Metabolomic analysis of biological material using LC-MS in the quest for urinary system cancer biomarkers – review

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ABSTRACT

Renal cell carcinoma (RCC) and bladder cancer (BC) are among the most frequently diagnosed urinary system cancers worldwide. They are characterized by high mortality and recurrence rates. In response to the rising incidence and mortality rates, scientists are exploring innovative diagnostic and therapeutic methods. Metabolomics, which analyzes metabolite levels, may enable early diagnosis and monitoring of therapy progress. Compared to other omics technologies, it focuses on the outcomes of metabolite activity, providing a unique perspective on processes occurring in cancer cells. Metabolomic analyses utilize techniques such as mass spectrometry. These methods allow the identification of biomarkers and precise determination of the chemical composition of biological samples. However, the most commonly used method is liquid chromatography-mass spectrometry (LC-MS), which enables the most comprehensive screening of cancer metabolomes. Recent studies show significant progress in recognizing characteristic metabolites associated with urological cancers, although this area remains partially unexplored. Research on circulating metabolites, especially those present in easily accessible samples like blood or urine, demonstrates promising potential in clinical practice. Study results reveal differences in metabolic profiles between various stages of cancer development, which may have clinical significance. The future of this field involves an increasing number of clinical cohorts, standardization of sample preparation, and further improvements in instrument sensitivity and speed. LC-MS-based metabolomics has the potential to contribute to the improvement of diagnostics, therapy, and the quality of life of patients with some urological cancers. However, challenges, such as the lack of uniform methodologies and understanding of metabolite determinants, require further research and innovation.

Keywords: mass spectrometry, metabolomics, LC-MS, kidney cancer, renal cell carcinoma, bladder cancer

1. Introduction

Cancers are one of the leading causes of death worldwide, prompting intensive research into effective diagnostic and therapeutic methods [1]. Renal cell carcinoma (RCC) and bladder cancer (BC) rank among the most prevalent urological malignancies and are consistently diagnosed as some of the most common cancers globally. They are marked by high mortality and recurrence [2, 3]. In response to the increasing statistics, scientists are seeking innovative tools for identifying cancer biomarkers that could improve the diagnosis and treatment of cancers [1]. At present, molecular analysis stands as a crucial field, especially in the context of complex diseases [4]. The metabolomics of cancer is highly significant for understanding the changes occurring in cancer cells compared to normal cells. Defining the role of mass spectrometry (MS) in the metabolomic analysis of cancer is essential for a comprehensive grasp of the subject and the achievement of groundbreaking research results [5]. Metabolomic analysis of cancer based on mass spectrometry is becoming an increasingly versatile tool, allowing for the precise identification and quantitative analysis of thousands of chemical compounds present in biological samples [4]. One of the techniques used in metabolomics is liquid chromatography coupled with mass

spectrometry (LC-MS). This analytical technique is employed for the separation of chemical compounds, including the identification of cancer biomarkers [6]. In this publication, we will focus on the role of liquid chromatography coupled with mass spectrometry in metabolomic studies of urinary system cancers and various aspects of the technology and its practical applications.

