Asian Journal of Urology 11 (2024) 221–241

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Review

Metabolomics for the diagnosis of bladder cancer: A systematic review

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Received 14 January 2022; accepted 29 November 2022
Available online 12 September 2023

KEYWORDS
Metabolite;
Metabolomics;
Bladder cancer;
Metabolomics profile

Abstract *Objective:* Metabolomics has been extensively utilized in bladder cancer (BCa) research, employing mass spectrometry and nuclear magnetic resonance spectroscopy to compare various variables (tissues, serum, blood, and urine). This study aimed to identify potential biomarkers for early BCa diagnosis.
Methods: A search strategy was designed to identify clinical trials, descriptive and analytical observational studies from databases such as Medline, Embase, Cochrane Central Register of Controlled Trials, and Latin American and Caribbean Literature in Health Sciences. Inclusion criteria comprised studies involving BCa tissue, serum, blood, or urine profiling using widely adopted metabolomics techniques like mass spectrometry and nuclear magnetic resonance. Primary outcomes included description of metabolites and metabolomics profiling in BCa patients and the association of metabolites and metabolomics profiling with BCa diagnosis compared to control patients. The risk of bias was assessed using the Quality Assessment of Studies of Diagnostic Accuracy.
Results: The search strategy yielded 2832 studies, of which 30 case-control studies were included. Urine was predominantly used as the primary sample for metabolite identification. Risk of bias was often unclear inpatient selection, blinding of the index test, and reference standard assessment, but no applicability concerns were observed. Metabolites and metabolomics profiles associated with BCa diagnosis were identified in glucose, amino acids, nucleotides, lipids, and aldehydes metabolism.
Conclusion: The identified metabolites in urine included citric acid, valine, tryptophan, taurine, aspartic acid, uridine, ribose, phosphocholine, and carnitine. Tissue samples exhibited elevated levels of lactic acid, amino acids, and lipids. Consistent findings across tissue,

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Peer review under responsibility of Tongji University.

https://doi.org/10.1016/j.ajur.2022.11.005
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urine, and serum samples revealed downregulation of citric acid and upregulation of lactic acid, valine, tryptophan, taurine, glutamine, aspartic acid, uridine, ribose, and phosphocholine.
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1. Introduction

Bladder cancer (BCa) is the sixth most common cancer in the United States, being the third most common cancer in men and the eleventh in women [1,2]. Most cancers derived from transitional epithelium are transitional cell carcinomas (urothelial carcinoma). These can be classified into low-grade and high-grade, distinguished by their propensities to invade the muscle and metastasize to other parts of the body, rather than by the risk of recurrence (as both often recur after treatment) [3].

This type of cancer is also divided into non-muscle invasive BCa (NMIBC), which includes carcinoma *in situ*, Ta, and T1 tumors; and muscle invasive BCa (MIBC), which refers to tumors with a stage of ≥T2, based on invasion of the muscularis propria (detrusor muscle). Most people with BCa (75%—85%) have NMIBC and frequently recur [4].

Regarding diagnostics, the macroscopic and microscopic hematuria is a clear sign to perform cystoscopy, which is an invasive diagnostic method. The preferred method for diagnosis and treatment of the BCa is transurethral bladder tumor resection, since it allows to have the surgical specimen including part of the detrusor muscle to analyze its compromise. In addition, urinary cytology is a noninvasive and low-cost test, with an especially high specificity and poor sensitivity especially in low-grade BCa [5].

Metabolomics could be useful in BCa research to identify potential non-invasive and highly sensitive biomarkers. This technique has been extensively used in oncologic research, with studies on BCa using mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy to compare different variables (tissue, serum, blood, and urine) between patients with and without this type of cancer, in order to identify differences [6,7].

Metabolomics is a rapidly growing field that was defined as the quantitative study of metabolites (molecules smaller than 1500 kDa) in a biological system and alterations of their concentrations due to environmental or genetic effects. Research has focused on areas such as toxicology, biomedical sciences, nutrition, genetics, innate errors of metabolism, diabetes, cancers, diagnostic tests, and neuronal diseases. These applications are based on the theory that metabolites are the functional outputs of an organism. This technique might identify a disturbance in the system's homeostasis that could occur before the appearance of symptoms of a particular disease, as a single metabolite may be the substrate for several different enzymes involved in complex metabolic pathways [6,8,9].

One of the main advantages of applying metabolomics is the ability to detect hundreds of metabolites in parallel [10], thereby efficiently monitoring biochemical alterations. Additionally, the metabolic profiles of biological specimens can be affected by factors such as diet, age, ethnicity, lifestyle, medications, or microbiota, and these factors should be controlled to obtain disease-specific information [11].

Two of the essential techniques currently used in metabolomics are NMR and MS. NMR requires little to no preparation, is rapid and non-invasive, does not destroy tissue, and has highly reproducible results (coefficient of variation 1%—2% for technical replicates). Combining NMR with MS might increase the diagnostic yield. However, the data obtained from NMR and/or MS experiments are quite complex. They provide qualitative and quantitative information on several metabolites; however, distinguishing statistically between disease and control markers could be challenging [6,10].

NMR has several advantages and disadvantages. It is known as one of the most relevant diagnostic methods used in metabolomics for a broad range of diseases. Among the advantages are the use of liquid, gassy, solid, and tissue samples, making it a more complete tool. Meanwhile, its disadvantages can be seen in its low sensitivity range, therefore, a challenge for upcoming developments in this matter. On the other hand, MS has higher specificity and sensitivity results, which makes it a more useful technique. It also allows the detection of non-carbon and non-protonic ions, resulting in a great analytical tool for metabolic detection in any sample (Table 1) [12—14].

Methodologies used in metabolomics may be separated into two big categories, the targeted and untargeted metabolomics. The former studies defined molecules, already known, and biochemically characterized metabolites and their measurement. Meanwhile, the latter analyzes all metabolites that can be measured within a sample including those chemically unknown [15—17].

There are few studies using different samples and analytical platforms with similar results for some metabolites; nonetheless, it is essential to standardize these two fundamental variables to establish a way to diagnose BCa nowadays. We aimed to describe metabolites and metabolomics profiling in patients with BCa that could serve as biomarkers for its early diagnosis.

2. Methods

We performed this review according to the recommendations of the Cochrane Collaboration and following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement [18]. The protocol was previously published [19].

Table 1 Advantages and limitations of NMR compared to MS in metabolomics applications.

Metabolomics technique	Advantage	Disadvantage	Applicable substance
NMR spectroscopy	- High reproducibility, straightforward sample preparation, preservation of molecular integrity, potential sample reuse, cost-effectiveness, and greater ease of identification compared to MS [14]	- Lower detectable metabolites in urine sample and lower sensitivity compared to MS [14]	- Body fluids, *in vivo* and *in situ* studies [13]
GC-MS	- Higher specificity and sensitivity, available for comparison with a standard, and can be used for both targeted and nontargeted analyses compared to NMR spectroscopy [12]	- The manipulation of low volatile molecules can present difficulties [12]	- Heat stable, volatile, medium, and low polar molecules [14]
LC-MS	- Higher specificity and sensitivity, available for comparison with a standard, and can be used for both targeted and nontargeted analyses compared to NMR spectroscopy [12,14]	- Low retention for hydrophilic molecules and no complete database to compare with a standard [14]	- Most compounds, including those that exhibit heat-lability, nonvolatility, and resistance to derivatization [12]

MS, mass spectrometry; GC, gas chromatography; LC, liquid chromatography; NMR, nuclear magnetic resonance.

2.1. Eligibility criteria

2.1.1. Study designs

We searched for clinical trials, descriptive and analytical observational studies. Also, we looked for molecular studies.

