Urine 5 (2023) 57–64

Contents lists available at ScienceDirect

Urine

journal homepage: www.keaipublishing.com/en/journals/urine/

Urine biomarkers can outperform serum biomarkers in certain diseases

Cheng Xue [a,1], Bo Yang [b,1], Lili Fu [a,1], Huihui Hou [a], Jihua Qiang [a], Chenchen Zhou [c], Youhe Gao [d,**], Zhiguo Mao [a,*]

[a] Division of Nephrology, Changzheng Hospital, Second Military Medical University (Naval Medical University), Shanghai, China
[b] Internal Medicine III (Nephrology), Naval Medical Center of PLA, Second Military Medical University, Shanghai, 200000, China
[c] Outpatient Department, Yangpu Third Military Retreat, Shanghai, China
[d] Gene Engineering Drug and Biotechnology Beijing Key Laboratory, College of Life Sciences, Beijing Normal University, Beijing, 100875, China

ARTICLE INFO

Keywords:
Urine
Biomarker
Diagnosis
Serum biomarker
Gene
Extracellular vesicle
Urinary biomarker

ABSTRACT

Urinary biomarkers offer a non-invasive and easily accessible means of assessing an individual's health and susceptibility to various diseases. Urine biomarkers have advantages in no need or mechanism for stability, specific biomarkers produced by tubules, and non-invasive nature compared with serum biomarkers. Urine biomarkers can provide critical insights into an individual's predisposition to certain conditions, disease progression, and therapeutic response. In this review, we summarized the currently reported urinary biomarkers that outperformed serum biomarkers, including urinary protein biomarkers, gene biomarkers, urinary metabolites, electrolytes, and urinary extracellular vesicles. Combining urinary and serum biomarkers can offer a more comprehensive approach to disease diagnosis, monitoring, and personalized medicine. Despite some challenges in standardization and expanding the repertoire of diseases that can be diagnosed using urinary biomarkers, urinary biomarkers hold immense promise in improving patient outcomes and transforming healthcare.

1. Introduction

Lots of biomarkers have been identified and confirmed over the past decades, but overall, the results of biomarker research have not been as promising as anticipated. Efforts have mostly been concentrated on blood. Nevertheless, blood biomarkers could not cover all clinically useful indicators, while urine is a good complement.[1] Besides, physicians apply a variety of lab tests or procedures to diagnose diseases or predict the outcomes of certain conditions. The majority of conclusive diagnostic procedures, such as biopsy, are invasive, which not only limits the use of the 'gold standard' tests but also increases the risk of performing the procedures.[2] Emerging biomarkers from urine have been investigated.[3] We conducted this review by searching relevant studies in Pubmed and EMBASE databases (until Sep 2023) (Fig. 1). In certain circumstances, urinary biomarkers other than blood may have better performances (Fig. 2).

2. Mechanisms of urine outperforming serum biomarkers in certain circumstances

Blood and urine are two distinct components of the human body's waste elimination and circulatory systems (Table 1). Blood is a complex fluid responsible for various physiological functions, including transportation and defense, while urine is the waste product produced by the kidneys as a result of filtration and plays a critical role in maintaining overall bodily homeostasis.[4] Because of the body's homeostatic systems, blood is comparatively steady. Urine, on the other hand, records changes in the body over time, making it a superior source of early biomarkers.[1,5] A stable internal environment is the survival advantage of higher organisms. Blood tends to be more stable, while urine is produced by blood passing through the kidneys, and urine has no need or mechanism for stability at all.[1] A change introduced into the blood will be reduced by organs such as the liver and kidney through various mechanisms, while urine may reflect this introduced change better than blood.[1] Of course,