2. Mass Spectrometry Techniques Applied in Research of Urinary System Cancers

Metabolomics enables early diagnosis and therapy progress monitoring of cancer by biomarker identification and tracking changes in the metabolome. Through the customization of treatment to the unique metabolic changes, we have the potential to enhance its effectiveness and minimize adverse side effects. Additionally, as a research tool, metabolomics supports clinical studies by patient selection and monitoring therapeutic effects. Its application may improve treatment and patients' quality of life. Metabolite levels are direct results of metabolic enzyme activity in cancer cells. This sets it apart from genomics, which identifies genetic mutations, and transcriptomics, which analyzes gene expression at the RNA level. In comparison to proteomics, metabolomics shows how proteins are metabolized and how changes in metabolites can impact their functions. Metabolomics can effectively be used with other omics technologies, such as genomics, transcriptomics, and proteomics, to provide comprehensive insights into cancer biology and identify potential therapeutic targets [7]. In metabolomic analyses, mass spectrometry coupled with gas chromatography (GC-MS) and liquid chromatography (LC-MS) are commonly many chemical compounds in biological samples. Both techniques allow for precise determination of sample chemical composition and the identification of changes in metabolic profiles associated with various physiological disorders and diseases. GC-MS shows high resolution, enabling the accurate separation and identification of chemical compounds in a sample, especially volatile substances with low molecular mass. However, it requires chemical transformations of some metabolites, which can increase the risk of errors, as well as the time and cost of analysis. This method is less effective for polar compounds. On the other hand, LC-MS allows for the analysis of both polar and nonpolar compounds. It exhibits higher sensitivity and analysis speed but may be less precise in identifying certain substances compared to GC-MS. Despite some drawbacks, both methods are valuable in understanding complex metabolic processes and identifying biomarkers associated with various health disorders and diseases [6]. However, LC-MS is more commonly used in metabolomic analyses due to its versatility and the ability to analyze a broad range of metabolites with different chemical properties. LC-MS enables the most comprehensive screening of cancer metabolites [2,6].

2.1. Metabolomic Analysis of Urine Using Liquid Chromatography-Mass Spectrometry

In 2007, a group of American scientists pioneered the application of three independent analytical techniques, including gas chromatography with time-of-flight (GC-TOF-MS), hydrophilic interaction liquid chromatography (HILIC-LC-MS) and ultra-performance liquid chromatography with reversed phases (RP-UPLC-MS), to assess their utility in detecting clear cell renal cell carcinoma (ccRCC) in the urine of patients (6 patients with ccRCC and 6 healthy controls). The feature selection process revealed less than 30 metabolites differentiating between the cancer and control group in each dataset [8]. One of the first studies on the metabolomic analysis of urine in patients with BC using LC-MS were carried out in 2008 [9]. In recent years, there has been a proliferation of studies employing LC-MS for urinary metabolomic profiling in search of potential biomarkers, which mainly focus on patients with bladder cancer. Selected studies have been compiled in Table 1.

Table 1.   Characteristics of the included studies.

Study	Cancer	Participant	External validation tests	Identified metabolites
2011, Huang et al., Bladder Cancer Determination Via Two Urinary Metabolites: A Biomarker Pattern Approach	BC	59	+	9
2011, Putluri et al., Metabolomic Profiling Reveals Potential Markers and Bioprocesses Altered in Bladder Cancer Progression	BC	134	-	35
2013, Alberice et al., Searching for urine biomarkers of bladder cancer recurrence using a liquid chromatography–mass spectrometry and capillary electrophoresis–mass spectrometry metabolomics approach	BC	48	-	12
2014, Jin et al., Diagnosis of bladder cancer and prediction of survival by urinary metabolomics	BC	138	-	12
2017, Shao et al., Metabolite marker discovery for the detection of bladder cancer by comparative metabolomics	BC	152	-	12
2021, Łuczykowski et al., Metabolic Evaluation of Urine from Patients Diagnosed with High Grade (HG) Bladder Cancer by SPME-LC-MS Method	BC	48	-	6
2020 Zhang, et al., A pilot investigation of a urinary metabolic biomarker discovery in renal cell carcinoma	RCC	129	+	9