2.1.2. Participants

We included studies with the following characteristics: profiling studies on BCa tissue, serum, blood, and urine; and profiling studies using techniques widely used in metabolomics, such as MS and NMR.

2.1.3. Primary outcomes

i. The description of metabolites and/or metabolomics profiling in patients with BCa that could serve as biomarkers for early diagnosis.
ii. To examine the association between metabolites and/or metabolomics profiling in patients diagnosed with BCa and control participants.

2.1.4. Exclusion criteria

We excluded recurrence BCa, benign bladder disease, and animal-related studies.

2.2. Information sources

We obtained our information through Medline, Embase, Caribbean Literature in Health Sciences, and the Cochrane Central Register of Controlled Trials from inception to nowadays (Appendix 1). To ensure literature saturation, we scanned references from the most relevant articles identified through our search, conferences, thesis databases, Open Grey, Google scholar, and ClinicalTrials.gov, among others. We contacted authors by e-mail in case of any missing information. There were not any language or timing restrictions.

2.3. Data collection and analysis

Two researchers (Dávila-Raigoza AM and García-Perdomo HA) reviewed each reference by the title and abstract, then scanned full texts of relevant studies, applied pre-specified inclusion and exclusion criteria, and extracted the data. Disagreements over the inclusion of papers were discussed and worked out, and whenever they could not be resolved, a third reviewer (Korkes F) mediated the conflict.

Two trained reviewers (Dávila-Raigoza AM and García-Perdomo HA) using a standardized form independently extracted the following information from each article: study design, geographic location, author names, title, objectives, inclusion and exclusion criteria, number of patients included, lost to follow-up, timing, definitions of outcomes, results and association measures, and funding sources.

2.4. Risk of bias

We assessed the risk of bias with the Quality Assessment of Studies of Diagnostic Accuracy tool [20]. We used RevMan 5.4 for the graphical representation.

2.5. Data analysis and synthesis of results

We qualitatively synthesized the results. We could not perform any statistical analysis due to the high heterogeneity of data.

3. Results

3.1. Selection of studies

The search strategy in the databases delivered 2832 records. After reviewing the titles, abstracts, and the full texts, we selected 30 studies for qualitative synthesis (Fig. 1) [21—50].

3.1.1. Characteristics of the included studies

All were case-control studies. They accounted for a total of 3269 patients. The biological samples used in metabolomics analyses included blood ($n=1$), tissue ($n=2$), serum ($n=4$), and urine ($n=23$) (Table 2).

3.2. Risk of bias assessment

We evaluated a large part of the studies as unclear risk due to the lack of information regarding patient selection, blinding of index test assessment, and blinding of the reference standard assessment. Nevertheless, there were no applicability concerns for this systematic review (Figs. 2 and 3).

3.3. Glucose metabolism

Glucose metabolism implies multiple processes including glycolysis, glycogenolysis, glycogenesis, and gluconeogenesis.

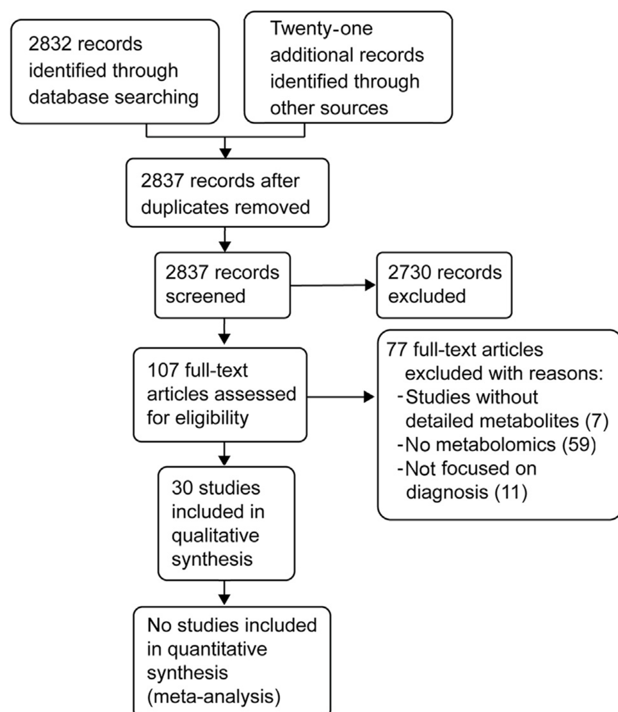

Figure 1 Flowchart of selected studies.

Glycolysis is the most crucial process. The end products of which are two molecules of pyruvic acid that turn into acetyl coenzyme A, which then decomposes and releases energy as ATP through Krebs's cycle or the tricarboxylic acid cycle [51,52]. The metabolites intermediate with significant variations in this pathway are shown in Table 3. Glucose levels were found to be upregulated in serum [27]. Conversely, fructose exhibited downregulation in two urine studies [22,36]. In terms of pyruvic acid, its regulation showed divergent patterns. One study reported an upregulation [33], whereas another study observed a downregulation [36]. These contrasting findings highlight the complexity of pyruvic acid's regulatory mechanisms. Moving on to lactose, it was consistently downregulated in plasma [38]. The regulation of lactic acid displayed inconsistent results. One study observed upregulation in serum [30], whereas another study reported downregulation [27]. Interestingly, in tissue, lactic acid was found to be upregulated according to one study [35], emphasizing the need for further investigation into the tissue-specific regulation of lactic acid. Several studies focused on urine regulation, revealing intriguing findings, and three of them reported upregulation [36,43,50]; however, another study noted changes in regulation without specifying the direction of regulation [45], underscoring the complexity of urine metabolite regulation (Table 3).

Citric acid emerged as a consistently regulated metabolite, particularly in urine. Four studies found downregulation [21,22,29,36] and another study reported changes in its regulation without specifying the trend [45]. It was also downregulated in serum [27]. Succinate exhibited inconsistent regulation. One study found upregulation in urine [33], while another study reported downregulation [36]. Another study described changes in succinate regulation without specifying the trend [45]. Regarding plasma metabolites, one study identified upregulation of malic acid and fumarate [38], offering insights into potential alterations in energy metabolism or cellular processes involving these metabolites.

3.4. Amino acid metabolites

Amino acids, the building blocks of protein, based on their physiological and nutritional roles, can be differentiated as essential and non-essential [53]. Table 4 shows the significant variations of amino acid metabolites in BCa samples. In terms of essential amino acids, leucine exhibited upregulation in urine [23,24,36] as well as in tissue [35], while it was downregulated in serum [27]. On the other hand, the regulation of lysine showed inconsistency. It was downregulated in urine in two studies [23,37] and upregulated in one [24]. As for tissue regulation, one study reported upregulation [35], while another [45] described changes without specifying the trend in both urine and tissue.

Valine, one of the metabolites, exhibited more consistent regulation. Four urine studies found upregulation [22—24,36], along with tissue [35] and serum [30]; however, one study indicated changes in regulation without specifying the trend in both urine and serum [45]. Isoleucine showed upregulation in urine in two studies [24,36], as well as in tissue [35], while it was downregulated in serum [27]. Phenylalanine exhibited downregulation in urine in two

Table 2 Characteristics of the included studies.

