Metabolomics and Bladder Cancer

Subjects: Biochemistry & Molecular Biology

Contributor: DANIEL CICERO

In the last decade, metabolomics has tried to assert its value in the bladder cancer field. Due to the current invasive diagnostic techniques, such as cystoscopy and the continuous monitoring patients must undergo, the introduction of non-invasive urinary biomarkers for monitoring this disease would be advantageous. This section represents a collection of all the metabolic information that various studies have obtained in recent years on bladder cancer, with particular attention to discovering biomarkers in urine for the diagnosis of this disease. In principle, they would complement cystoscopy or, at best, replace it. However, evaluating the different degrees of reproducibility that the experiments have shown in the indication of biomarkers, a synthesis was proposed to obtain a consensus list that is more reliable to become a guideline for clinical practice.

bladder cancer metabolomics biomarkers urine

1. The Critical Challenge of Bladder Cancer Diagnosis

Bladder cancer (BC) is the most common urinary tract cancer and a leading cause of mortality worldwide, with approximately 550,000 new cases and 160,000 deaths per year [1]. The incidence of bladder cancer differs according to the geographical region considered: the age-standardized incidence (ASI) is one-third less in undeveloped with respect to high-developed countries. The World Health Organization (WHO) predicts a rise in cases and deaths for the near future due to increased life expectancy [2].

BC encompasses a wide range of histologies: urothelial carcinoma (UC), which represent the majority (~90–95%) of bladder tumors, squamous cell carcinoma (SCC) (2–5%), adenocarcinoma (0.5–2%), and small cell carcinoma (<1%). BC's risk factors include occupational factors, age, sex, race, socioeconomic status, personal health, diet, and infection by pathogens [3][4][5]. BC tumors are divided into two classes depending on whether they invade the detrusor muscle (muscle-invasive bladder cancer, MIBC) or not (non-muscle-invasive bladder cancer, NMIBC). The first presents a higher risk of metastasis of lymph nodes or other organs but, fortunately, represents only 25% of diagnosticated BC cases [2]. NMIBC generally involves the fibroblast growth factor receptor 3 (*FGFR3*) mutation, producing cancer with a high recurrence rate but a low risk of progression. By contrast, MIBC and carcinoma in situ exhibit deletions or mutations of *TP53*, RB transcriptional corepressor 1 (*RB1*), erb-b2 receptor tyrosine kinase 2 or *PTEN*, leading to metastatic cancer [6]. A link between some of these genotypes and cell phenotypes was recently observed, leading to the result that cell lines associated with a low risk of progression present an activated oxidative metabolic state, while those associated with a high risk present a non-oxidative state and high glycolytic activity [7].

Evaluation of patients suspected of having BC is performed using cystoscopy, an invasive endoscopic procedure performed with a flexible scope and with local anesthesia [8]. Histological evaluation is required if reddish flat papillary or solid lesions are observed because benign conditions like inflammatory diseases can mimic BC. Trans Urethral Resection of Bladder Tumor (TURBT) or resection of the entire area is used to obtain information about the histology of tumors. In addition, an inspection of cells in the urine (cytology) can be performed to detect missed cancer. Cells with a malignant appearance indicate cancerous lesions in the bladder and warrant cystoscopy and histological investigation.

2. Metabolomics of Bladder Cancer

The introduction of metabolic markers for an accurate diagnosis of BC and its risk of progression may decrease disease management costs and increase patients' quality of life [9]. The availability of non-invasive markers for diagnosis would also improve patients' susceptibility to routine screening, thereby increasing the effectiveness of preventive diagnostics. Therefore, it is essential to render prevention non-invasive and thus more efficient, even without apparent symptoms. In addition, the biochemical interpretation of the metabolic unbalances that can result from these screenings can open new opportunities for development of more effective therapies and monitoring of treatment and disease evolution.

Metabolomics may be the most appropriate way to achieve this goal. In the particular case of BC, the direct contact of the tumor with urine makes it feasible that specific biomarkers can be present in this fluid. Many specific reviews have been published about urinary markers of BC, testifying to the great interest in this field [10][11][12][13][14][15][16][17][18][19][20]. We have revised most available data on urinary metabolomics and bladder cancer to answer three key questions: (i) is it possible to use the urinary metabolic profile to detect BC? (ii) In the case of a positive answer, what are the metabolites responsible for this difference? (iii) What is the origin of the observed metabolic imbalances?

2.1. Can We Diagnose Bladder Cancer by Analyzing the Urinary Profile?

Twenty-five papers about discovering BC diagnostical metabolic markers in urine have been published in the literature (**Table 1**). Almost all the studies have used MS coupled with gas or liquid chromatography for the quantification of metabolites. The relatively low number of works using NMR reflects the complexity of urine as a biofluid because it contains many compounds and highly variable composition.

Table 1. List of research works on urinary metabolomics for the discovery of BC diagnostic biomarkers.