In 2022, a research team by Oto, J., Fernández-Pardo, Á., Roca, M. et al. conducted a study based on LC-MS metabolomic analysis of urine, revealing distinct profiles between non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC)'. The study included 198 BC patients and 98 healthy volunteers, matched for age and gender (BC patients: 27 females and 171 males, aged 62-77; volunteers: 15 females and 83 males, aged 52-68), with no urinary system tumors. p-Cresol glucuronide was identified as a potential diagnostic biomarker in BC patients compared to the control group. For NMIBC, p-cresol glucuronide emerged as a significant biomarker for determining the stage, while p-coumaric acid may serve as a potential specific biomarker for advanced NMIBC. For MIBC, spermine was identified as a potential specific biomarker for determining the stage. This study represents the first metabolomic investigation conducted on precisely characterized urine samples including all stages of NMIBC, MIBC, and a healthy control group, identifying non-invasive diagnostic biomarkers and assessing the stage of advancement [10]. Yang M. in 2023 performed untargeted LC-MS-based metabolomic analyses of urine to identify and classify urothelial cancer (UC)'. The analysis involved 35 samples from patients with upper urinary tract urothelial carcinoma (UTUC), 44 with BC, and 53 healthy individuals as the control group (HC), matched for gender and age. Diverse metabolites and disrupted metabolic pathways were examined in different groups. These studies not only allowed the differentiation of patients with UC from HC (prostaglandin I2, 5-methylodeoxycytidine, 2,6-dimethylheptanoylcarnitine, and deoxyinosine) but also distinguished UTUC from BC. UTUC and BC without hematuria were differentiated using a panel of 5'-methylthioadenosine, L-beta-aspartyl-L-serine, dehydroepiandrosterone sulfate, and *N'*-formylkynurenine. Meanwhile, a metabolite panel encompassing aspartyl methionine, 7-methylinosine, and alpha-CEHC glucuronide allowed for the differentiation of UTUC from BC with hematuria [11].

2.2.   Metabolomic Analysis of Blood Serum with Liquid Chromatography-Mass Spectrometry

In 2010, the first study on the blood serum of RCC patients was conducted. Serum samples from both kidney cancer patients and control volunteers were analyzed using LC-MS with high-resolution ESI-Q-TOF-MS, resulting in 48 variables [12]. Similarly, the first study on the serum of BC patients dates back to 2012. BC profiles in serum were analyzed using LC-MS, revealing five potential biomarkers for diagnosing various types of urogenital cancers [13]. Over the years, a series of studies have been conducted on this issue, although the majority of them were primarily focused on BC. Some of these studies have been compiled in Table 2.

Table 2.   Characteristics of the included studies.

Study	Cancer	Participant	External validation tests	Identified metabolites
2017, Tan et al., Three serum metabolite signatures for diagnosing low-grade and high-grade bladder cancer	BC	172	-	13
2017, Sahu et al., Metabolomics analysis reveals distinct profiles of nonmuscle-invasive and muscle-invasive bladder cancer	BC	103	-	513
2019, Vantaku et al., Large-scale profiling of serum metabolites in African American and European American patients with bladder cancer reveals metabolic pathways associated with patient survival	BC	72	-	53
2019, Amara et al., Serum Metabolic Profiling Identified a Distinct Metabolic Signature in Bladder Cancer Smokers: A Key Metabolic Enzyme Associated with Patient Survival	BC	87	-	40
2020, Liu et al., LC-MS-Based Plasma Metabolomics and Lipidomics Analyses for Differential Diagnosis of Bladder Cancer and Renal Cell Carcinoma	BC and RCC	279	+	8
2022, Nizioł et al., Untargeted ultra-high-resolution mass spectrometry metabolomic profiling of blood serum in bladder cancer	BC	98	+	23

