M. A., Galsky, M. D., Teoh, J., & Theodorescu, D. (2023). Bladder cancer. *Nature reviews Disease primers*, *9*(1), 58.

Feretzakis, G., Juliebø-Jones, P., Tsaturyan, A., Sener, T. E., Verykios, V. S., Karapiperis, D., Bellos, T., Katsimperis, S., Angelopoulos, P., & Varkarakis, I. (2024). Emerging trends

in AI and radiomics for bladder, kidney, and prostate cancer: a critical review. *Cancers*, *16*(4), 810.

GLOBOCAN. (2024). *World Bladder Cancer Patient Coalition*. https://worldbladdercancer.org/news_events/globocan-2022-bladder-cancer-is-the-9th-most-commonly-diagnosed-worldwide/

Hentschel, A. E., van der Toom, E. E., Vis, A. N., Ket, J. C., Bosschieter, J., Heymans, M. W., van Moorselaar, R. J. A., Steenbergen, R. D., & Nieuwenhuijzen, J. A. (2021). A systematic review on mutation markers for bladder cancer diagnosis in urine. *BJU international*, *127*(1), 12-27.

Jacob, L., Freyn, M., Kalder, M., Dinas, K., & Kostev, K. (2018). Impact of tobacco smoking on the risk of developing 25 different cancers in the UK: a retrospective study of 422,010 patients followed for up to 30 years. *Oncotarget*, *9*(25), 17420.

Khetrapal, P., Lee, M. W. L., Tan, W. S., Dong, L., de Winter, P., Feber, A., & Kelly, J. D. (2018). The role of circulating tumour cells and nucleic acids in blood for the detection of bladder cancer: A systematic review. *Cancer Treatment Reviews*, *66*, 56-63.

Kirkali, Z., Chan, T., Manoharan, M., Algaba, F., Busch, C., Cheng, L., Kiemeney, L., Kriegmair, M., Montironi, R., & Murphy, W. M. (2005). Bladder cancer: epidemiology, staging and grading, and diagnosis. *Urology*, *66*(6), 4-34.

Kutwin, P., Konecki, T., Borkowska, E. M., Traczyk-Borszyńska, M., & Jabłonowski, Z. (2018). Urine miRNA as a potential biomarker for bladder cancer detection–a meta-analysis. *Central European journal of urology*, *71*(2), 177.

Larsson, S. C., Andersson, S.-O., Johansson, J.-E., & Wolk, A. (2008). Fruit and vegetable consumption and risk of bladder cancer: a prospective cohort study. *Cancer Epidemiology Biomarkers & Prevention*, *17*(9), 2519-2522.

Leal, J., Luengo-Fernandez, R., Sullivan, R., & Witjes, J. A. (2016). Economic burden of bladder cancer across the European Union. *European urology*, *69*(3), 438-447.

Lenis, A. T., Lec, P. M., Chamie, K., & Mshs, M. (2020). Bladder cancer: a review. *Jama*, *324*(19), 1980-1991.

Li, F., An, S., Hou, L., Chen, P., Lei, C., & Tan, W. (2014). Red and processed meat intake and risk of bladder cancer: a meta-analysis. *International Journal of Clinical and Experimental Medicine*, *7*(8), 2100.

Li, Y., Tindle, H. A., Hendryx, M. S., Xun, P., He, K., Liang, X., & Luo, J. (2019). Smoking cessation and the risk of bladder cancer among postmenopausal women. *Cancer Prevention Research*, *12*(5), 305-314.

Lin, J., Forman, M. R., Wang, J., Grossman, H. B., Chen, M., Dinney, C. P., Hawk, E. T., & Wu, X. (2012). Intake of red meat and heterocyclic amines, metabolic pathway genes and bladder cancer risk. *International journal of cancer*, *131*(8), 1892-1903.

Lopez-Beltran, A., Cookson, M. S., Guercio, B. J., & Cheng, L. (2024). Advances in diagnosis and treatment of bladder cancer. *Bmj*, *384*.

Lozano, F., Raventos, C. X., Carrion, A., Trilla, E., & Morote, J. (2020). Current status of genetic urinary biomarkers for surveillance of non-muscle invasive bladder cancer: A systematic review. *BMC urology*, *20*, 1-11.