References	Platform	Control Group (CTRL)			Bladder Cancer Patients (BC)		
		Type	%M [a]	Age	Type	%M [a]	Age
Issaq et al., 2008 [21]	LC-MS	Healthy (48)	44	59 (20–86)	MIBC + NMIBC (41)	88	76 (51–93)

References	Platform	Control Group (CTRL)			Bladder Cancer Patients (BC)		
		Type	%M [a]	Age	Type	%M [a]	Age
Pasikanti et al., 2010 [22]	GC-TOF	non-BC (51)	55	67 ± 12	NMIBC (24)	83	61 ± 12
Srivastava et al., 2010 [23]	^{1}H-NMR	Healthy (37), UTI [b] (31) bladder stone (2)	41	33 ± 15	NMIBC (33)	100	45 ± 25
Kim et al., 2010 [24]	GC-MS	Healthy (8)	100	NR [c]	NMIBC (8)	100	47–78
Huang et al., 2011 [25]	LC-MS	Healthy (32)	56	53 (46–67)	NMIBC (27)	70	56 (42–71)
Putluri et al., 2011 [26]	LC-MS	Healthy (13)	62	53 ± 11	MIBC + NMIBC (13)	85	61 ± 14
	LC-MS	Benign patients (16)	75	69 ± 12	MIBC + NMIBC (28)	82	66 ± 13
	LC-MS	Benign patients (11)	NR	68 ± 14	MIBC + NMIBC (34)	NR	71 ± 10
	LC-MS	Healthy (11)	45	NR	MIBC + NMIBC (8)	50	NR
Gamagedara et al., 2012 [27]	LC-MS/MS	No-evidence-of-malignancy (NEM) (12)	NR	NR	BC [d] (11)	NR	NR
Huang et al., 2013 [28]	LC-MS	Healthy (24)	62	50 (26–65)	MIBC + NMIBC (19)	74	60 (45–74)
	LC-MS	Kidney cancer (25)	60	55 (27–71)	MIBC + NMIBC (19)	74	60 (45–74)
Pasikanti et al., 2013 [29]	GC-TOF	non-BC (61)	59	60 ± 13	NMIBC (38)	84	68 ± 11
Wittmann et al., 2014 [30]	LC and CG MS	non-BC (266)	64	64	MIBC + NMIBC (66)	85	67

References	Platform	Control Group (CTRL)			Bladder Cancer Patients (BC)		
		Type	%M [a]	Age	Type	%M [a]	Age
Jin et al., 2014 [31]	LC-MS	Healthy (69), benign HU [e] (52)	64	64 ± 9	MIBC + NMIBC (138)	81	66 ± 13
Peng et al., 2014 [32]	LC-QTOFMS	Hernia (68), UTI [b] or HU (31)	91	62 ± 12	MIBC + NMIBC (91)	70	68 ± 13
Shen et al., 2015 [33]	LC-MS	Healthy (21)	57	54 ± 19	MIBC + NMIBC (23)	78	65 ± 13
Shao et al., 2017 [34]	UPLC-TOF	Hernia (65)	95	65 ± 13	MIBC + NMIBC (87)	62	68 ± 14
Zhou et al., 2017 [35]	GC-MS	Healthy (35)	66	63 ± 8	MIBC + NMIBC (50)	70	63 ± 12
Mpanga et al., 2018 [36]	LC-MS	Healthy (40)	55	60 (53–81)	BC (40)	50	62 (50–87)
Cheng et al., 2018 [37]	LC-HRMS	Healthy (78)	78	59 ± 11	NMIBC (54)	78	62 ± 13
Liu et al., 2018 [38]	LC-HRMS	Healthy (203)	48	20–60	NMIBC (110)	64	64 ± 13
Loras et al., 2018 [39]	UPLC-TOF-MS	NMIBC after TURBT (18)	53	67 ± 11	NMIBC before TURBT (18)	53	67 ± 11
Loras et al., 2019 [40]	^{1}H-NMR	MIBC + NMIBC after TURBT (21)	67	69 ± 10	MIBC + NMIBC before TURBT (12)		
Loras et al., 2019 [41]	^{1}H-NMR	MIBC + NMIBC after TURBT (17)	59	71 ± 9	MIBC + NMIBC before TURBT (13)		
Jacyna et al.,	^{1}H-NMR, GC-	Healthy (24)	75	64 ± 10	MIBC (24)	75	65 ± 12

References	Platform	Control Group (CTRL)			Bladder Cancer Patients (BC)		
		Type	%M [a]	Age	Type	%M [a]	Age
2019 [42]	MS, HPLC-MS						
Wang et al., 2019 [43]	UPLC-MS	Healthy (98)	59	55 (20–91)	NMIBC (53)	77	62 (33–87)
		RCC [f] (64)	75	53 (14–82)	NMIBC (146)	77	62 (33–87)
Łuczykowski et al., 2021 [44]	SPME-LC-MS	Healthy (24) [28]	75	64 ± 10	MIBC (24)	76	65 ± 13
Pinto et al., 2021 [45]	HS-SPME-GC-MS	Cancer-free (56) [31]	71	52 (45–66)	MIBC + NMIBC (53)	74	69 (43–87)
Lin et al., 2021 [46]	GC-MS	Hernia (61)	95	65 ± 12	NMIBC (63)	71	67 ± 13 [2]

[a] Male percentage. [b] Urinary Tract Infection. [c] Not reported. [d] Tumor type not specified. [e] Hematuria. [f] Renal cell carcinoma.

ic profiles
ufficiently
marker of
evant that
f healthy
ncer (KC),
oups with
s used to
t included
aturia is a
ore recent
s, both in

the presence or absence of hematuria [43]. Peng et al. [32] found that ten metabolites were responsible for the significant distinction between BC patients and a control group formed by subjects with urinary tract infection (UTI) and HUP. On the contrary, no urinary metabolite was found to differentiate BC from prostate cancer (PCa).