* Corresponding author. Division of Nephrology, Shanghai Changzheng Hospital, Second Military Medical University (Naval Medical University), Shanghai, 200003, China.
** Corresponding author. Gene Engineering Drug and Biotechnology Beijing Key Laboratory, College of Life Sciences, Beijing Normal University, Beijing 100875, China.
E-mail addresses: chengxia1568@126.com, xuecheng@smmu.edu.cn (C. Xue), gaoyouhe@bnu.edu.cn (Y. Gao), maozhiguo93@126.com (Z. Mao).
[1] Equal contributors.

https://doi.org/10.1016/j.urine.2023.10.001
Received 1 August 2023; Accepted 1 October 2023
Available online 2 October 2023

this also determines that there will be many factors affecting urine. In addition to the markers of the disease studied, there are many possible interference factors affecting urine, and more samples will be required for urinary biomarker validation.

Secondly, due to the direct relationship between urine and the urinary system, finding biomarkers of urological diseases from urine may be the easiest place to make a breakthrough in the field of biomarkers. The process of urine formation starts in nephrons. Blood flows through the glomerulus; water and certain small molecules from the bloodstream are filtered into capsules while blood cells and large proteins are kept in the blood. For example, large molecules such as IgM are typically not filtered by healthy glomeruli, the levels in urine are a better predictor of kidney injury than serum molecules like albuminin a variety of glomerular disorders.[6] Compared with blood, urine has direct contact with epithelial cells in the urinary system.[7] The concentration of specific biomarkers of the urinary system such as kidney injury molecule-1 (KIM-1) may be higher in urine than in blood. Besides, certain small molecule biomarkers that could be filtered into the capsule and not be reabsorbed may also be concentrated in the urine, which made it easier to detect. With tubular reabsorption and secretion, nutrients, ions, and water move between capillaries and urine in the tubules. In the collecting duct, urine formation is completed. It passes out of the kidneys through ureters and down to the bladder.

Thirdly, the ease of urine collection and its non-invasive nature make it an attractive biofluid for biomarker discovery and monitoring, particularly in diseases that affect the urinary system, such as kidney disorders and urological malignancies.[7] Due to the completely non-invasive characteristics of urine, obtaining samples technically and ethically should not be a bottleneck. Urinary proteins can be adsorbed on a piece of membrane and kept dry in a vacuum bag for long-term preservation and archiving of urine samples.[8] Utilizing a fluff pulp diaper, urine protein could be collected and preserved.[9] Like recording and keeping medical records, the comprehensive and systematic preservation of patient biological samples provides the possibility to improve the efficiency of subsequent large-scale verification of markers and the quality of output markers.

Recently, lots of groundbreaking studies have shed light on the superiority of urinary biomarkers over serum biomarkers in identifying certain diseases.[10] This significant advancement has the potential to transform the field of medical diagnostics, enabling more accurate and timely detection of diseases, and leading to better patient outcomes. In the current review, we summarized better performances of urinary biomarkers compared with plasma biomarkers for disease detection.

3. Protein urinary biomarkers

A molecule that can be reliably tested and assessed as a sign of healthy biological activities, harmful processes, or pharmacologic reactions to therapeutic intervention is known as a biomarker.[11] Numerous categories, including proteins, glycoproteins, hormones, oncofetal antigens, receptors, genetic markers, and DNA/RNA molecules, can be used to classify current biomarkers.[12] Among them, protein biomarkers account for the main part. Unlike serum protein biomarkers, which may only provide a limited snapshot of the body's molecular