Study	Country	Participant, *n*	Gender	Age, year	Analytical platform	Sample type	Cancer Pt, *n*	Control, *n*	Metabolite up-regulated in BCa	Metabolite down-regulated in BCa
Srivastava et al., 2010 [21]	India	103	• Male and female	• BCa: 45±25[a] (range 20–70) • Control: 35±15[a] (range 20–50)	• H-NMR	Urine	33	• 70 - HC: 37 - UTI: 31 - BS: 2	• Tau	• Hippuric acid, citrate, and Phe
Kim et al., 2010 [23]	Republic of Korea	19	• Male	• BCa: range 47–78 • Control: range 42–78	• GC-MS	Urine	11	• HC: 8	• Val, Leu, pyroglutamic acid, Thr, Phe, Asp, glutamic acid, asparagine, alpha-aminoadipic acid, Tyr, and Trp	• Ala, Gly, a-amino-butyric acid, serine, ornithine, Gln, and Lys
Pasikanti et al., 2010 [22]	Singapore	75	• Male and female	• BCa: 67.2±12.3[a] • Control: 61.3±12.4[a]	• GC/TOFMS	Urine	24	• Non-BCa Pts: 51	• Melibiose, uridine, and Val	• Senecioic acid, 2-butenedioic acid, ribonic acid, 2,5-furandicarboxylic acid, sumiki's acid, 2-propenoic acid, glycerol, gluconic acid, valerate, fructose, citric acid, and ribitol
Putluri et al., 2011 [24]	USA	134	• Male and female	NR	• LC-MS	Urine	83	• HC: 51	• Val, carnitine, Tyr, creatine, histidine, Leu/Ile, kynurenine, Phe, Lys, serine, thymine, guanine, and uracil	• Histamine and palmitic acid
Gamagedara et al., 2012 [26]	USA	23	• NR	NR	• LC-MS	Urine	11	• Non-BCa Pts: 12	• Tau	• NR
Huang et al, 2011 [25]	China	59	• Male and female	• BCa: mean 56 (range 42–71) • Control: mean 53 (range 46–67)	• LC-MS	Urine	27	• HC: 32	• NR	• Carnitine and hippuric acid

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Table 2 (*continued*)

Study	Country	Participant, *n*	Gender	Age, year	Analytical platform	Sample type	Cancer Pt, *n*	Control, *n*	Metabolite up-regulated in BCa	Metabolite down-regulated in BCa
Cao et al., 2012 [27]	China	110	• Male	• HC: 67.43±8.75[a] • Calculi: 55.04±11.34[a] • LBC: 60.26±14.52[a] • HBC: 70.27±11.0[a] • Post-TURBT: 66.65±9.42[a]	• H-NMR	Serum	37	• 73 - HC: 25 - CP: 28 - Post-TURBT: 20	• Glucose, acetoacetate, and very low-density lipoprotein	• Ile, Leu, Tyr, Phe, choline, lactate, Gly, and citrate
Huang et al., 2013 [31]	China	43	• Male and female	• BCa: mean 60.5 (range 45–74) • Control: mean 50.5 (range 26–65)	• LC-MS	Urine	19	• HC: 24	• Tau	• Carnitine and hippuric acid
Jobu et al., 2012 [32]	Japan	16	• NR	• NR	• GC-MS	Urine	9	• HC: 7	• Ethylbenzene, nonanoyl chloride, dodecanal, (Z)-2-nonenal, and 5-dimethyl-3(2H)-isoxazolone	• NR
Lin et al., 2012 [28]	China	68	• Male and female	• HC: mean 45 (range 21–68) • Nephrolithiasis: mean 54; range (31–74) • BPH: mean 70 (range 65–82) • BCa: mean 61 (range 45–74)	• LC-MS	Serum	20	• 48 - HC: 20 - Nephrolithiasis: 18 - BPH: 10	• Eicosatrienol, azaprostanoic acid, docosatrienol, retinol, and 14-apo-beta-carotenal	• NR
Tripathi et al., 2013 [35]	USA	59	• Male and female	• HC: 69.4±10.6[a] (range 51–87) • BCa: - Ta-T1: 60.1±11.5[a] (range 33–78) - ≥T2: 68.8±11.7[a] (range 43–87)	• HR-MAS-NMR GC-MS	Tissue	33	• HC: 26	• Triglycerides, Leu, Ile, Val, lactate, Ala, acetate, Lys, glutamate, glutathione, Gln, aspartate, creatine, choline, phosphocholine, glycerophosphocholine, Tau, Gly,	• NR

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Table 2 (*continued*)

Study	Country	Participant, *n*	Gender	Age, year	Analytical platform	Sample type	Cancer Pt, *n*	Control, *n*	Metabolite up-regulated in BCa	Metabolite down-regulated in BCa
									inositol, uridine diphosphate sugars, Phe, and Tyr	
Bansal et al., 2013 [30]	India	99	• Male	• >40	• H-NMR	Serum	67	• HC: 32	• Dimethylamine, malonate, lactate, histidine, Val, and Gln	• NR
Pasikanti et al., 2013 [29]	Singapore	99	• Male and female	• BCa: 68.3±10.9[a] • Control: 60.5±12.7[a]	• GC×GC-TOFMS	Urine	38	• Non-BCa Pts: 61	• Adipic acid, anthranilic acid, coumaric acid derivative, cyclopentane-1, 2-diamine, erythritol, erythro-pentonic acid, ethylmalonic acid, gluconic acid derivative, heptadecanoic acid, hydroxybutyric acid, itaconic acid, lactic acid, melibiose, N-acetylanthranilic acid, p-cresol, pseudouridine, uridine, vanillylmandelic acid, 2,3,4,5-tetrahydroxypentanoic acid-1,4-lactone, 2-aminoisobutyric acid, 2-hydroxymalonic acid, 2-pentadecanol, 3,4-dihydroxyphenyl pyruvate, 3-hydroxysebacic acid, 3-methyl-	• Citric acid, dihydroxyacetone, ethyl tartrate, gluconic acid, glycerol, levulinic acid enol, pinene, ribitol, ribonic acid, sebacic acid, Sumiki's acid, 2,5-furandicarboxylic acid, 2-butanedioic acid, and 2-hydroxyglutaric acid

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Table 2 (*continued*)

Study	Country	Participant, *n*	Gender	Age, year	Analytical platform	Sample type	Cancer Pt, *n*	Control, *n*	Metabolite up-regulated in BCa	Metabolite down-regulated in BCa
									adipic acid, and 4-methoxycinnamic acid	
Jin et al., 2014 [33]	Republic of Korea	259	• Male and female	• BCa: 65.64±12.65[a] • Control: 64.31±9.18[a]	• HPLC-QTOFMS	Urine	138	• 121 - HC: 69 - HU: 52	• Succinate, pyruvate, oxoglutarate, carnitine, phosphoenolpyruvate, trimethyllysine, isovalerylcarnitine, octenoylcarnitine, acetyl coenzyme A, carnitine palmitoyltransferase, and carnitine acylcarnitine	• Melatonin, glutarylcarnitine, decanoylcarnitine, and dihydrolipoyl dehydrogenase (pyruvate dehydrogenase complex)
Peng et al., 2014 [34]	China and Canada	285	• Male and female	• BCa: 68±14[a] • Control: 61±5[a]	• LC-MS	Urine	135	• 150 - Hernia: 82; - UTI or HU: 68	• 5-hydroxyindoleacetic acid, phosphoethanolamine, and pyroglutamic acid	• Uridine and histamine
Wittmann et al, 2014 [36]	USA	440	• Male and female	• BCa: mean 67.4 • Control: mean 64.2	• UHPLC-MS/MS-GC-MS	Urine	95	• Non-BCa Pts: 345	• Lactate, acetylcarnitine, palmitoyl sphingomyelin, adipate, Trp, gluconate, Ile, Val, Leu, phosphocholine, choline, aspartate, beta-hydroxypyruvate, and guanine	• 3-hydroxyphenylaceate, fructose, pyridoxate, succinate, xanthurenate, itaconate, 2-methylbutyrylglycine, pyruvic acid, citrate, tyramine, guanidinoacetate, anserine, gamma-aminobutyrate, creatine, adenosine, and ethanolamine