2.2. Which Are the Metabolites Responsible for the Difference in the Urinary Profile?

If the first question has a unanimous affirmative answer, the markers proposed in each study are very different, most of the time, from each other, which prevents a clear consensus on which metabolites are responsible for the metabolic differentiation of urine from BC patients. The combined results of all the studies in **Table 1** generate a list of 352 putative urine markers for the presence of BC, but only 20 (6%) were found in at least three studies to have a significantly altered level (**Table 2**). Even the metabolite with the most significant consensus, hippuric acid, showed a concentration change in less than half of the studies. The variation direction was also poorly reproduced: only eight metabolites (2%) of the list in **Table 2** showed the same variation between control and BC groups among the different studies, further reducing the consensus list. If we consider only those markers proposed in at least three studies, the occurrence of BC will potentially cause a decrease in the levels of hippuric and citric acids and an increase in lactic acid, taurine, valine, glutamine, histidine, and erythritol.

Table 2. Urinary metabolites proposed for bladder cancer diagnosis that were found to be perturbed in at least three studies.

Metabolite	BC/CTRL	References
hippuric acid	↓↓↓↓↓↓↓↓↓. [a]	[23][25][26][27][28][35][39][40][41][42][44]

Metabolite	BC/CTRL	References
citric acid	↓↓↓↓.	[22][23][29][39][40][41]
gluconic acid	↑↑↓↓↓	[22][29][30][36][44]
lactic acid	↑↑↑↑?	[29][30][35][40][42]
taurine	↑↑↑.	[23][27][28][40][41]
uridine	↑↑↑↓?	[22][24][29][32][42]
valine	↑↑↑.	[26][22][30][40][41]
phenylacetylglutamine	↑↓↓↓	[25][28][39][42]
succinate	↑↓↓?	[30][31][35][40]
tyrosine	↑↑↑↓	[26][35][39][39][42]
carnitine	↑↑↓↓	[26][31][39][25]
ribitol	↑↓↓↓	[22][29][35][46]
creatine	↑↓↓?	[26][30][39][41]
p-cresol	↑↑↓	[29][35][45]
acetyl-carnitine	↑↑↓	[25][39][30]
5-hydroxyindoleacetic acid	↑↓↓	[32][39][42]
fructose	↑↓↓	[22][30][46]
glutamine	↑.	[40][41][42]
histidine	↑.	[26][41][42]
erythritol	↑↑↑	[29][42] [46]

[a] Each symbol refers to a single study; “↑” “↓” symbols represent higher and lower alterations in metabolite concentrations in cancer versus healthy control group, respectively; “?”: direction of variation not reported.

These disappointing numbers are not unique characteristics of metabolomics applied to BC. For example, in PCa, thirteen studies published from 2015 to 2020 proposed a total of 179 different putative urinary biomarkers. Of these, only four (2%) were repeated in at least three studies showing the same variation [48]. This lack of consistency among the results is undoubtedly multicausal, and in the following chapters, we will address some of the potential problems affecting the reproducibility of results.

2.2.1. Sample Size

One possible reason for the lack of reproducibility is the use of small cohorts in the experiments. The most frequently proposed limit of published metabolomics studies is the insufficient number of samples analyzed and, as a result, the number of population studies is still insignificant compared to the overabundance of pilot experiments. The appropriate sample size calculation is not easy in metabolic phenotyping studies because of their top-down hypothesis-free characteristic (the so-called untargeted approach), complicating the experimental setup [49]. Nevertheless, the lack of reproducibility in the results should be a potent incentive to improve the significance of the experimental results by recruiting more participants.

2.2.2. Geographical Origin, Economic Status, and Diet

The heterogeneity among the different studies regarding the participants' geographic origins, economic status, and diet may contribute to the distinct metabolic alterations observed. Urine's metabolic composition is strongly dependent on lifestyle and diet [50][51][52], two factors highly related to the country and even the different cities where samples are collected [51]. The analysis of 2732 urine samples from 1391 subjects across five European countries revealed systematic variation in the metabolic profiles, especially in terms of gender, country, and, to a lesser extent, economic status [50]. Even if socioeconomic status's effect was generally less marked, the two primary metabolite variations associated with this factor are those of hippurate and citrate [50], two compounds among the most repeated as biomarkers of BC in the different studies (**Table 2**). The impoverishment in the quality and quantity of food consumed by populations at risk of poverty may to some extent influence these alterations [50], as was previously observed for Brazilian children [53]. The relationship with diet may become an important confounding factor that detracts from the observed variations concerning BC. For this reason, all the people participating in a metabolomics study must follow a standardized diet at least 24 h before collecting the samples [54].

2.2.3. The Control Group

The definition of a proper control group is challenging, especially when used as a reference to BC patients since they constitute an elderly population with significant comorbidities. The higher incidence of BC in males is another characteristic to consider when defining the control group. Given that gender is one of the most critical determinants of urinary composition [55], results may be biased if this is not adequately considered. For example, the urinary citric acid concentration is more elevated in females [56], and this difference can partially explain the observed decrease of this metabolite level for the BC group when more males than the control group form it. The importance of matching both sex and age has already been pointed out in a metabolomics study of urinary BC biomarkers: tryptophan metabolism, the citrate cycle, and pantothenate and CoA biosynthesis heavily contribute to inter-individual variations. In this case, and to obtain meaningful results, the authors proceeded with cohorts strictly matched in age and sex [38].