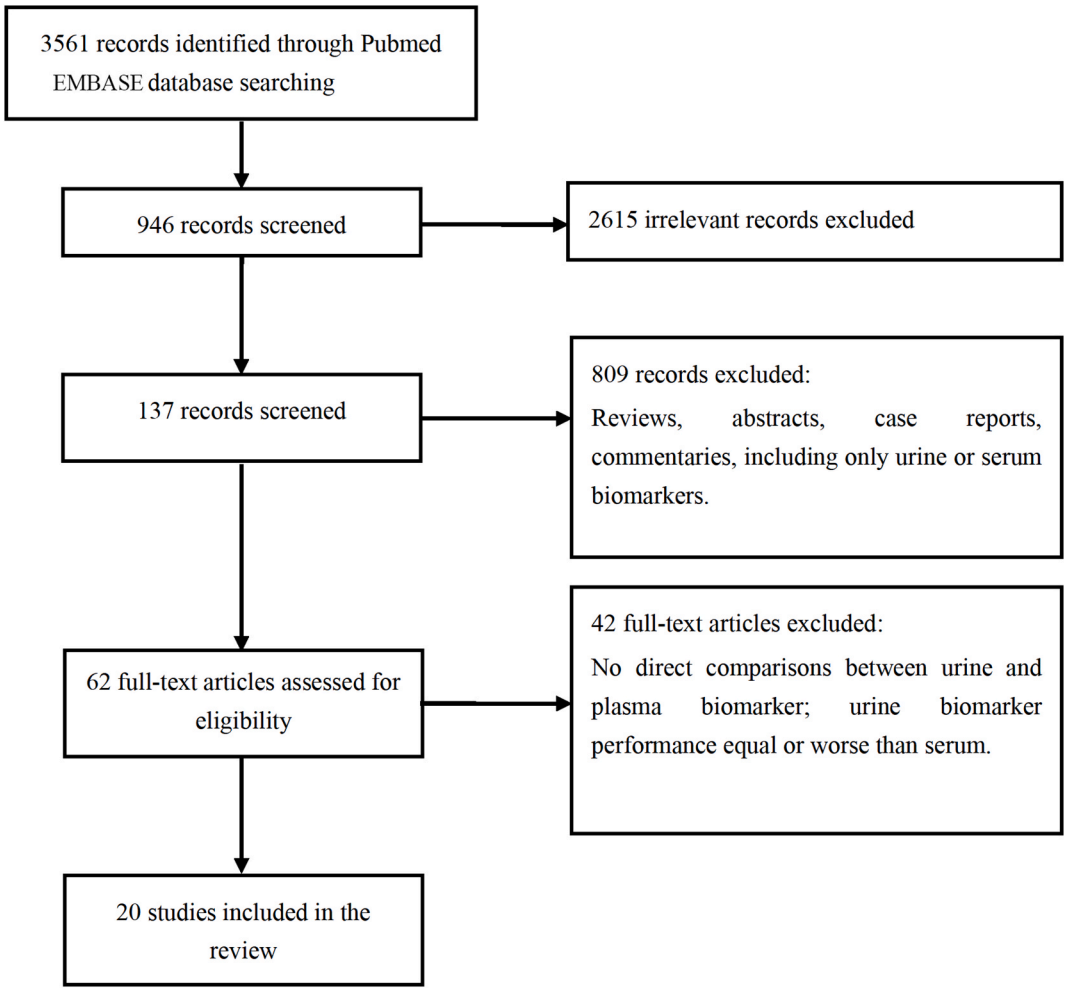

Fig. 1. The flowchart of included studies in this review.