Maas, M., Todenhöfer, T., & Black, P. C. (2023). Urine biomarkers in bladder cancer—current status and future perspectives. *Nature Reviews Urology*, *20*(10), 597-614.

Malinaric, R., Mantica, G., Lo Monaco, L., Mariano, F., Leonardi, R., Simonato, A., Van der Merwe, A., & Terrone, C. (2022). The role of novel bladder cancer diagnostic and surveillance biomarkers—what should a urologist really know? *International Journal of Environmental Research and Public Health*, *19*(15), 9648.

Masuda, N., Ogawa, O., Park, M., Liu, A. Y., Goodison, S., Dai, Y., Kozai, L., Furuya, H., Lotan, Y., & Rosser, C. J. (2018). Meta-analysis of a 10-plex urine-based biomarker assay for the detection of bladder cancer. *Oncotarget*, *9*(6), 7101.

Michaud, D., Pietinen, P., Taylor, P., Virtanen, M., Virtamo, J., & Albanes, D. (2002). Intakes of fruits and vegetables, carotenoids and vitamins A, E, C in relation to the risk of bladder cancer in the ATBC cohort study. *British journal of cancer*, *87*(9), 960-965.

NIH. (2024). *National Cancer Institute*. https://www.cancer.gov/types/bladder

Papavasiliou, E., Sills, V. A., Calanzani, N., Harrison, H., Snudden, C., di Martino, E., Cowan, A., Behiyat, D., Boscott, R., & Tan, S. (2023). Diagnostic Performance of Biomarkers for Bladder Cancer Detection Suitable for Community and Primary Care Settings: A Systematic Review and Meta-Analysis. *Cancers*, *15*(3), 709.

Saginala, K., Barsouk, A., Aluru, J. S., Rawla, P., Padala, S. A., & Barsouk, A. (2020). Epidemiology of bladder cancer. *Medical sciences*, *8*(1), 15.

Sathianathen, N. J., Butaney, M., Weight, C. J., Kumar, R., & Konety, B. R. (2018). Urinary biomarkers in the evaluation of primary hematuria: a systematic review and meta-analysis. *Bladder Cancer*, *4*(4), 353-363.

Shi, H.-B., Yu, J.-X., Yu, J.-X., Feng, Z., Zhang, C., Li, G.-Y., Zhao, R.-N., & Yang, X.-B. (2017). Diagnostic significance of microRNAs as novel biomarkers for bladder cancer: a meta-analysis of ten articles. *World Journal of Surgical Oncology*, *15*, 1-10.

Sieverink, C. A., Batista, R. P., Prazeres, H. J., Vinagre, J., Sampaio, C., Leão, R. R., Máximo, V., Witjes, J. A., & Soares, P. (2020). Clinical validation of a urine test (Uromonitor-V2®) for the surveillance of non-muscle-invasive bladder cancer patients. *Diagnostics*, *10*(10), 745.

Silberstein, J. L., & Parsons, J. K. (2010). Evidence-based principles of bladder cancer and diet. *Urology*, *75*(2), 340-346.

Soputro, N. A., Gracias, D. N., Dias, B. H., Nzenza, T., O'Connell, H., & Sethi, K. (2022). Utility of urinary biomarkers in primary haematuria: systematic review and meta-analysis. *BJUI compass*, *3*(5), 334-343.

Su, Q., Wu, H., Zhang, Z., Lu, C., Zhang, L., & Zuo, L. (2021). Exosome-derived long non-coding RNAs as non-invasive biomarkers of bladder cancer. *Frontiers in Oncology*, *11*, 719863.

Vedula, R., & Iyengar, A. A. (2020). Approach to diagnosis and management of hematuria. *The Indian Journal of Pediatrics*, *87*(8), 618-624.

Wang, J., Gao, Y., Wang, X., Gao, Y., Li, L., Zhang, J., Zhang, L., & Che, F. (2020). Circulating lncRNAs as noninvasive biomarkers in bladder cancer: a diagnostic meta-analysis based on 15 published articles. *The International Journal of Biological Markers*, *35*(2), 40-48.

Willis, G. C., & Tewelde, S. Z. (2019). The approach to the patient with hematuria. *Emergency Medicine Clinics*, *37*(4), 755-769.

Xiao, S., Wang, J., & Xiao, N. (2016). MicroRNAs as noninvasive biomarkers in bladder cancer detection: A diagnostic meta-analysis based on qRT-PCR data. *The International Journal of Biological Markers*, *31*(3), 276-285.