2.2.4. BC Heterogeneity

Disease heterogeneity is also a strong element that can lead to disparate results when looking at urinary biomarkers. BC presents one of the highest mutational burdens, only exceeded by lung and skin cancer [57]. This enormous genetic variability causes a vast heterogeneity that is manifested in at least five different levels: interpatient (different subjects present tumors with different genotypes); intratumoral (different spatial regions of the primary tumor do not share the same genetic alterations); intertumoral (differences in the genotype among multiple primary tumors or metastatic sites); circulation (difference between tissue-based and circulating markers) and temporal (genetic changes in the tumor over time and/or during treatment) [58].

In addition to the complexity and instability of the BC tumor genotype landscape, cancer cell metabolism itself is also highly variable. It is subject to environmental signals, mainly generated by the tumor microenvironment. Variations in the levels of oxygen and nutrients can induce metabolic heterogeneity, and the cell metabolic phenotype can further change during tumor progression because of a higher limitation in nutrients [59]. Different studies probably searched for biomarkers almost like they were characterizing distinct diseases due to all these changes in the genotype and phenotype. For example, in some studies, patients with NMIBC and MIBC were considered together, although their metabolic profiles are expected to be very different, as shown in tissue samples studies [60]. Three major pathways were found altered in MIBC, including increased eicosanoid signaling, enhanced de novo synthesis of NAD+, and increased heme catabolism. Even if a study considers only one of these two cancer categories, it is important to note that these broad classifications include carcinomas presenting different stages and thus representing a different phase of cancer progression. For example, it was observed that tryptophan metabolism is upregulated in the urine of high-grade NMIBC patients when compared with low-grade NMIBC patients [37]. In the study by Alberice et al., a series of markers for the BC diagnosis specific to the grade and stage were proposed [61]. The authors also considered cases of recurrence for patient classification and found prognostic markers specific to BC stability. The differences found in this work demonstrate that, as the control, the patient group chosen for a metabolomic study should also be homogeneous. Although it is still a pending issue in this field, a large cohort is the only way to mediate the heterogeneities mentioned above when searching for universal BC urinary biomarkers.

2.2.5. Technical Issues about Biomarkers Identification and Quantification

The identification and quantification of metabolites in biofluids also face a series of technical difficulties. LC-MS is the most diffuse platform in these untargeted studies, and problems such as detector saturation and matrix effect can alter the signal intensity in particular samples [62]. Compound identification without the use of labeled standards is a further challenge. According to the Metabolomics Standard Initiative, this corresponds to level 2 identification (putative annotation); for MS, the scientific community agrees that a direct comparison of the experimental data with an authentic reference standard is essential for level 1 identification using MS data. Level 1 was only granted in very few cases among the studies in **Table 1** [24][27][30], while in all other LC-MS-based analyses, metabolite structure determination was only putative and based on comparison with MS libraries or MS/MS data. This may add ambiguity about the actual chemical structure of the quantified features and can, in part, contribute to the lack of agreement among the different studies. A dataset composed of accurately identified and quantified metabolites yields more robust and, therefore, more comparable results across different studies for biochemical information

and diagnostic/prognostic purposes. A possible way to reach this has been recently proposed by the synergic use of NMR and UHPLC-HRMS [62].

References

1. J. Ferlay; M. Colombet; I. Soerjomataram; C. Mathers; D.M. Parkin; Marion Piñeros; A. Znaor; F. Bray; Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *International Journal of Cancer* **2018**, *144*, 1941-1953, 10.1002/ijc.31937.
2. Oner Sanli; Jakub Dobruch; Margaret A. Knowles; Maximilian Burger; Mehrdad Alemozaffar; Matthew E. Nielsen; Yair Lotan; Bladder cancer. *Nature Reviews Disease Primers* **2017**, *3*, 17022, 10.1038/nrdp.2017.22.
3. Bogdan Czerniak; Colin Dinney; David McConkey; Origins of Bladder Cancer. *Annual Review of Pathology: Mechanisms of Disease* **2016**, *11*, 149-174, 10.1146/annurev-pathol-012513-104703.
4. Karl Gruber; Coffee consumption and bladder cancer are linked, analysis shows. *BMJ* **2015**, *350*, h1477-h1477, 10.1136/bmj.h1477.
5. Mark C. Markowski; Stephen A. Boorjian; Jeremy Burton; Noah M. Hahn; Molly A. Ingersoll; Saman Maleki Vareki; Sumanta K. Pal; Karen S. Sfanos; The Microbiome and Genitourinary Cancer: A Collaborative Review. *European Urology* **2019**, *75*, 637-646, 10.1016/j.eururo.2018.12.043.
6. Margaret A. Knowles; Carolyn D. Hurst; Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. *Nature Reviews Cancer* **2014**, *15*, 25-41, 10.1038/nrc3817.
7. Greta Petrella; Giorgia Ciufolini; Riccardo Vago; Daniel Oscar Cicero; The Interplay between Oxidative Phosphorylation and Glycolysis as a Potential Marker of Bladder Cancer Progression. *International Journal of Molecular Sciences* **2020**, *21*, 8107, 10.3390/ijms21218107.
8. Tahlita C.M. Zuiverloon; Willemien Beukers; Kirstin A. van der Keur; Jesrael R. Munoz; Chris H. Bangma; Hester F. Lingsma; Marinus J.C. Eijkemans; Jan P. Schouten; Ellen C. Zwarthoff; A methylation assay for the detection of non-muscle-invasive bladder cancer (NMIBC) recurrences in voided urine. *BJU International* **2011**, *109*, 941-948, 10.1111/j.1464-410x.2011.10428.x.
9. Robert S. Svatek; Brent K. Hollenbeck; Sten Holmäng; Richard Lee; Simon P. Kim; Arnulf Stenzl; Yair Lotan; The Economics of Bladder Cancer: Costs and Considerations of Caring for This Disease. *European Urology* **2014**, *66*, 253-262, 10.1016/j.eururo.2014.01.006.
10. Matthew E. Hyndman; Jeffrey K. Mullins; Trinity J. Bivalacqua; Metabolomics and bladder cancer. *Urologic Oncology: Seminars and Original Investigations* **2011**, *29*, 558-561, 10.1016/j.urolonc.2011.05.014.