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Table 2 (*continued*)

Study	Country	Participant, *n*	Gender	Age, year	Analytical platform	Sample type	Cancer Pt, *n*	Control, *n*	Metabolite up-regulated in BCa	Metabolite down-regulated in BCa
Shen et al., 2015 [37]	China	44	• Male and female	• BCa: 65.14±13.27[a] • Control: 53.76 ±19.47[a]	• UPLC -HRMS	Urine	23	• HC: 21	• Nicotinuric acid, Asp, Gly, Trp, and trehalose	• Gly, Cys, Ala, Lys, inosinic acid, and ureidosuccinic acid
Zhou et al., 2016 [38]	China	140	• Male and female	• BCa: 66±12.6[a] • Control: 65.6±6.8[a]	• GC-MS-SIM	Plasma	92	• HC: 48	• Hypotaurine, uridine, malic acid, fumaric acid, creatinine, ribose, glycolic acid, gluconic acid, kynurenine, erythritol, ribonic acid, and serine	• Hippuric acid, hypoxanthine, lactose, xylitol, eicosenoic acid, and glyoxylic acid
Shao et al., 2017 [40]	China	122	• Male and female	• BCa: 68.2±14.5[a] • Control: 64.6±13.2[a]	• UPLCTOF-MS	Urine	87	• Hernia: 65	• Imidazoleacetic acid	• NR
Tan et al., 2017 [39]	China	172	• Male and female	• NR	• UHPLC-Q-TOFMS	Serum	120	• HC: 52	• 5-aminoimidazole ribonucleotide, hypoxanthine, inosine, acetyl-N-formyl-5-methoxykynurenamine, Indoleacetic acid, glycocholic acid, phytosphingosine, sphinganine, and linolenyl carnitine	• 3-hydroxyoctanoyl carnitine and 3-hydroxydecanoyl carnitine
Cheng et al, 2018 [42]	China	284	• Male and female	• BCa: 62.2±13.2[a] • Control: 59.5±11.2[a]	• LC-HRMS	Urine	167	• HC: 117	• Dopamine 4-sulfate, doxazosin, dihydroferulic acid 4-O-glucuronide, ofloxacin, 15-dehydro-prostaglandin E1(1-), 4-(ethoxymethyl) phenol, ovalicin, flunisolide, (Z)-13-hexadecenoic	• Aspartyl-histidine, tyrosyl-methionine, 3-hydroxycarbofuran, 1,2-dehydrosalsolinol, ecabet, gossyvertin, avocadyne 4-acetate, 13-hydroxy-9-methoxy-10-oxo-11-octadecenoic acid,

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Table 2 (*continued*)

Study	Country	Participant, *n*	Gender	Age, year	Analytical platform	Sample type	Cancer Pt, *n*	Control, *n*	Metabolite up-regulated in BCa	Metabolite down-regulated in BCa
									acid, and alpha-ionol O-[arabinosyl-(1->6)-glucoside]	fluoxymesterone, and 1-acetoxy-2-hydroxy-16-heptadecen-4-one
Yumba Mpanga et al., 2018 [41]	Poland	80	• Male and female	• BCa: median 62; • Control: median 60	• RP-HPLC-QQQ/MS	Urine	40	• HC: 40	• Trimethyllysine, Tau, citrulline, acetyllysine, glucuronic acid, gluconic acid, N-acetylneuraminic acid, pseudouridine, xanthine, uridine, 7-methylguanine, aconitic acid, and Trp	• 3,7-dimethyluric acid, 2-furoyglycine, 1,7-dimethylxanthine, and hippuric acid
Wei et al., 2019 [44]	China	30	• NR	• NR	• HS-GC-MS	Blood	15	• HC: 15	• Formaldehyde, acetaldehyde, propanal, butanal, pentanal, hexanal, and heptanal	• NR
Jacyna et al, 2019 [43]	Poland	48	• Male	• NR	• HPLC-TOF/MS, GC-QQQ/MS, and HNMR	Urine	24	• HC: 24	• 2-deoxy-ribonic acid, diacetylspermine, meso-erythritol, Gln, lactic acid, pentanedioic acid, phenylacetylglutamine, propanoic acid, threonic acid, Tyr, uric acid, and uridine	• S-adenosylmethionine, benzenediol, glycolic acid, hippuric acid, and pipecolic acid
Loras et al., 2019 [45]	Spain	34	• Male and female	• NR	• H-NMR	Tissue and urine	21	• Non-BCa Pts: 13	• Ala, Gln, glutamate, Tau, Pro, Gly, Thr, Phe, Val, Lys, methanol, creanine, choline,	• Ala, Gln, glutamate, Tau, Pro, Gly, Thr, Phe, Val, Lys, methanol, creanine, choline,

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Table 2 (*continued*)

Study	Country	Participant, *n*	Gender	Age, year	Analytical platform	Sample type	Cancer Pt, *n*	Control, *n*	Metabolite up-regulated in BCa	Metabolite down-regulated in BCa
									glycerol, glycerophosphocholine, phosphocholine, citrate, succinate, hippuric acid, lactic acid, Tau, and sucrose	glycerol, glycerophosphocholine, phosphocholine, citrate, succinate, hippuric acid, lactic acid, Tau, and sucrose
Lin et al, 2021 [46]	China	124	• Male and female	• BCa: 67.4±13.5[a] • Control: 65±12[a]	• GC-MS	Urine	63	• Hernia: 61	• Desaminotyrosine, erythritol, d-ribose, ribitol, d-fructose, d-mannose, and d-galactose	• NR
Luczykowski et al, 2021 [47]	Poland	48	• Male and female	• BCa: 65±12[a] • Control: 64±10.4[a]	• LC-MS	Urine	24	• HC: 24	• 2-acetyl-1-alkyl-sn-glycero-3, phosphocholine, and adenine	• 3-dehydroxycarnitine, 3-methylxanthine, 4-hydroxycinnamic acid, 5-hydroxyindoleacetic acid, benzoic acid, carnosine, epinephrine, hippuric acid, histidine, isoniazid, n-acetyl-Phe, p-aminobenzoic acid, retinol, theophylline, gluconic acid, and indolelactic acid
Pinto et al., 2021 [48]	Portugal	109	• Male and female	• BCa: 68.9±10.6[a] • Control: 51±5.2[a]	• HS-SPME-GC-MS	Urine	53	• Non-BCa Pts: 56	• 2-methylnonane, 2,4-dimethylheptane, 2,6-dimethylnonane,	• 2-furaldehyde, 2-methylbutanal, formaldehyde, hexanal, 2-butanone, 4-

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Table 2 (*continued*)