Yafi, F. A., Brimo, F., Steinberg, J., Aprikian, A. G., Tanguay, S., & Kassouf, W. (2015). Prospective analysis of sensitivity and specificity of urinary cytology and other urinary biomarkers for bladder cancer. Urologic oncology: seminars and original investigations,

Yan, H., Ying, Y., Xie, H., Li, J., Wang, X., He, L., Jin, K., Tang, J., Xu, X., & Zheng, X. (2018). Secondhand smoking increases bladder cancer risk in nonsmoking population: a meta-analysis. *Cancer management and research*, 3781-3791.

Zhao, X., Wang, Y., & Liang, C. (2022). Cigarette smoking and risk of bladder cancer: a dose–response meta-analysis. *International urology and nephrology*, *54*(6), 1169-1185.
Zhou, J., Kelsey, K. T., Smith, S., Giovannucci, E., & Michaud, D. S. (2015). Lower urinary tract symptoms and risk of bladder cancer in men: results from the health professionals follow-up study. *Urology*, *85*(6), 1312-1318.
Zhu, C.-Z., Ting, H.-N., Ng, K.-H., & Ong, T.-A. (2019). A review on the accuracy of bladder cancer detection methods. *Journal of Cancer*, *10*(17), 4038.
Zingg, E. J., & Wallace, D. M. A. (2012). *Bladder cancer*. Springer Science & Business Media.

Appendix A

Table 3: Novel diagnostic biomarkers that were extracted from the enrolled studies

Novel Diagnostic Biomarker	Biomarker Description	Sample size	Specimen	Sensitivity	Specificity	No. Studies
116 peptides panel (>collagen fragments, APO-I peptides, basement-membrane specific heparan proteoglycan fragments)	metabolomics and metabonomics	1357	Blood and Urine	88–91%	51–68%	1
6 ions panel (>imidazoleacetic acid)	metabolomics and metabonomics	87	Blood and Urine	82%	85–90%	1
ACTB-106	cell-free circulating DNA	227	Serum	91.6%	43.3%	1
ADXBLADDER, Mcm5	protein expression	2980	Urine	63%–97%	65–88%	7
AIB1	protein	135	Blood and Urine	80%	86%	1
AIB1, EIF5A2, NPM22	protein	135	Blood and Urine	89%	91%	1
AKT1, ARID1A, BRAF, CDKN1A, CDKN2A, EP300, ERBB2, ERBB3, FBXW7, FGFR3, KDM6A, KRAS, MED12, PIK3CA, PLEKHS1, RB1,STAG2,TERT,TP53,TSC1	mutation markers	185	Urine	83.3%	97.1%	1
ANG, APOE, A1AT, CA9, IL8, MMP9, MMP10, PAI1, SDC1, VEGF	protein	1295	Urine	87%-93%	93%	3
APE1/REF1	protein	277	Blood and Urine	81.7%	79.6%	1
ARID1A, CDKN2A, CREBBP,	mutation markers	162	Urine	93.7%	43.3%	1
ERBB2, ERBB3, FGFR1,						
FGFR3, HRAS, KTM2D,						
NF1, PIK3CA, STAG2,						
TP53, TSC1						
AssureMDx	urine-based test	508	Urine	95%-96.7%	82.1% - 85%	2
Base model (age and grade of haematuria) + UroVysion + Cytology	Aneuploidy of Chromosomes 3, 7 and 17 and loss of chromosome locus 9p21 + protein expression of Carcinoembryonic antigen and Sulphate mucin glycoproteins	808	Urine	78.3%	81.1%	1
Base model (age and grade of haematuria) + UroVysion + uCyt+	Aneuploidy of Chromosomes 3, 7 and 17 and loss of chromosome locus 9p21 + protein expression of Carcinoembryonic	808	Urine	71.3%	86.3%	1