11. V. Urquidi; C. J. Rosser; S. Goodison; Molecular diagnostic trends in urological cancer: biomarkers for non-invasive diagnosis.. *Current Medicinal Chemistry* **2012**, *19*, 3653-63, 10.2174/092986712801661103.
12. Sungyong Jung; Jayoung Kim; Biomarker discovery and beyond for diagnosis of bladder diseases. *Bladder* **2020**, *7*, e40, 10.14440/bladder.2020.813.
13. Cheng Y, Yang X, Deng X, Zhang X, Li P, Tao J, Qin C, Wei J, Lu Q.; Metabolomics in bladder cancer: A systematic review. *International journal of clinical and experimental medicine* **2015**, *8(7)*, 11052–11063.
14. Eric Chun Yong Chan; Kishore Kumar Pasikanti; Yanjun Hong; Paul C. Ho; Ratha Mahendran; Lata Raman Nee Mani; Edmund Chiong; Kesavan Esuvaranathan; Metabonomic Profiling of Bladder Cancer. *Journal of Proteome Research* **2014**, *14*, 587-602, 10.1021/pr500966h.
15. Hangchuan Shi; Xiang Li; Qingyang Zhang; Hongmei Yang; Xiaoping Zhang; Discovery of urine biomarkers for bladder cancer via global metabolomics. *Biomarkers* **2016**, *21*, 578-588, 10.3109/1354750x.2016.1171903.
16. Daniela Rodrigues; Carmen Jeronimo; Rui Henrique; Luís Belo; Maria De Lourdes Bastos; Paula Guedes de Pinho; Márcia Carvalho; Biomarkers in bladder cancer: A metabolomic approach usingin vitroandex vivomodel systems. *International Journal of Cancer* **2016**, *139*, 256-268, 10.1002/ijc.30016.
17. Wen-Tao Zhang; Zi-Wei Zhang; Ya-Dong Guo; Long-Sheng Wang; Shi-Yu Mao; Jun-Feng Zhang; Meng-Nan Liu; Xu-Dong Yao; Discovering biomarkers in bladder cancer by metabolomics. *Biomarkers in Medicine* **2018**, *12*, 1347-1359, 10.2217/bmm-2018-0229.
18. Abhishek Bhat; Chad R. Ritch; Urinary biomarkers in bladder cancer. *Current Opinion in Urology* **2019**, *29*, 203-209, 10.1097/mou.0000000000000605.
19. Chandra Sekhar Amara; Venkatrao Vantaku; Yair Lotan; Nagireddy Putluri; Recent advances in the metabolomic study of bladder cancer. *Expert Review of Proteomics* **2019**, *16*, 315-324, 10.1080/14789450.2019.1583105.
20. Muhammad Shahid; Austin Yeon; Jayoung Kim; Metabolomic and lipidomic approaches to identify biomarkers for bladder cancer and interstitial cystitis (Review). *Molecular Medicine Reports* **2020**, *22*, 5003-5011, 10.3892/mmr.2020.11627.
21. Haleem J. Issaq; Ofer Nativ; Timothy Waybright; Brian Luke; Timothy D. Veenstra; Elias J. Issaq; Alexander Kravstov; Michael Mullerad; Detection of Bladder Cancer in Human Urine by Metabolomic Profiling Using High Performance Liquid Chromatography/Mass Spectrometry. *Journal of Urology* **2008**, *179*, 2422-2426, 10.1016/j.juro.2008.01.084.
22. Kishore Kumar Pasikanti; Kesavan Esuvaranathan; Paul C. Ho; Ratha Mahendran; Revathi Kamaraj; Qing Hui Wu; Edmund Chiong; Eric Chun Yong Chan; Noninvasive Urinary

Metabonomic Diagnosis of Human Bladder Cancer. *Journal of Proteome Research* **2010**, *9*, 2988-2995, 10.1021/pr901173v.