Study	Country	Participant, *n*	Gender	Age, year	Analytical platform	Sample type	Cancer Pt, *n*	Control, *n*	Metabolite up-regulated in BCa	Metabolite down-regulated in BCa
									1-methylnaphthalene, 2-methylnaphthalene, 1,2,4-trimethylbenzene, and p-cresol	heptanone, carvone, piperitone, and 1,5-dimethyl-6,8-dioxabicycloctane
Li et al, 2022 [49]	China	95	• Male and female	• BCa: 65.8±3.5[a] • Control: 61±4.1[a]	• LC-MS	Urine	57	• Non-BCa Pts: 38	• Isoleucyl-Phe, choline, 1-methylhistidine, n-undecanoylglycine, linoleyl carnitine, adenosine-monophosphate, niacinamide, phenylalanyl-asparagine, and Tau	• Mesobilirubinogen, 2-hydroxycaproic acid, urobilin, 6-hydroxyhexanoic acid, cytidine, dihydrotestosterone, histidine, flavin mononucleotide, valylserine, 4-acetamidobutanoic acid, (5R)-5-hydroxyhexanoic acid, adenosine, arginine, 1,11-undeanedicarboxylic acid, ubiquinone-2, butenylcarnitine, sebacic acid, and alanyl-tyrosine
Jacyna et al., 2022 [50]	Poland	48	• Male and female	• 69.6±5.6[a]	• HPLC-TOF/MS, GC-QqQ/MS and H-NMR	Urine	24	• HC: 24	• Benzenediol, 2-deoxy-ribonic acid, diacetylspermine, meso-erythritol, Gln, hippuric acid, lactic acid, pentanedioic acid, phe-	• Benzenediol, 2-deoxy-ribonic acid, diacetylspermine, meso-erythritol, Gln, hippuric acid, lactic acid, pentanedioic acid,

(*continued on next page*)

Table 2 (*continued*)

Study	Country	Participant, *n*	Gender	Age, year	Analytical platform	Sample type	Cancer Pt, *n*	Control, *n*	Metabolite up-regulated in BCa	Metabolite down-regulated in BCa
									nylacetylglut-amine, pipecolic acid, propanoic acid, threonic acid, Tyr, uric acid, and uridine	phenylacetylglut-amine, pipecolic acid, propanoic acid, threonic acid, Tyr, uric acid, and uridine

Pt, patient; BCa, bladder cancer; UTI, urinary tract infection; HU, hematuria; BS, bladder stone; NMR, nuclear magnetic resonance; H-NMR, proton NMR; MS, mass spectrometry; GC, gas chromatography; TOFMS, time-of-flight MS; NR, not reported; LC, liquid chromatography; HC, healthy controls; CP, calculi patient; TURBT, transurethral resection of bladder tumor; BPH, benign prostatic hyperplasia, ; HR-MAS-NMR, high resolution-magic angle spinning-NMR; GC×GC-TOFMS, two-dimensional GC TOFMS; UHPLC-MS/MS, ultrahigh-performance liquid chromatography/tandem MS; UPLC-HRMS, ultra-performance liquid chromatography and direct infusion HR MS; SIM, selected ion monitoring; UPLCTOF, ultra-performance LC TOF; UHPLC-QTOFMS, UHPLC-quadrupole TOFMS; HPLC, high-performance liquid chromatography; RP-HPLC-QQQ/MS, reverse-phase HPLC coupled with triple quadrupole MS; HS-GC-MS, chromatography-MS coupled with a headspace generator sampler; HS-SPME-GC-MS, headspace solid-phase microextraction GC MS; LBC, low-grade bladder cancer; HBC, high-grade bladder cancer; Asp, aspartic acid; Gly, glycine; Trp, tryptophan; Cys, cysteine; Ala, alanine; Lys, lysine; Thr, threonine; Gln, glutamine; Tyr, tyrosine; Leu, leucine; Ile, isoleucine; Phe, phenylalanine; Val, valine; Pro, proline; Tau, taurine.

[a] Values are presented as mean±standard deviation.

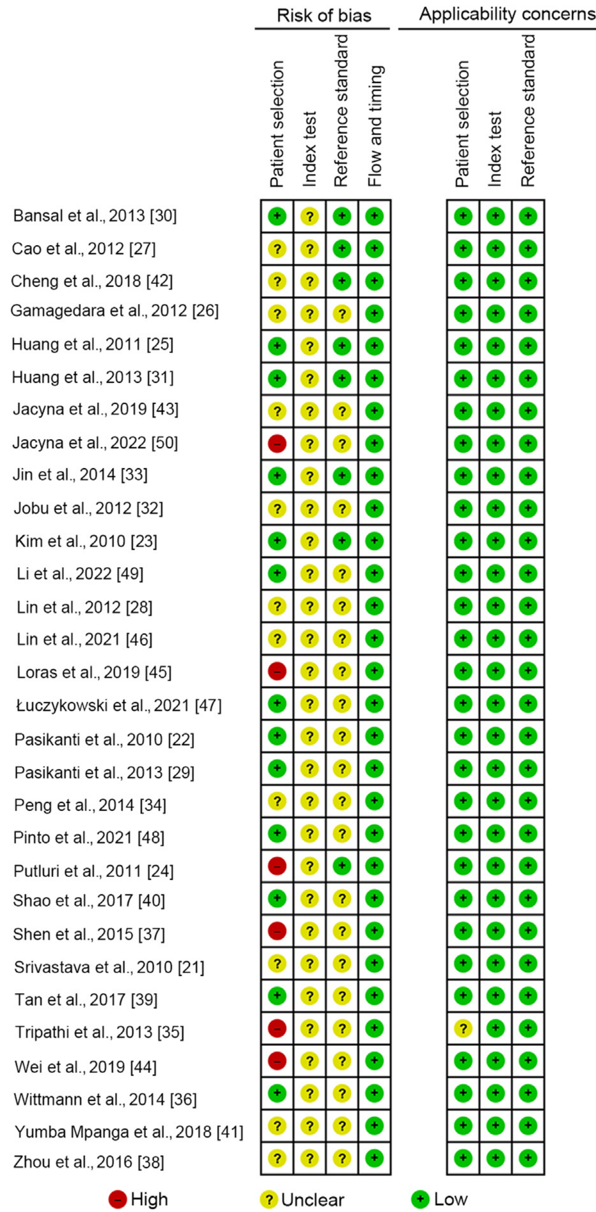

Figure 2 Risk of bias assessment within studies.

studies [21,47], as well as in serum [27], while it was upregulated in tissue [35]. Tryptophan consistently showed upregulation in urine [23,36,37,41]. Lastly, threonine was found to be upregulated in urine in one study [23], while another study indicated an indeterminate regulation [45].

When it comes to non-essential amino acids, serine exhibited inconsistent regulation in urine. One study reported downregulation [23], while another study indicated upregulation [24]. Cysteine showed downregulation in urine [37]. Aspartic acid demonstrated upregulation in urine [23,37] and tissue [35]. Proline showed changes in regulation without a specific trend in both urine and tissue [45]. Arginine was downregulated in urine [49].

Regarding tyrosine, consistent upregulation was observed in urine in three studies [23,24,43] and one study reported changes in regulation without specifying the trend [50]. In tissue, it was upregulated [35], while in

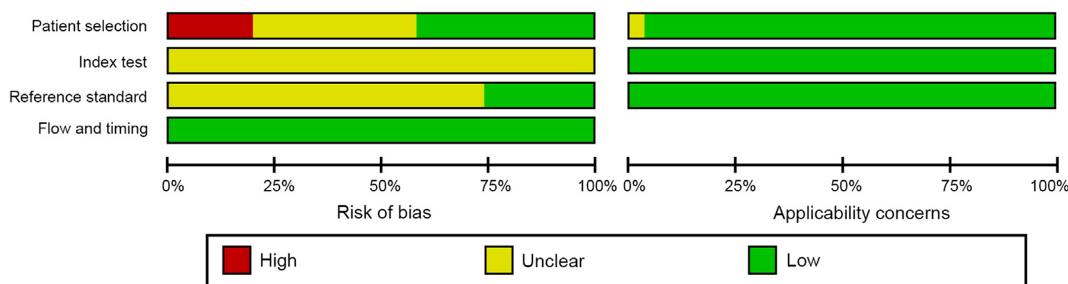

Figure 3 Risk of bias assessment across studies.

serum, it was downregulated [27]. Arginine was downregulated in urine [49], while asparagine exhibited upregulation [23].