	antigen and Sulphate mucin glycoproteins					
BCL2	gene methylation	475	Urine	97.0%	76.9%	1
Bladder EpiCheck	DNA methylation test of 15 (unpublished) genes (Bladder EpiCheck)	1274	Blood and Urine	57–85%	80–88%	5
Bladder wash (>resectisol, urea, creatinine, uric acid, different types of cells, cylinders, crystals) analyzed by FTIR	metabolomics and metabonomics	71	Blood and Urine	81.8–100%	52.9–80.9%	1
CCNA1, ONECUT2, BCL2, EOMES, SALL3, VIM	gene methylation	475	Urine	97.0%	79.5%	1
CCNA1	gene methylation	475	Urine	97%	76.9%	1
CDKN2A, ERBB2, FGFR3, HRAS,KRAS,MET,MLL, PIK3CA,TP53,VHL	mutation markers	446	Urine	88.0%	96.6%	1
CFTR, SALL3, TWIST 1, NID2, TWIST1	Altered methylation of CFTR, SALL3, TWIST 1, NID2, TWIST1 in adjunct to Cytology	897	Blood and Urine	57–96%	40–72%	2
Concentration matrices	metabolomics and metabonomics	155	Blood and Urine	55%	74.7%	1
CRH, IGF2, UPK1B, ANXA10, ABL1 (Xpert Bladder)	mRNAs panel	3019	Blood and Urine	63–80%	73–81%	10
CxBladder	urine-based test	970	Blood and Urine	81.3%-97.7%	61%-85.1%	5
Cytokeratin 20	cytokeratin	147	Blood and Urine	56–76%	NM	2
CxBladder Triage (CxBT) + Imaging	mRNA expression of CDK1, HOXA13, MDK, IGFBP5, CXCR2	884	Urine	98.1%	NM	1
Cytokeratins 8 and 18 (UBC rapid test)	cytokeratin	754	Blood and Urine	30-87%	63-87%	5
Cytology + CK20 + p53 immunostaining	NM	123	Blood and Urine	91.1%	74.3%	2
Dimethyl amine, malonate, glutamine, lactate, histidine and valine metabolites panel	metabolomics and metabonomics	160	Blood and Urine	80.8–98.1%	66.7–80.3%	1
Cytology enriched with p53, ki67 coloration	Improving cytology	252	Blood and Urine	68.9%	97.5%	2
Cytology combined with p16/ki-67 dual-labeling	Improving cytology	208	Blood and Urine	80%	71.4%	1
EIF5A2	protein	135	Blood and Urine	74%	78%	1
ELNAT1	lncRNA	408	Serum	74%	76%	1

Extended Model consisting of: Existing model (univariate analysis incl. age, mutation, methylation) + type of haematuria + gender	Mutation of FGFR3, TERT and HRAS and methylation of OTX1, ONECUT2 and TWIST1	838	Urine	96%	73%	1
FEGFR3, Cyclin D3	genomic mutation	471	Blood and Urine	73%	90%	1
FGFR3 + Cytology	Mutation of FGFR3 gene + Cytology	748	Urine	58.3%	99.5%	1
FGFR3, HRAS, TERT	mutation markers	354	Urine	72.1-77.3%	93.2-96.9%	2
FGFR3, HRAS, PIK3CA, RXRA, TERT, TP53	mutation markers	231	Urine	70.5%	97.2%	1
FGFR3, TP53, PIK3CA, ARID1A, STAG2, KTM2D	genomic mutation	162	Urine	73–95%	85–90%	1
FGFR3, TERT, HRAS, OTX1, ONECUT2, TWIST1	Mutation of FGFR3, TERT and HRAS genes and methylation of OTX1, ONECUT2 and TWIST1 genes	1038	Urine	93%	86%	2
FGFR3, TERT	mutation markers	1854	Urine	69.5 - 88.9	82.2	3
GAS5	lncRNA	160	Urine	74%	76%	1
H19	lncRNA	204	Serum and Urine	74%-86%	76%-81%	2
HOXA9	gene methylation	111	Urine	50.9%	93.1%	1
hsa-miR-144 5p	miRNA	58	Blood	70.0%	82.4%	1
hsa-miR-374-5p	miRNA	58	Blood	60.0%	94.0%	1
HOXA9, PCDH17, POU4F2, ONECUT2	gene methylation	111	Urine	90.5%	73.2%	1
hsa-miR-652, hsa-miR-199a-3p, hsa-miR-140-5p, hsa-miR-93, hsa-miR-142-5p, has-miR-1305, hsa-miR-30a, hsa-miR-224, hsa-miR-96, hsa-miR-766, hsa-miR-223, hsa-miR-99b, hsa-miR-140-3p, hsa-let-7b , hsa-miR-141 , hsa-miR-191, hsa-miR-146b-5p,	miRNA	121	Urine	87%	100%	1