23. Shatakshi Srivastava; Raja Roy; Sudhir Singh; Praveen Kumar; Diwakar Dalela; Satya N. Sankhwar; Apul Goel; Abhinav A. Sonkar; Taurine – a possible fingerprint biomarker in non-muscle invasive bladder cancer: A pilot study by 1H NMR spectroscopy. *Cancer Biomarkers* **2010**, *6*, 11-20, 10.3233/CBM-2009-0115.
24. Jeong-Whun Kim; Gwang Lee; Seung-Min Moon; Myung-June Park; Sung Kyu Hong; Young-Hwan Ahn; Kyoung-Rae Kim; Man-Jeong Paik; Metabolomic screening and star pattern recognition by urinary amino acid profile analysis from bladder cancer patients. *Metabolomics* **2010**, *6*, 202-206, 10.1007/s11306-010-0199-6.
25. Zhenzhen Huang; Lin Lin; Yao Gao; Yongjing Chen; Xiaomei Yan; Jinchun Xing; Wei Hang; Bladder Cancer Determination Via Two Urinary Metabolites: A Biomarker Pattern Approach. *Molecular & Cellular Proteomics* **2011**, *10*, M111.007922, 10.1074/mcp.m111.007922.
26. Nagireddy Putluri; Ali Shojaie; Vihas Vasu; Shaiju K. Vareed; Srilatha Nalluri; Vasanta Putluri; Gagan Singh Thangjam; Katrin Panzitt; Christopher Tallman; Charles Butler; et al.Theodore R. SanaSteven M. FischerGabriel SicaDaniel J. BratHuidong ShiGanesh S. PalapattuYair LotanAlon Z. WeizerMartha TerrisShahrokh F. ShariatGeorge MichailidisArun Sreekumar Metabolomic Profiling Reveals Potential Markers and Bioprocesses Altered in Bladder Cancer Progression. *Cancer Research* **2011**, *71*, 7376-7386, 10.1158/0008-5472.can-11-1154.
27. Sanjeewa Gamagedara; Honglan Shi; Yinfa Ma; Quantitative determination of taurine and related biomarkers in urine by liquid chromatography–tandem mass spectrometry. *Analytical and Bioanalytical Chemistry* **2011**, *402*, 763-770, 10.1007/s00216-011-5491-4.
28. Zhenzhen Huang; Yongjing Chen; Wei Hang; Yao Gao; Lin Lin; Daniel Y. Li; Jinchun Xing; Xiaomei Yan; Holistic metabonomic profiling of urine affords potential early diagnosis for bladder and kidney cancers. *Metabolomics* **2012**, *9*, 119-129, 10.1007/s11306-012-0433-5.
29. Kishore Kumar Pasikanti; Kesavan Esuvaranathan; Yanjun Hong; Paul C. Ho; Ratha Mahendran; Lata Raman Nee Mani; Edmund Chiong; Eric Chun Yong Chan; Urinary Metabotyping of Bladder Cancer Using Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry. *Journal of Proteome Research* **2013**, *12*, 3865-3873, 10.1021/pr4000448.
30. Bryan M. Wittmann; Steven M. Stirdivant; Matthew W. Mitchell; Jacob E. Wulff; Jonathan E. McDunn; Zhen Li; Aphrihl Dennis-Barrie; Bruce P. Neri; Michael V. Milburn; Yair Lotan; et al.Robert L. Wolfert Bladder Cancer Biomarker Discovery Using Global Metabolomic Profiling of Urine. *PLoS ONE* **2014**, *9*, e115870, 10.1371/journal.pone.0115870.
31. Xing Jin; Seok Joong Yun; Pildu Jeong; Isaac Yi Kim; Wun-Jae Kim; Sunghyouk Park; Diagnosis of bladder cancer and prediction of survival by urinary metabolomics. *Oncotarget* **2014**, *5*, 1635-1645, 10.18632/oncotarget.1744.

32. Jun Peng; Yi-Ting Chen; Chien-Lun Chen; Liang Li; Development of a Universal Metabolome-Standard Method for Long-Term LC–MS Metabolome Profiling and Its Application for Bladder Cancer Urine-Metabolite-Biomarker Discovery. *Analytical Chemistry* **2014**, *86*, 6540-6547, 10.1021/ac5011684.
33. Chong Shen; Zeyu Sun; Deying Chen; Xiaoling Su; Jing Jiang; Gonghui Li; Biaoyang Lin; Lin Biaoyang; Developing Urinary Metabolomic Signatures as Early Bladder Cancer Diagnostic Markers. *OMICS: A Journal of Integrative Biology* **2015**, *19*, 1-11, 10.1089/omi.2014.0116.
34. Chi-Hung Shao; Chien-Lun Chen; Jia-You Lin; Chao-Jung Chen; Shu-Hsuan Fu; Yi-Ting Chen; Yu-Sun Chang; Jau-Song Yu; Ke-Hung Tsui; Chiun-Gung Juo; et al.Kun-Pin Wu Metabolite marker discovery for the detection of bladder cancer by comparative metabolomics. *Oncotarget* **2017**, *8*, 38802-38810, 10.18632/oncotarget.16393.
35. Yang Zhou; Ruixiang Song; Chong Ma; Lina Zhou; Xinyu Liu; Peiyuan Yin; Zhensheng Zhang; Yinghao Sun; Chuanliang Xu; Xin Lu; et al.Guowang Xu Discovery and validation of potential urinary biomarkers for bladder cancer diagnosis using a pseudotargeted GC-MS metabolomics method. *Oncotarget* **2017**, *8*, 20719-20728, 10.18632/oncotarget.14988.
36. Arlette Yumba Mpanga; Danuta Siluk; Julia Jacyna; Oliwia Szerkus; Renata Wawrzyniak; Marcin Markuszewski; Roman Kaliszan; Michał Jan Markuszewski; Targeted metabolomics in bladder cancer: From analytical methods development and validation towards application to clinical samples. *Analytica Chimica Acta* **2018**, *1037*, 188-199, 10.1016/j.aca.2018.01.055.
37. Xiangming Cheng; Xiaoyan Liu; Xiang Liu; Zhengguang Guo; Haidan Sun; Mingxin Zhang; Zhigang Ji; Wei Sun; Metabolomics of Non-muscle Invasive Bladder Cancer: Biomarkers for Early Detection of Bladder Cancer. *Frontiers in Oncology* **2018**, *8*, 494, 10.3389/fonc.2018.00494.
38. Xiaoyan Liu; Xiangming Cheng; Xiang Liu; Lu He; Wenli Zhang; Yajie Wang; Wei Sun; Zhigang Ji; Investigation of the urinary metabolic variations and the application in bladder cancer biomarker discovery. *International Journal of Cancer* **2018**, *143*, 408-418, 10.1002/ijc.31323.
39. A. Loras; M. Trassierra; D. Sanjuan-Herráez; M. C. Martínez-Bisbal; Jose V. Castell; G. Quintás; J. L. Ruiz-Cerdá; Bladder cancer recurrence surveillance by urine metabolomics analysis. *Scientific Reports* **2018**, *8*, 1-10, 10.1038/s41598-018-27538-3.
40. Alba Loras; Cristian Suárez-Cabrera; M. Carmen Martínez-Bisbal; Guillermo Quintás; Jesús M. Paramio; Ramón Martínez-Máñez; Salvador Gil; José Luis Ruiz-Cerdá; Integrative Metabolomic and Transcriptomic Analysis for the Study of Bladder Cancer. *Cancers* **2019**, *11*, 686, 10.3390/cancers11050686.
41. Alba Loras; M. Carmen Martínez-Bisbal; Guillermo Quintás; Salvador Gil; Ramón Martínez-Máñez; José Luis Ruiz-Cerdá; Urinary Metabolic Signatures Detect Recurrences in Non-Muscle Invasive Bladder Cancer.. *Cancers* **2019**, *11*, 914, 10.3390/cancers11070914.