Glutamate showed upregulation in tissue [35]. However, another study described changes in regulation without specifying the trend in both urine and tissue [45]. Glycine yielded inconsistent results, with upregulation in urine in one study [37] and downregulation in another [23]. In serum, it was downregulated [27], while in tissue, it was upregulated [35].

Alanine displayed inconsistent regulation in urine, with upregulation in one study [23] and downregulation in another [37]. In tissue, it showed upregulation [35]. However, both urine and tissue studies described changes in regulation without a specific trend [45]. Similarly, histidine showed inconsistent results. In urine, two studies reported upregulation [24,36], while two studies reported downregulation [47,49]. It was upregulated in serum [30].

Taurine exhibited consistent regulation, with upregulation in urine in five studies [21,25,26,41,49], and one study reported upregulation in tissue [35]. However, one study indicated changes in regulation without specifying the trend [45]. Glutamine showed upregulation in urine in two studies [36,43], while one study reported downregulation [23]. It was upregulated in serum [30] and tissue [35], while two studies described changes in regulation without specifying the trend [45,50] (Table 4).

3.5. Nucleotide metabolites

Nucleotides are the building blocks of nucleic acids. They have main roles in many cellular processes. These building blocks are composed by a five-carbon sugar (ribose or deoxyribose), a nitrogen base, and one or more phosphate groups. Nitrogenous bases are classified into purines and pyrimidines. The purines include adenine and guanine, while the pyrimidines are cytosine, thymine, and uracil [54]. Table 5 shows the significant variations of nucleotide metabolites in BCa samples.

Regarding purines, adenine was downregulated in urine [36,49], while guanine was upregulated [24,36]. In terms of pyrimidines, uridine exhibited the most consistent regulation. In urine, four studies reported upregulation [22,29,41,43]. However, one study observed downregulation [34], and another study reported a change in regulation without specifying the trend [50]. Additionally, uridine was upregulated in tissue [35] and plasma [38]. Continuing with pyrimidines, thymine and uracil were upregulated in urine [24]. Furthermore, inosine showed upregulation in serum [39], while hypoxanthine exhibited upregulation in serum [39] and downregulation in plasma [38]. Ribose also demonstrated consistent regulation, being upregulated in urine [29,46] and in plasma [38].

3.6. Lipid metabolites

The BCa patients showed significant variations in the concentration of lipid metabolites in urine, serum, and tissue, as depicted in Supplementary Table 1. In urine, choline [36,49], phosphocholine [36,47], and carnitine [24,33,36] exhibited upregulation, while ethanolamine [36] and glycerol [22,29] showed downregulation. In tissue, choline, phosphocholine, glycerophosphocholine, inositol, and triglycerides were upregulated [35]. Conversely, in serum, choline was downregulated, while acetoacetate was upregulated [27]. Furthermore, a study was conducted in urine and tissue describing changes in the regulation of choline, phosphocholine, and glycerophosphocholine, without specifying their trends [45].

3.7. Aldehydes metabolites

Aldehydes, the carbonyl compounds derived from both natural and artificial origin, can be found everywhere [55]. Endogen aldehydes are part of lipidic, amino acids, vitamins, and carbohydrates metabolism. Regarding cancer, human tumoral cells produce large amounts of free radicals that increase the aldehyde concentration. Consequently, these molecules can be considered as possible cancer biomarkers [56]. Supplementary Table 2 presents the findings concerning aldehyde-derived metabolites. Formaldehyde showed an upregulation in blood [44]; however, in urine, it exhibited downregulation [48]. As for ethanal, propanal, butanal, pentanal, heptanal, and hexanal, they displayed upregulation in blood [44]. However, hexanal also showed downregulation in urine [48]. On the other hand, nonenal and dodecanal demonstrated upregulation in urine [32].

4. Discussion

4.1. Summary of findings

As a result, we found endogen aldehydes in blood samples. In urine were citric acid, valine, tryptophan, taurine, aspartic acid, uridine, ribose, phosphocholine, and

Table 3 Glucose metabolism-related metabolites in bladder cancer.

Study	Sample type	Analytical platform	Anaerobic oxidation (glycolysis)					Aerobic oxidation (the TCA cycle)				
			Glucose	Fructose	Pyruvic acid	Lactose	Lactic acid	Citrate or citric acid	Succinate	Malic acid	Fumaric acid	Itaconate
Srivastava et al., 2010 [21]	Urine	H-NMR						↓				
Kim et al., 2010 [23]	Urine	GC-MS										
Pasikanti et al., 2010 [22]	Urine	GC/TOFMS		↓				↓				
Cao et al., 2012 [27]	Serum	H-NMR	↑				↓	↓				
Bansal et al., 2013 [30]	Serum	H-NMR					↑					
Pasikanti et al., 2013 [29]	Urine	GC×GC-TOFMS						↓				
Tripathi et al., 2013 [35]	Tissue	HR-MAS-NMR GC-MS					↑					
Jin et al., 2014 [33]	Urine	HPLC-QTOFMS			↑				↑			
Wittmann et al., 2014 [36]	Urine	UHPLC-MS/MS GC-MS		↓	↓		↑	↓	↓			↓
Zhou et al., 2016 [38]	Plasma	GC-MS-SIM				↓				↑	↑	
Jacyna et al., 2019 [43]	Urine	MS and H-NMR					↑					
Loras et al., 2019 [45]	Urine	NMR					↓↑	↓↑	↓↑			
Jacyna et al., 2022 [50]	Urine	MS and H-NMR					↑					

TCA, tricarboxylic acid cycle; NMR, nuclear magnetic resonance; H-NMR, proton NMR; GC, gas chromatography; MS, mass spectrometry; TOFMS, time-of-flight MS; GC×GC-TOFMS, two-dimensional GC-TOFMS; HR-MAS-NMR, high resolution-magic angle spinning NMR; HPLC-QTOFMS, high-performance liquid chromatography-quadrupole TOFMS; UHPLC-MS/MS, ultrahigh-performance liquid chromatography/tandem MS; SIM, selected ion monitoring. ↓, downregulated; ↑, upregulated; ↓↑, undetermined trend.

carnitine. In addition, there was an increase in lactic acid, amino acid, and lipidic metabolites in tissue samples. Besides, the most consistent results in all samples (tissue, urine, serum, and blood) were downregulation of citric acid and upregulation of lactic acid, valine, tryptophan, taurine, glutamine, aspartic acid, uridine, ribose, and phosphocholine.

4.2. Clinical applications of metabolomics

Nowadays the clinical application of metabolomics is limited; however, the number of potential applications has been increasing and might change the clinical practice in the future [57]. In oncology, the metabolomics profiling has been associated with the diagnosis of several kinds of tumors, more noticeable in breast cancer, where there have been observed over 30 endogenous metabolites that might be used as biomarkers [57,58].

In addition to its potential in diagnosis, metabolomics has also been used to guide oncologic surgeries. Inglese et al. [59] managed to couple rapid ionization MS with electrosurgical tools to characterize the margins while performing the tumor dissection. This approach enables a real-time metabolomics and surgical process with promising live results, although at high-cost, resulting in an important limitation of its use. Besides the already mentioned applications in oncology [57], Tenori et al. [60] found the relationship between the metastatic breast cancer survival rate and phenylalanine serum levels prior treatment.

In neurology, it allows to obtain the metabolomics profiling to identify patients who have suffered an ischemic event; due to studies carried out on its most common etiologies and the correlation with specific metabolites, metabolomics has the potential to achieve a significant role in the study of cryptogenic ictus [61]. In endocrinology, it has been used to quantify type 2 diabetes mellitus risk. Wang et al. [62], suggested that the 2-aminoadipic acid metabolite is a diabetes risk marker as they found that its concentration in the highest quartile elevated four times diabetes risk, and this could be identified 12 years before the disease becomes clinically evident.