hsa-miR-491-5p, hsa-miR-339-3p, hsa-miR-200c, hsa-miR-106b, hsa-miR-143, hsa-miR-429, hsa-miR-222, hsa-miR-200a						
HURP + Cytology	mRNA expression of Hepatoma Up-regulated Protein + Cytology	334	Urine	77.3 %	NM	2
HURP	mRNA expression	334	Urine	78.67 %	94 %	2
IGF2, MAGEA3	genes panel transcriptomic alterations	789	Blood and Urine	81%	91%	1
IGFBP7, SNX16, CSPG6, CTSD, CHD2, NELL2, TNFRSF7	mRNAs panel	73	Plasma	83.0%	93.0%%	1
LASP1	protein	261	Blood and Urine	59%	80%	1
IQGAP3	circulating free nucleid acids	212	Blood and Urine	80–96.2%	60.2–90.7%	1
KLHDC7B, CASP14, PRSS1 and MIR205HG and GAS5	3 mRNAs: KLHDC7B, CASP14 and PRSS1; lncRNAs: MIR205HG and GAS5	180	Blood and Urine	87.2%	83.3%	1
Let-7c, miR-135a, miR-135b, miR-148a, miR-204, miR-345	miRNAs panel	202	Urine	88.3%	NM	1
MALAT1, MEG, SNHG16	lncRNAs panel	172	Blood and Urine	82%	73%	1
LINC00355, UCA1-201, UCA1-203, MALAT1	lncRNAs panel	83	Blood and Urine	92%	91.7%	1
MALAT1	lncRNA	556	Serum and Urine	67%-74%	74%-76%	3
MADR Assay	Methylation of TWIST1 and NID2 genes, mutation of FGFR3 gene and protein expression of matrix metalloproteinase 2	748	Urine	94%	NM	1
MEG3	lncRNA	172	serum	70%	75%	1
MALAT1, PCAT1, SPRY4-IT1	lncRNAs panel	160	Blood and Urine	62.5%-74%	76%-85%	1
Mcm5 + NMP-22	protein expression	1677	Urine	67%	72%	1
miR- 18a, let 7a	miRNA	96	Urine	74%	78%	1

Microsatellite instability (MIS) or Loss of heterozigosity (LOH) of D16S476, D9S171, FGA, ACTBP2	genomic mutation	30	Blood and Urine	96.7%	30%	1
miR -16, -21, -34a, -99a, -106b, -126, -129, -133a, -145, -200c, -205, -218, -221/222, -331	miRNA	81	Blood and Urine	88%	48%	1
miR-100	miRNA	121	Urine	60.4%	78.7%	1
miR-106b	miRNA	190	Urine	74%-83%	64.1%-78%	1
miR-1224	miRNA	121	Urine	74%	78%	1
miR-125	miRNA	59	Urine	59.3	95.7	1
miR-1255b-5p	miRNA	55	Urine	85%	68.4%	1
miR-125b	miRNA	71	Urine	84.8%	76.2%	1
miR-133b	miRNA	121	Urine	74%	78%	1
miR-135b	miRNA	121	Urine	71.2%	74.4%	1
miR-135b, -15b, -1224-3p	miRNA	121	Urine	94.1%	51%	1
miR-140-5p, -142-5p, -199a-3p, -93, -652, -20a, -106b, -1305, -223, -18a, -191, -126, -26b, -26a, -145, -146a, -30a-3p, -96, -573, -221, -182,-142-3p, -19b, -224, -181a, -766, -146b-5p, -429, -200a, -200c, -20b, -324-3p, -19a, -106a, -143, -99b, -140-3p, -491-5p, -151-3p, -671-3, -222, -339-3p, -141, -200b, -7b, -21	miRNAs panel	121	Blood and Urine	87%	100%	1
miR-137/124-2/124-3/9-3	miRNA	106	Urine	81	89	1
miR-141, miR-34b, miR-10b ,miR-103	miRNA	119	Urine	75	63.5	1
miR-141-3p	miRNA	133	Urine	71%	71%	2
miR-145	miRNA	487	Urine	77.8%	61.1%	2
miR-146a-5p	miRNA	280	Urine	100%	53.5%	2
miR-152, -148b-3p, -3187-3p,-15b-5p, -27a-3p, -30a-5p	miRNA	240	Serum	90.0%	90.0%	1
miR-155	miRNA	314	Urine	80.2%	84.6%	1
miR-15a	miRNA	121	Urine	51.7	72	1
miR-15b	miRNA	121	Urine	67.8	81.3	1
miR-187, -18a, -25, -142-3p, -140-5p, -204	miRNA	277	Urine	84.4	86.5	1
miR-18a, miR-25, miR-140-5p, miR-187, miR-142-3p, miR-204	miRNAs panel	317	Urine	84.8%	86.5%	1
miR-192	miRNA	238	Urine	78%	76.7%	1