In 2020, a serum metabolomics analysis based on LC-MS was shown by Liu X, Zhang M, Cheng X, et. al. Potential biomarkers of (BC and RCC) differentiating cancer and non-cancer cases were analyzed for early detection of these types of cancer. The tumor-specific biomarkers for BC and RCC were investigated for differential diagnosis. This led to the discovery of 8 common differential metabolites of BC, RCC, and control serum samples [14]. In 2022, metabolic profiles of serum were examined among low-grade BC (n=54), papillary urothelial neoplasm of low malignant potential (PUNLMP) (n=3), high-grade BC (n=41), non-invasive bladder cancer (pTa/pT1), invasive muscle bladder cancer (MIBC, pT2), and healthy individuals (NC). The average age of BC and NC patients was 74±10 and 64±12 years, respectively. Metabolic profiles of 100 bladder cancer patients were characterized to develop specific compounds of the studied material, enabling early and specific detection of bladder cancer. Twenty-three serum metabolites were identified, allowing for differentiation between BC patients and the control group. Lipids and lipid-like molecules were the major class of metabolites differentiating NCs and BC patients. The 10 glycerophospholipids level was significantly higher in NC than in BC patients' serum. The results suggest that measuring serum metabolites may provide a more direct and less invasive diagnostic and treatment approach for bladder cancer [2].

3.   Perspectives and Future Directions

Mass spectrometry is regarded as a useful primary technique in cancer research. The advancements in liquid chromatography, mass spectrometry, and molecular biology have increased interest in liquid biopsies as tools for early cancer detection. Different biological samples, such as serum and urine, can be effectively analyzed using LC-MS [4]. Although it represents a minimally invasive method for diagnosing cancer, there are several challenges associated with this approach. Most importantly, there is a lack of uniform methodologies in the quality and quantity of metabolites. Targeted and untargeted methods differ in both quality and quantity. Untargeted metabolomics identifies substances based on spectral information, while targeted confirmation of metabolites requires quantitative standards. Differences in detection efficiency also depend on the instrument platform. There is also heterogeneity in metabolites across different tumors, dependent on genotype and tissue. Tumor cells, especially tumor stem cells, exhibit metabolic differences impacting metastasis and treatment resistance [1]. Furthermore, circulating metabolites are influenced by various factors such as changes in enzymatic patterns, resulting from genetic variability, the influence of the gut microbiome, and lifestyle factors like stimulants (smoking, alcohol, drugs) or diet. Nevertheless, key factors determining the level of metabolites remain poorly understood [15]. Through the combination of deep learning models and integrated multi-omics data analysis, a conceptual biological regulatory network focused on metabolomics is being created, enabling the exploration of potential biomarkers and therapeutic targets. Recognition of characteristic metabolic changes at the transcription and protein levels is crucial for identifying new biomarkers and potential therapeutic targets [1, 15]. A rapid increase in the size of clinical cohorts is expected in the future due to standardized, high-throughput sample preparation techniques. This will reduce the number of studies where statistical significance is insufficient and enhance the effectiveness of identifying biomarkers as well as the clinical application of drugs. Further improvements in the sensitivity of mass spectrometry instruments and measurement speed will result in more precise substance identification, eliminating the need for preliminary sample fractionation. Improvements in detection/quantification levels will facilitate the development of metabolomics. Data analysis will provide greater diagnostic and prognostic accuracy compared to individual markers. All these advancements will be necessary for mass spectrometry-based metabolomics to realize its full potential in translating research findings into clinical practice [5].

4.   Conclusions

Metabolomics research with liquid chromatography-mass spectrometry techniques represents a crucial area of development in identifying urinary system cancer biomarkers. LC-MS enables the analysis of complex metabolite profiles, providing valuable insights into the stage, degree of advancement, and even the development of tumors themselves. Studies from recent years reveal progress in the identification of specific metabolites associated with urinary system cancers. The analysis of circulating metabolites, especially in easily accessible samples like blood or urine, is promising for clinical applications. The potential of rapid and non-invasive tests based on circulating metabolites can significantly enhance diagnostic processes and prognostication for urinary system cancers. Further research in metabolomics, especially in the context of identifying new biomarkers and refining data analysis techniques is needed. The development of these studies has the potential to introduce innovative diagnostic tools into clinical practice, contributing to the improvement of the effectiveness and precision of diagnosing urinary system cancers. Fast and non-invasive tests based on circulating metabolites have the potential to revolutionize diagnostic processes.

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