42. Julia Jacyna; Renata Wawrzyniak; Stéphane Balayssac; Véronique Gilard; Myriam Malet-Martino; Aleksandra Sawicka; Marta Kordalewska; Łukasz Nowicki; Eliza Kurek; Ewa Bulska; et al.Małgorzata PatejkoMarcin MarkuszewskiPiotr GutknechtJanusz SiebertRoman KaliszanMichał J. Markuszewski Urinary metabolomic signature of muscle-invasive bladder cancer: A multiplatform approach. *Talanta* **2019**, *202*, 572-579, 10.1016/j.talanta.2019.05.039.

43. Zhan Wang; Xiaoyan Liu; Xiang Liu; Haidan Sun; Zhengguang Guo; Guoyang Zheng; Yushi Zhang; Wei Sun; UPLC-MS based urine untargeted metabolomic analyses to differentiate bladder cancer from renal cell carcinoma. *BMC Cancer* **2019**, *19*, 1-11, 10.1186/s12885-019-6354-1.

44. Kamil Łuczykowski; Natalia Warmuzińska; Sylwia Operacz; Iga Stryjak; Joanna Bogusiewicz; Julia Jacyna; Renata Wawrzyniak; Wiktoria Struck-Lewicka; Michał Markuszewski; Barbara Bojko; et al. Metabolic Evaluation of Urine from Patients Diagnosed with High Grade (HG) Bladder Cancer by SPME-LC-MS Method. *Molecules* **2021**, *26*, 2194, 10.3390/molecules26082194.

45. Joana Pinto; Ângela Carapito; Filipa Amaro; Ana Lima; Carina Carvalho-Maia; Maria Martins; Carmen Jerónimo; Rui Henrique; Maria Bastos; Paula Guedes de Pinho; et al. Discovery of Volatile Biomarkers for Bladder Cancer Detection and Staging through Urine Metabolomics. *Metabolites* **2021**, *11*, 199, 10.3390/metabo11040199.

46. Jia-You Lin; Bao-Rong Juo; Yu-Hsuan Yeh; Shu-Hsuan Fu; Yi-Ting Chen; Chien-Lun Chen; Kun-Pin Wu; Putative markers for the detection of early-stage bladder cancer selected by urine metabolomics. *BMC Bioinformatics* **2021**, *22*, 1-16, 10.1186/s12859-021-04235-z.

47. Sanjeewa Gamagedara; Anthony T Kaczmarek; Yongqing Jiang; Xiaoliang Cheng; Maduka Rupasinghe; Yinfa Ma; Validation study of urinary metabolites as potential biomarkers for prostate cancer detection. *Bioanalysis* **2012**, *4*, 1175-1183, 10.4155/bio.12.92.

48. Ana Lima; Joana Pinto; Filipa Amaro; Maria Bastos; Márcia Carvalho; Paula Guedes de Pinho; Advances and Perspectives in Prostate Cancer Biomarker Discovery in the Last 5 Years through Tissue and Urine Metabolomics. *Metabolites* **2021**, *11*, 181, 10.3390/metabo11030181.

49. Elise Billoir; Vincent Navratil; Benjamin J. Blaise; Sample size calculation in metabolic phenotyping studies. *Briefings in Bioinformatics* **2015**, *16*, 813-819, 10.1093/bib/bbu052.