miR-200	miRNA	136	Urine	46.2%	100.0%	1
miR-200a	miRNA	706	Urine	74%-100%	52.6%-78%	3
miR-203	miRNA	121	Urine	66.1	66	1
miR-205-5p	miRNA	133	Urine	82%	62%	2
MIR205HG	lncRNA	160	Urine	72%	78%	1
MIR205HG, GAS5	lncRNA	160	Urine	72%	78%	1
miR-21	miRNA	257	Urine	74%	78%	2
miR-20a	miRNA	166	Urine	72.1%	87.5%	1
miR-210	miRNA	850	Serum and Urine	97.6%	69.2%	3
miR-211	miRNA	121	Urine	74%	78%	1
miR-212	miRNA	121	Urine	54.2%	64%	1
miR-210, -10b, -29c	miRNA	360	Urine	95.2%	NM	1
miR-214	miRNA	643	Urine	54.7%-90.5%	65.6%-93.5%	2
miR-21-5p	miRNA	87	Urine	84%	59%	1
miR-23b	miRNA	121	Urine	74%	78%	1
miR-24-1	miRNA	121	Urine	74%	78%	1
miR-25, miR-18a, miR-187 miR-204, miR-142-3p, miR-140-5p	miRNA	277	Urine	70%	72%	1
miR-26-a/93/191/940	miRNA	130	Urine	70%	84%	1
miR-26b-5p	miRNA	58	Blood	65. 0%	94.1%	1
miR-27b	miRNA	121	Urine	60.3%	81.1%	1
miR-31-5p, -93-5p	miRNA	304	Blood and Urine	82%	70%	1
miR-328	miRNA	121	Urine	74%	78%	1
miR-34b, miR-10b	miRNA	119	Urine	59.1%	78.8%	1
miR-519a	miRNA	262	Urine	74%	78%	1
miR-497, -663b	miRNA	224	Plasma	97.6%	69.2%	1
miR-520e	miRNA	55	Urine	70%	63.2%	1
miR-6087, -6724, -3960, -1343-5p, -1185-1-3p, -6831-5p, -4695-5p	miRNAs panel	401	Blood and Urine	95–98%	87–91%	2
miR-6124/miR-4511	miRNA	214	Urine	91.5%	74.2%-76.2%	1
miR-618	miRNA	55	Urine	70%	68.4%	1