In conclusion, metabolomics is becoming a widely used tool in multiple areas of medical practices and specializations as mentioned before. Its main utility is seeing in novel research with the characterization of metabolic pathways in order to find, as shown in this review, specific biomarkers identified different types of diseases in early stages.

4.3. Contrast with literature

The BCa prognosis might improve if an early diagnosis of the disease is made by the identification of biomarkers and especially if it is possible to be done in a non-invasive way, therefore reducing the morbidity of the tests. Urine is the ideal sample to perform the tests due to the proximity of the bladder to the urine itself; however, blood, tissue, and serum are also useful. It is known that disturbances of several metabolic routes are involved in BCa, which represents an essential tool for its diagnosis [63].

Table 4 Metabolites of amino acid metabolism in bladder cancer.

Study	Sample type	Analytical platform	Essential amino acid								Non-essential amino acid												
			Leu	Met	Lys	Val	Ile	Phe	Trp	Thr	Ser	Cys	Asp	Pro	Arg	Tyr	Asn	Glu	Gly	Ala	His	Tau	Gln
Srivastava et al., 2010 [21]	Urine	H-NMR						↓														↑	
Kim et al., 2010 [23]	Urine	GC-MS	↑		↓	↑			↑	↑	↓		↑			↑	↑		↓	↑			↓
Pasikanti et al., 2010 [22]	Urine	GC/TOFMS				↑																	
Gamagedara et al., 2012 [26]	Urine	LC-MS																				↑	
Putluri et al., 2011 [24]	Urine	LC-MS	↑		↑	↑	↑				↑					↑					↑		
Huang et al., 2011 [25]	Urine	LC-MS																				↑	
Cao et al., 2012 [27]	Serum	H-NMR	↓				↓	↓								↓			↓				
Bansal et al., 2013 [30]	Serum	H-NMR				↑															↑		↑
Tripathi et al., 2013 [35]	Tissue	HR-MAS-NMR GC-MS	↑		↑	↑	↑	↑					↑			↑		↑	↑	↑		↑	↑
Wittmann et al., 2014 [36]	Urine	UHPLC-MS/MS GC-MS	↑			↑	↑		↑												↑		↑
Shen et al., 2015 [37]	Urine	UPLC-HRMS			↓				↑			↓	↑						↑	↓			
Yumba Mpanga et al., 2018 [41]	Urine	RP-HPLC-QQQ/MS							↑													↑	
Jacyna et al., 2019 [43]	Urine	MS and H-NMR														↑							↑
Loras et al., 2019 [45]	Tissue and urine	H-NMR			↑↓	↑↓				↑↓				↑↓				↑↓		↑↓		↑↓	↑↓
Łuczykowski et al., 2021 [47]	Urine	LC-MS						↓													↓		
Li et al., 2022 [49]	Urine	LC-MS													↓						↓	↑	
Jacyna et al., 2022 [50]	Urine	MS and H-NMR														↑↓							↑↓

Leu, leucine; Met, methionine; Lys, lysine; Ile, isoleucine; Val, valine; Phe, phenylalanine; Trp, tryptophan; Thr, threonine; Ser, serine; Cys, cysteine; Asp, aspartic acid; Pro, proline; Arg, arginine; Tyr, tyrosine; Asn, asparagine; Glu, glutamate; Gly, glycine; Ala, alanine; His, histidine; Tau, taurine; Gln, glutamine; MS, mass spectrometry; NMR, nuclear magnetic resonance; H-NMR, proton NMR; GC, gas chromatography; TOFMS, time-of-flight MS; LC, liquid chromatography; HR-MAS-NMR, high resolution magic angle spinning NMR; UHPLC-MS/MS, ultrahigh-performance liquid chromatography/tandem MS; UPLC-HRMS, ultra-performance liquid chromatography and direct infusion high-resolution MS; RP-HPLC-QQQ/MS, reverse-phase high-performance liquid chromatography coupled with triple quadrupole MS. ↓, downregulated; ↑, upregulated; ↓↑, undetermined trend.

Table 5 Nucleotide metabolites in bladder cancer.

Study	Sample type	Analytical platform	Purine		Pyrimidine						
			Adenine	Guanine	Cytosine	Thymine	Uridine	Uracil	Inosine	Hypoxanthine	Ribose
Pasikanti et al., 2010 [22]	Urine	GC/TOFMS					↑				
Putluri et al., 2011 [24]	Urine	LC-MS		↑		↑		↑			
Tripathi et al., 2013 [35]	Tissue	H-NMR, GC-MS					↑				
Pasikanti et al., 2013 [29]	Urine	GC×GC-TOFMS					↑				↑
Peng et al., 2014 [34]	Urine	LC-MS					↓				
Wittmann et al., 2014 [36]	Urine	UHPLC-MS/MS GC-MS	↓	↑							
Zhou et al., 2016 [38]	Plasma	GC-MS-SIM					↑			↓	↑
Tan et al., 2017 [39]	Serum	UHPLC-Q-TOFMS							↑	↑	
Yumba Mpanga et al., 2018 [41]	Urine	RP-HPLC-QQQ/MS					↑				
Jacyna et al., 2019 [43]	Urine	MS, H-NMR					↑				
Lin et al., 2021 [46]	Urine	GC-MS									↑
Li et al., 2022 [49]	Urine	LC-MS	↓								
Jacyna et al., 2022 [50]	Urine	MS, H-NMR					↑↓				

GC, gas chromatography; MS, mass spectrometry; TOFMS, time-of-flight MS; LC, liquid chromatography; H-NMR, proton nuclear magnetic resonance; UHPLC-MS/MS, ultrahigh-performance liquid chromatography/tandem MS; SIM, selected ion monitoring; UHPLC-QTOFMS, UHPLC-quadrupole TOFMS; RP-HPLC-QQQ/MS, reverse-phase high-performance liquid chromatography coupled with triple quadrupole MS. ↓, downregulated; ↑, upregulated; ↓↑, undetermined trend.

Cheng et al. [64] pooled 11 studies which described metabolites to detect BCa in a systematic review, with different techniques, methodological limitations, and high heterogeneity. They found significant changes in metabolite concentrations of glucose, amino acids, nucleotides, and lipidic pathways [64]. In our study, we identified metabolites involved in a novel metabolic pathway, known as the endogenous aldehyde pathway [32,44,48].

Regarding the altered expression of glucose metabolites, their results were inconclusive about glucose, fructose, or lactic acid, since they were found up in some studies and down in others [64]. Nevertheless, citric acid was found down in different studies. Among BCa patients, the low concentration of citric acid and high concentration of lactic acid in urine were the most representative metabolites which were altered in at least three studies from the same kind of samples in our review, making them potential biomarkers for the diagnose of this kind of cancer.

One of the main characteristics of cancerous cells is the energy metabolism disturbance. Normal cells produce ATP using two mechanisms: glycolysis and the tricarboxylic acid cycle (the Krebs cycle). The representative disturbance of the energy metabolism in cancerous cells is the increase of glucose absorption and its fermentation into lactate. This metabolic change towards anaerobic glycolysis, even in an environment with a normal oxygen concentration, is what can be called an "aerobic glycolysis", also known as Warburg effect [65]. This process results in the biosynthesis of necessary precursors to trigger growth and cellular division, such as ribose, acetyl-coenzyme A, and glycolytic intermediates to produce nucleotides, fat acids, and non-essential amino acids, respectively. Furthermore, the lactic acid increase reduces the pH of the microenvironment leading to the death of non-cancerous cells because they do not possess the mechanisms to adapt the extracellular acidity (such as *p53* mutation), choosing those resistant cells and allowing the tumoral progression. As glycolysis heightens, it produces not only an increase in lactic acid but also a corresponding decrease in the metabolites related to the tricarboxylic acid cycle, therefore reducing the synthesis of citric acid, which is compatible with the findings from a systematic review [66].