50. Alessia Trimigno; Bekzod Khakimov; Francesco Savorani; Leonardo Tenori; Vaiva Hendrixson; Alminas Čivilis; Marija Glibetic; Mirjana Gurinović; Saara Pentikäinen; Janne Sallinen; et al.Sara Garduno DiazFrancesca PasquiSantosh KhokharClaudio LuchinatAlessandra BordoniFrancesco CapozziSøren Balling Engelsen Investigation of Variations in the Human Urine Metabolome amongst European Populations: An Exploratory Search for Biomarkers of People at Risk-of-Poverty. *Molecular Nutrition & Food Research* **2018**, *63*, e1800216, 10.1002/mnfr.201800216.

51. Jing Li; Haidan Sun; Xiaoyan Liu; Qinghong Shi; Lu He; Yulin Sun; Chengyan He; Yajie Wang; Xiaohang Zhao; Lihua Fan; et al.Zhengguang GuoShirley X. TsangWei Sun Evaluation of the

multicenter variations of urinary metabolomics. *URINE* **2019**, *1*, 29-34, 10.1016/j.urine.2020.05.004.

52. Joram M. Posma; Isabel Garcia-Perez; Gary Frost; Ghadeer S. Aljuraiban; Queenie Chan; Linda Van Horn; Martha Daviglus; Jeremiah Stamler; Elaine Holmes; Paul Elliott; et al.Jeremy K. Nicholson Nutriome–metabolome relationships provide insights into dietary intake and metabolism. *Nature Food* **2020**, *1*, 426-436, 10.1038/s43016-020-0093-y.
53. Adam Drewnowski; Nicole Darmon; Food Choices and Diet Costs: an Economic Analysis. *The Journal of Nutrition* **2005**, *135*, 900-904, 10.1093/jn/135.4.900.
54. Jason H Winnike; Marjorie G Busby; Paul B Watkins; Thomas M O'Connell; Effects of a prolonged standardized diet on normalizing the human metabolome. *The American Journal of Clinical Nutrition* **2009**, *90*, 1496-1501, 10.3945/ajcn.2009.28234.
55. Martin Fitzpatrick; Stephen P. Young; Metabolomics--a novel window into inflammatory disease.. *Swiss Medical Weekly* **2013**, *143*, w13743-w13743, 10.4414/smw.2013.13743.
56. Sunil Kochhar; Doris M. Jacobs; Ziad Ramadan; France Berruex; Andreas Fuerholz; Laurent B. Fay; Probing gender-specific metabolism differences in humans by nuclear magnetic resonance-based metabonomics. *Analytical Biochemistry* **2006**, *352*, 274-281, 10.1016/j.ab.2006.02.033.
57. Zachary R. Chalmers; Caitlin F. Connelly; David Fabrizio; Laurie Gay; Siraj M. Ali; Riley Ennis; Alexa Schrock; Brittany Campbell; Adam Shlien; Juliann Chmielecki; et al.Franklin HuangYuting HeJames SunUri TaboriMark KennedyDaniel S. LieberSteven RoelsJared WhiteGeoffrey A. OttoJeffrey S. RossLevi GarrawayVincent A. MillerPhillip J. StephensGarrett M. Frampton Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Medicine* **2017**, *9*, 1-14, 10.1186/s13073-017-0424-2.
58. Joshua J. Meeks; Hikmat Al-Ahmadie; Bishoy M. Faltas; John A. Taylor; Thomas W. Flaig; David J. DeGraff; Emil Christensen; Benjamin L. Woolbright; David J. McConkey; Lars Dyrskjøt; et al. Genomic heterogeneity in bladder cancer: challenges and possible solutions to improve outcomes. *Nature Reviews Urology* **2020**, *17*, 259-270, 10.1038/s41585-020-0304-1.
59. Christian Frezza; Metabolism and cancer: the future is now. *British Journal of Cancer* **2019**, *122*, 133-135, 10.1038/s41416-019-0667-3.
60. Divya Sahu; Yair Lotan; Bryan Wittmann; Bruce Neri; Donna E. Hansel; Metabolomics analysis reveals distinct profiles of nonmuscle-invasive and muscle-invasive bladder cancer. *Cancer Medicine* **2017**, *6*, 2106-2120, 10.1002/cam4.1109.
61. Juliana Alberice; Andre F. S. Amaral; Emily Armitage; José Antonio Lorente; Ferrán Algaba; Emanuel Carrilho; Mirari Márquez; Antonia García; Nuria Malats; Coral Barbas; et al. Searching for urine biomarkers of bladder cancer recurrence using a liquid chromatography–mass

spectrometry and capillary electrophoresis–mass spectrometry metabolomics approach. *Journal of Chromatography A* **2013**, *1318*, 163-170, 10.1016/j.chroma.2013.10.002.

62. Greta Petrella; Camilla Montesano; Sara Lentini; Giorgia Ciufolini; Domitilla Vanni; Roberto Speziale; Andrea Salonia; Francesco Montorsi; Vincenzo Summa; Riccardo Vago; et al.Laura OrsattiEdith MonteagudoDaniel Oscar Cicero Personalized Metabolic Profile by Synergic Use of NMR and HRMS. *Molecules* **2021**, *26*, 4167, 10.3390/molecules26144167.
63. Ana Lima; Joana Pinto; Filipa Amaro; Maria Bastos; Márcia Carvalho; Paula Guedes de Pinho; Advances and Perspectives in Prostate Cancer Biomarker Discovery in the Last 5 Years through Tissue and Urine Metabolomics. *Metabolites* **2021**, *11*, 181, 10.3390/metabo11030181.