miR-7-5p, -22-3p, -29a-3p, -126-5p, -200a-3p, -375, -423-5p	miRNA	552	Blood and Urine	80.88%	91.67%	1
miR-652, -199a-3p, -140-5p, -93, -142-5p, -1305, -30a, -224, -96, -766	miRNAs panel	121	Blood and Urine	84%	87%	1
miR-99a	miRNA	130	Urine	74.1%-78%	82.6%-85.7%	2
miR-99a, miR-125b	miRNA	71	Urine	86.7%-90%	81.1%-90%	1
miRNA 192	miRNA	118	Blood and Urine	76.7%	78%	1
miRNA 192, 2D ultrasound	miRNA	118	Blood and Urine	93.2%	96.7%	1
miRNA-125b, -30b, -204, -99a, -532-3p	miRNAs panel	109	Blood and Urine	59%	96%	1
miRNA-130a-3p, -130b-3p, -301a-3p	miRNA 130 family	164	Blood	87.8%	93.3%	1
miRNA-4-1	miRNA	121	Urine	60%	58.5%	1
miRNAs: -19b1-5p, 21-5p, 136-3p, -139-5p, 210-3p combined with BLCA-4, NMP22, APE1/Ref1, CRK, VIM, and creatinine urinary concentrations	miRNA	93	Blood and Urine	80%	88%	1
miRNA-663, VIM	miRNA, VIM hypermethylation	51	Blood and Urine	92.6%	90%	1
MMP-2, MMP-9 , TIMP-2	MMP-2, MMP-9 and TIMP-2 urinary and serum proteins' and genes' expression levels	50	Blood and Urine	100%	100%	1
NMP22	protein	1318	Blood and Urine	37.9–88.5%	65.2–96.9%	1
N-Myc	circulating free nucleid acids	224	Blood and Urine	85.5%	81.4%	1
NMP-22 + Cytology	protein expression	1188	Urine	67.8%	87.5%	2
NMP-52	protein expression	160	Urine	87%	83%	1
ONECUT2	gene methylation	740	Urine	56.6%	91.4%	3
Optimal (age + FGFR3, TERT, HRAS, ONECUT2 probes 1 + 4, OTX1 probe 2, TWIST)	Methylation of ONECUT2 and OXT1 genes and mutation of FGFR3, TERT and HRAS genes	154	Urine	97%	83%	1
Optimal model consisting of: Existing model (univariate analysis incl. age, mutation, methylation) + type of haematuria	Mutation of FGFR3, TERT and HRAS and methylation of OTX1, ONECUT2 and TWIST2	840	Urine	96%	73%	1
Orosomucoid-1	protein	165	Urine	92%	94%	1
p14ARF, p16INK4A, DAPK, RASSF1A, APC	Methylation of tumor suppressor	112	Blood and Urine	91–100%	NM	1

	genes (p14ARF, p16INK4A, DAPK, RASSF1A, APC)					
OTX1, ONECUT2, TWIST1, SEPTIN9, PCDH17, POU4F2, HS3ST2, SLIT2, FGFR3, CFTR, SALL3, GHSR, MAL, HRAS, TERT, FGFR3	Hypermethylation of OTX1, ONECUT2, TWIST1, SEPTIN9, PCDH17, POU4F2, HS3ST2, SLIT2, FGFR3, CFTR, SALL3, GHSR, MAL, mutation of HRAS, TERT, FGFR3	1722	Blood and Urine	93–98%	40–86%	6
PCAT-1, UBC1, SNHG16	lncRNAs panel	320	Urine	80%	75%	1
p53, MCM5, MCM2, ki-67 coloration in adjunct to cytology	???	152	Blood and Urine	67.3–90.4%	72–80%	1
PCAT-1	lncRNA	248	Urine	72%	78%	2
Phosphatidylinositol, nucleic acids, collagen, aromatic amino acids, cholesterol fatty acids, glycogen, monosaccarides and carotenoids' changes (Rametrix)	metabolomics and metabonomics	56	Blood and Urine	82.4%	79.5%	1
PTENP1	lncRNA	110	Urine	72%	78%	1
RAB-B2	gene methylation	216	Urine	53%	90.5%	1
RAB-B2 + Cytology	Methylation of RAB-B2 gene + Cytology	216	Urine	82%	88.8%	1
RAB-B2 + HYAL-1 + Cytology	Methylation of RAB-B2 gene + Hyaluronidase activity + Cytology	216	Urine	95%	81.9%	1
RAB-B2 + HYAL-1	Methylation of RAB-B2 + Hyaluronidase 1 activity	216	Urine	92%	81.9%	1
S100A4	gene	150	Serum	90.0%	92.0%	1
S100A6	gene	150	Serum	86.7%	84.0%	1
S100A7	gene	150	Serum	73.3%	93.3%	1
S100A8	gene	150	Serum	85.0%	92.0%	1
S100A9	gene	150	Serum	81.7%	92.0%	1
S100A11	gene	150	Serum	83.3%	91.0%	1
Soluble FAS	protein	191	Urine	88.03%	89.19%	1
Serum Irisin	protein	150	Blood	74.7%	90.7%	1
Serum exosomes	circulating free nucleid acids	82	Blood and Urine	82.4%	100%	1
SALL3, ONECUT2, CCNA1,BCL2, EOMES, VIM,TERT, FGFR3	Altered methylation of SALL3,ONECUT2, CCNA1,BCL2, EOMES, VIMcombined with	475	Blood and Urine	97%	77%	1