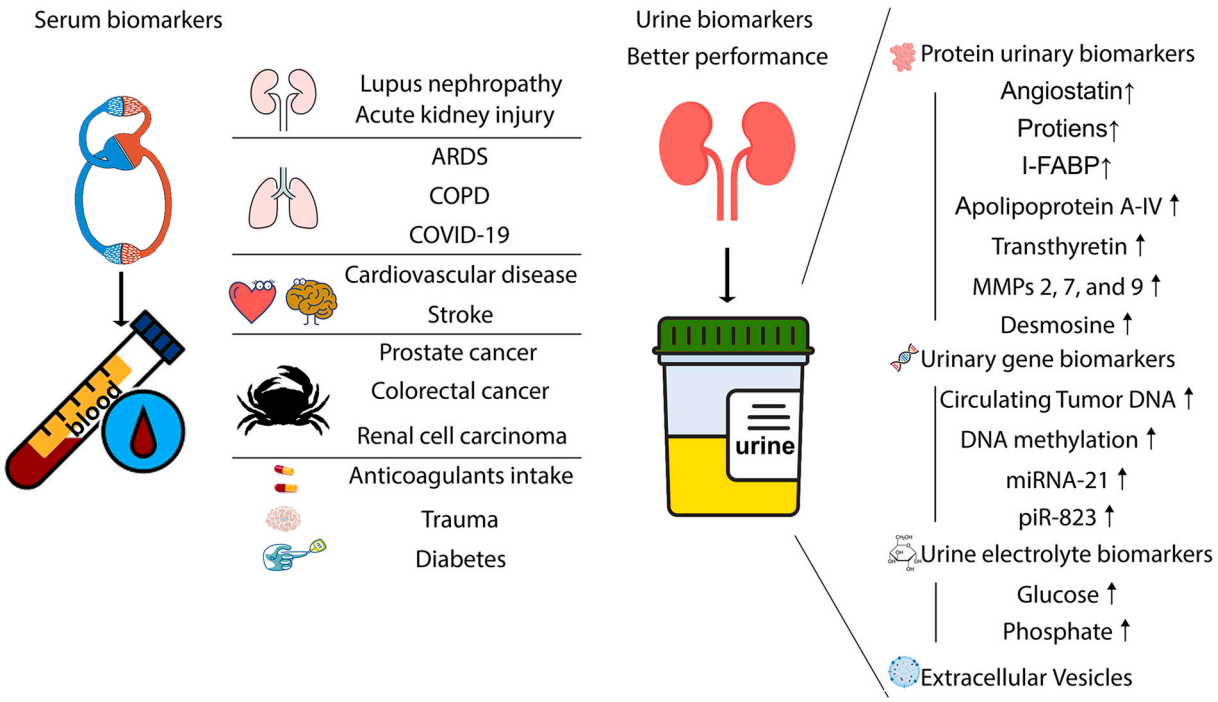

Fig. 2. Urine biomarkers can outperform serum biomarkers in certain diseases.
ARDS, acute respiratory distress syndrome; COPD, Chronic obstructive pulmonary disease.

Table 1
Comparative advantages and disadvantages between urinary biomarkers and serum biomarkers.

Aspect	Urinary Biomarkers	Serum Biomarkers
Collection Process	**Advantage:** Non-invasive and relatively easy to collect.	**Advantage**: Convenient and widely used for routine testing.
Sensitivity	**Advantage:** Some markers may be more sensitive and detect early disease or organ damage.	**Advantage**: Well-established markers for certain diseases, providing accurate measurements.
Specificity	**Advantage:** Can be highly specific for certain organ systems or diseases.	**Advantage:** Established specificity for specific diseases.
Proximity to Source	**Advantage:** Reflects proximal changes in organ function or damage.	**Disadvantage:** May not reflect early changes or subtle dysfunction of the organ or system.
Non-specificity	**Disadvantage:** Urine may contain non-specific biomarkers due to filtration processes.	**Advantage:** Serum markers are often more stable and less influenced by diet or hydration status.
Biomarker Availability	**Disadvantage:** Availability of specific urinary biomarkers may be limited.	**Advantage:** Wider availability of serum biomarkers for various diseases.
Cost-Efficiency	**Advantage:** Collection and analysis of urine biomarkers can be cost-effective.	**Disadvantage**: May require specialized equipment and higher costs.
Variability	**Disadvantage:** Urine composition may vary due to factors like diet or hydration.	**Disadvantage**: Serum biomarkers tend to show less variability.
Validation and Research	**Disadvantage:** May require further validation and research for clinical application.	**Advantage:** Serum biomarkers often have a longer history of research and validation.

profile, urinary protein biomarkers have unique access to the urinary system and its associated organs.[13] For example, in rat pregnancy, the urine proteome can reveal some maternal and embryonic developmental changes.[14] Consequently, they may offer a more comprehensive molecular profile, with disease-specific molecules concentrated in the urine. This proximity to the affected organs enhances the sensitivity and specificity of urinary protein biomarkers, making them more reliable in detecting certain diseases (Table 2).