Concerning the altered expression of amino acid metabolites, essential amino acids (threonine, phenylalanine, valine, isoleucine, lysine, methionine, and leucine) and non-essential amino acids (glutamate, histidine, arginine, aspartic acid, tyrosine, glutamine, and serine) were upregulated. In our review, the amino acids found upregulated in at least three studies were leucine, taurine, valine, isoleucine, tryptophan, aspartic acid, tyrosine, and glutamine, making them potential biomarkers for the diagnose of BCa. Histidine was upregulated in three studies [24,30,36]; nonetheless, it was downregulated in other two, therefore considered an inconsistent result [47,49].

The findings are consistent with the fact that the glutaminolysis rate increases within cancerous cells as an energy source for its proliferation [67]. In addition, tryptophan metabolism alterations have been broadly associated with BCa. Serotonin, indole, and kynurenine metabolic pathways use tryptophan. If there is an

excessive absorption of tryptophan plus a vitamin B6 deficiency, it might result in an accumulation of the mentioned kynurenine pathway metabolites within the bladder which can combine with nitrite becoming an oncogenic promoter as mutagenic nitrosamines and, in this location, can produce BCa. They can also form reactive oxygen species when interacting with transition metals such as copper and iron, and when tryptophan is exposed to ultraviolet light or visible light, it can also form more carcinogenic products [68]. Lee et al. [69] established that kynurenine to tryptophan proportions in both plasma and urine were significantly higher in patients with BCa. Cheng et al. [64] found that metabolites involved in tryptophan metabolism were upregulated in the high-grade NMIBC patients when compared with the metabolites for the low-grade NMIBC ones. In addition, expression of tryptophan 2,3-dioxygenase has been reported as a potential target in immunotherapy of BCa [70].

About nucleotides, the previously mentioned authors found elevated levels of guanine, cytosine, thymine, hypoxanthine, uracil, and ribose in the urine of BCa patients. In our review, the most representative metabolites were uridine and ribose which were upregulated in at least three studies, while in two separate studies, adenine was found to be downregulated [36,49] and guanine was upregulated [24,36]. Both studies utilized urine samples. The levels of hypoxanthine were inconsistent [38,39].

Damaged metabolic pathways develop within the tumor as they grow. Recent discoveries showed that tumor growth and immune system inhibition will occur whenever there is an aberrant nucleotide metabolism. There are a few studies about this matter but they are on a fast-experimenting development. The more discoveries about these pathways and mechanisms, the more strategies can be generated to intervene, regulate, and alter them to prevent or treat the tumors and their development. According to studies from Tan et al. [39], hypoxanthine and inosine serum levels increased in a significant way alongside cancer progression. For instance, in patients with low degree BCa, the increment was higher than that in healthy patients. Additionally, significantly higher levels of hypoxanthine and inosine were encountered in the serum of patients with advanced-stage BCa compared to those with a lower stage. These results suggest an upregulation of purines metabolism [39].

We observed in our study the rise in most of the intermediate lipidic metabolites, which was consistent with findings by Cheng et al. [64]. In our study, the metabolites found to be upregulated in at least three studies were choline, phosphocholine, and carnitine. It has been observed in multiple studies that lipidic metabolism has a vital role in the carcinogenesis and metastasis of cancer. Growth and tumor proliferation demand a critical amount of energy which translates into an increase in lipidic synthesis to ensure the survival of cancerous cells [71]. The importance of the lipidic metabolism is such that in the carcinogenesis simvastatin observed by Wang et al. [72], one of the 3-hydroxy-3-methyl glutaryl coenzyme A reductase inhibitors could reduce BCa cell proliferation and inhibit metastasis producing a stop in the cell cycle.

Endogen aldehydes are intermediate or final metabolic products of a broad spectrum of biochemical and physiological processes, such as oxidative stress and cellular activities. These compounds are generated from free radical induced reactions with cellular lipids. Biochemical processes that contribute to their formation are glycation, aminoacidic, alcohol, and sugar metabolism, with lipidic peroxidation being the main endogen source for them [73]. Due to the increased oxidative stress produced by human tumor cells, the upregulation of endogen aldehydes has been considered as a tumor biomarker.

In addition, Wei et al. [44] identified that average aldehyde concentrations in cancer patients' blood were several times higher than those in healthy controls. This resulted in a remarkable significant difference according to what is described for methanal, ethanal, propanal, pentanal, hexanal, and heptanal ($p<0.001$) and a significant difference for hexanal ($p<0.01$). Jobu et al. [32] found five substances in urine with the potential to become biomarkers for BCa; two of them belong to endogen aldehyde metabolism (nonenal and dodecanal).

4.4. Strengths and limitations

Regarding the strengths, we followed the standard recommendation of Cochrane and Preferred Reporting Items for Systematic reviews and Meta-Analyses. We found this study as a very focused, comprehensive systematic review that serves as a starting point to diagnose BCa non-invasively. In our review, the inclusion of endogenous aldehydes as components of novel metabolic pathways implicated in cancer development proved to be of utmost significance. These findings, in particular, garnered significant attention and are regarded as potentially valuable tumor biomarkers.

On the other side, as limitations, the results were heterogeneous due to diverse samples and techniques. The discrepancy on patients' origins within the studies, as well as their economic status and diet may be responsible for resulting in different metabolic alterations. Urine samples, as the most used ones among the studies, vary drastically depending on lifestyle and diet [74,75]. Besides, there is also another factor making this tool even more complicated and is the high variation of a cancer cell metabolism which is subject to different signals produced by the tumor itself. In addition, some researches were carried out listing NMIBC and MIBC in the same category when examining different samples even though their metabolic profilings are most likely different from each other, all these resulting in a quite heterogenic metabolic profiling. Besides, there was not enough information to correctly classify the risk of bias regarding the patient selection, the blinding of the index test, or the reference standard assessment.

Based on the current literature, it is not possible to determine whether the metabolites detected are predisposing factors or a consequence of BCa. Further epidemiologic studies investigating this issue would be of great value.

5. Conclusion

BCa has disturbances in multiple metabolic pathways, mainly those involved in energy synthesis. In our review, the most consistent metabolites found in urine were citric acid, valine, tryptophan, taurine, aspartic acid, uridine, ribose, phosphocholine, and carnitine. In blood samples, endogen aldehydes are highlighted as potential biomarkers. In tissue, there was an increase in lactic acid, amino acid, and lipidic metabolites. The most consistent results in all samples (tissue, urine, and serum) were downregulation of citric acid and upregulation of lactic acid, valine, tryptophan, taurine, glutamine, aspartic acid, uridine, ribose, and phosphocholine, which led to consider them as BCa biomarkers. Nevertheless, the clinical applicability of metabolomics in the diagnostic of BCa requires further studies.

Author contributions

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Data acquisition: Herney Andrés García-Perdomo, Angélica María Dávila-Raigoza, Fernando Korkes.
Data analysis: Herney Andrés García-Perdomo, Angélica María Dávila-Raigoza, Fernando Korkes.
Drafting of manuscript: Herney Andrés García-Perdomo, Angélica María Dávila-Raigoza, Fernando Korkes.
Critical revision of the manuscript: Herney Andrés García-Perdomo, Angélica María Dávila-Raigoza, Fernando Korkes.

Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajur.2022.11.005.

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