	altered mutations of TERT, FGFR3					
SNCG	protein expression	276	Urine	68.4%	97.4%	1
TERT	mutation marker	3861	Urine	46.7–90%	90–100%	11
SPARC	protein	571	Blood and Urine	39–43%	70–78%	1
SPRY4-IT1	lncRNA	208	Urine	72%	78%	1
TERT promoter	genomic mutation	255	Blood and Urine	46.7–90%	90–100%	1
TERT, FGFR3, CCNA1, ONECUT2	Mutation of TERT and FGFR3 genes plus methylation of CCNA1 and ONECUT2 genes	475	Urine	97.0%	79.5%	1
TERT, FGFR3, CCNA1, ONECUT2, BCL2	Mutation of TERT and FGFR3 genes plus methylation of CCNA1, ONECUT2 and BCL2 genes	475	Urine	97.0%	76.9%	1
TERT, FGFR3, KRAS	genomic mutation	428	Blood and Urine	79–98%	62–90%	2
TERT, FGFR3, OTX1	genomic mutation	977	Blood and Urine	72%	59%	1
TF	Unphosphorylated protein	83	Blood and Urine	70.6%	97.8%	1
TF-pSer258	Phosphorylated protein	83	Blood and Urine	88.2%	93.3%	1
TF-pSer253	Phosphorylated protein	83	Blood and Urine	88.2%	24.4%	1
TERT, PLEKHS1 promoters	genomic mutation	185	Urine	91–95%	96–100%	1
TIMP3, APC, RARB, TIG1, GSTP1, p14, p16, PTGS2, RASSF1A	Hypermethylation TIMP3, APC, RARB, TIG1, GSTP1, p14, p16, PTGS2 and RASSF1A	78	Serum	80.3%	80.0%	1
uc004cox.4	lncRNA	460	Blood and Urine	80%	85%	1
Urine-derived fc-DNA	circulating free nucleid acids	100	Blood and Urine	20.7%	91.2%	1
Urine exosomes	circulating free nucleid acids	82	Blood and Urine	92.6%	83.3%	1
UCA1	lncRNA	60	Serum	72%	78%	1
UCA1-201	lncRNA	108	Urine	72%	78%	1
UCA1-203	lncRNA	108	Urine	72%	78%	1
uCyt+ (+) NMP-22	protein expression	808	Urine	90.4%	35.9%	1
UroMark	DNA hypermethylation of 150 loci panel (UroMark)	116	Urine	96%	97%	2

UroVysion + Cytology	Aneuploidy of Chromosomes 3, 7 and 17 and loss of chromosome locus 9p21	808	Urine	67.8%-86.4%	83.3%-87.5%	2
UroVysion + NMP-22 + Cytology	Aneuploidy of Chromosomes 3, 7 and 17 and loss of chromosome locus 9p21 + protein expression of nuclear matrix protein 22 + Cytology	808	Urine	75.7%	84.7%	1
UroVysion + NMP-22	Aneuploidy of Chromosomes 3, 7 and 17 and loss of chromosome locus 9p21 + protein expression of nuclear matrix protein 22	808	Urine	71.3%	86.3%	1
UroVysion + uCyt+ (+) Cytology	Aneuploidy of Chromosomes 3, 7 and 17 and loss of chromosome locus 9p21 + protein expression of Carcinoembryonic antigen and sulphate mucin glycoproteins + Cytology	808	Urine	76.5%	84.4%	1
UroVysion + uCyt+ (+) NMP-22 + Cytology	Aneuploidy of Chromosomes 3, 7 and 17 and loss of chromosome locus 9p21 + protein expression of Carcinoembryonic antigen, Sulphate mucin glycoproteins and nuclear matrix protein 22 + Cytology	808	Urine	74.8%	86.2%	1
UroVysion + uCyt+ (+) NMP-22	Aneuploidy of Chromosomes 3, 7 and 17 and loss of chromosome locus 9p21 + protein expression of Carcinoembryonic antigen and Sulphate mucin glycoproteins and nuclear matrix protein 22	808	Urine	83.5%	74.1%	1
UroVysion + uCyt+	Aneuploidy of Chromosomes 3, 7	808	Urine	71.3%	86.3%	1

	and 17 and loss of chromosome locus 9p21 + protein expression of Carcinoembryonic antigen and Sulphate mucin glycoproteins					