3.1. *Urinary protein biomarkers in acute illnesses*

Acute illnesses, characterized by rapid onset and often severe symptoms, pose significant challenges to healthcare providers in their diagnosis, management, and treatment. The timely and accurate identification of these conditions is critical to ensure appropriate medical interventions and optimal patient outcomes. In recent years, the exploration of urinary biomarkers has gained considerable attention as a promising avenue for enhancing the early detection and prognostication of acute illnesses.

3.1.1. *Kidney disease*

Oliguria or an increase in serum creatinine (sCr) is typically used to make the diagnosis of acute kidney injury (AKI), formerly known as acute renal failure. Because several non-renal factors have a significant impact on serum concentration, serum creatinine is a poor indicator of early renal impairment. A systematic review showed that urine KIM-1, urine interleukin-18 (IL-18), and serum cystatin C worked best for the differential diagnosis of established AKI.[15] The best markers for the early diagnosis of AKI were urine neutrophil gelatinase-associated lipocalin, IL-18, glutathione-*S*-transferase-π, γ-glutathione-*S*-transferase, and cystatin C.[15] The best markers for predicting mortality risk following AKI were urine N-acetyl-D-glucosaminidase, KIM-1, and IL-18.[15] The field of critical care and nephrology may benefit greatly from the development of biomarkers, according to investigations of urine biomarkers. In Ueta

Table 2
Comparisons of urinary biomarkers outperforming serum biomarkers.

Study	Year	Number	Diseases	Biomarker	Outcomes	Method	Applications
Wu[33]	2013	100	Lupus nephropathy	Angiostatin	Urine AUC = 0.93, serum no significant difference	ELISA	Diagnosis
Parikh[17]	2013	311	AKI after cardiac surgery	NGAL	Urine AUC 0.72 vs. plasma AUC 0.56	ELISA	Diagnosis
Tuladhar[18]	2009	50	AKI after cardiac surgery	NGAL	Urine AUC 0.96 vs. plasma AUC 0.85	ELISA	Diagnosis
Li[24]	2014	6	Anticoagulants intake	Proteins	Transferrin and hemopexin were detected in urine but not in plasma.	LC-MS/MS, WB	Disease research
Bi[21]	2022	71	COVID-19	Proteins	Protein number 2.5 times in urine than serum	Proteomes and metabolomes	Disease research
Batra[22]	2023	59	ARDS	150 metabolites and 70 proteins	60 significant proteins linked with mortality in urine vs. 0 in serum	Proteomes and metabolomes	Risk prediction
Vollrath[25]	2022	16	Trauma (animals)	I-FABP	Longer high level after the advanced trauma than in urine than serum	ELISA	Disease monitoring
Sironi[23]	2001	18	Stroke (animals)	Apolipoprotein A-IV, transthyretin	Predict stroke in urine but not in serum	Electrophoresis	Risk prediction
Peng[31]	2023	141	Colorectal cancer	MMPs 2, 7, and 9	Better diagnostic values for early CRC in urine than serum	Chemiluminescence, chemiluminescence	Diagnosis
Huang[35]	2012	390	Chronic obstructive pulmonary disease	Desmosine	Urinary desmosine levels are raised by exacerbations of COPD whereas blood desmosine levels are not.	Validated isotopic dilution liquid Chromatography-tandem mass spectrometry methods	Disease monitoring
Chen[37]	2022	48	Prostate cancer	Circulating tumor DNA	More cfDNA alterations in urine than in blood, including TP53, AR, ATM, MYC, and SPOP mutations	Hybrid-capture-based next-generation sequencing assay	Diagnosis
Payne[38]	2009	50	Prostate cancer	GSTP1, RASSF2, HIST1H4K, and TFAP2E DNA methylation	All biomarkers showed greater sensitivity for prostate cancer in urine than in plasma DNA	Real-time PCR	Diagnosis
Du[39]	2013	120	Acute kidney injury	miRNA-21	Urine miR-21 was a better outcome predictor than plasma miR-21 with higher (1.4–2.6-fold) unadjusted odds ratio for progression of AKI and other outcomes.	RT-qPCR	Risk prediction
Iliev[41]	2016	588	renal cell carcinoma	piR-823	Urine AUC 0.74 vs. serum AUC 0.62	RT-qPCR	Diagnosis
Yin[43]	2018	12	Diabetes (animals)	Glucose	Urine glucose rose 10 weeks before their blood	Glucometer	Diagnosis
Donat-Vargas[45]	2023	1625	Cardiovascular disease	Phosphate	Urinary P predicts CVD while plasma P does not.	Vanadomolybdophosphoric method	Risk prediction

ARDS, acute respiratory distress syndrome; I-FABP, intestinal fatty acid-binding protein; MMP, Matrix metalloproteinases; AKI, Acute kidney injury; NGAL, neutrophil gelatinase-associated lipocalin.

et al.'s study,[16] 47 patients who had stent graft repair of aortic aneurysms had samples of urine and serum taken as biomarkers. Before the sCr detection of AKI, the values of urinary neutrophil gelatinase-associated lipocalin (NGAL)/Cr, N-acetyl-β-d-glucosaminidase (NAG), and liver fatty acid-binding protein (L-FABP) peaked. Parikh et al. found that urine IL-18 and urine NGAL, but not plasma NGAL (AUC 0.71 vs 0.85), were linked to AKI and poor outcomes in pediatric heart surgery patients.[17] Tuladhar et al. also found that the AUC for plasma NGAL seemed inferior to urine obtained from 50 patients undergoing cardiopulmonary bypass-requiring surgery (AUC 0.96 vs 0.85).[18]

Leukocytes, blood arteries, and renal tubules all release the pro-inflammatory cytokine IL-18, but renal tubule production of IL-18 significantly increases with acute renal damage. Therefore, elevated urine IL-18 is a relatively sensitive and specific indicator of acute tubular necrosis and delayed graft function in the post-ischaemic kidney.[19] The AUC of urinary IL-18 level to predict AKI was about 0.70[19], while serum IL-18 could not predict AKI.[20]

3.1.2. Respiratory disease

Bi et al.[21] examined proteomes and metabolomes in serum and urine samples from coronavirus disease 2019 (COVID-19) patients. Overall, there were 2.5 times as many proteins found in urine as there were in sera. Eighty percent of the 1195 proteins found in sera were also found in urine, proving that the majority of detectable serum proteins were present in urine. Moreover, COVID-19 severity levels are effectively categorized by urine proteins. Comparatively to the serum, the assessed urine proteome contained more intracellular compartment proteins discharged from tissues.[21] Using TMT-based proteomics, they found 197 cytokines and receptors in urine but only 124 in serum.[21] To assess the immunological pathobiology and clinical trajectory of COVID-19 and other infectious diseases, the urine proteome should be included in a set of multi-omics analytes.

A typical COVID-19 consequence is acute respiratory distress syndrome (ARDS), a disease that can be fatal during critical illness. Richa Batra et al.[22] performed a comparative analysis of serum and urine-based metabolomics and proteomics profiles from 43 COVID-19 ARDS patients and 17 sepsis-induced ARDS patients. Proteins with differing protein abundances were detected in the urine and plasma of COVID-19 survivors and non-survivors, according to a proteomics-based mortality profile.[22] While none of the proteins evaluated in the plasma of the same patients were linked with mortality, 60 proteins were significant in urine proteomic profiles.[22]

3.1.3. Cerebrovascular disease

In the animal model of stroke-prone spontaneously hypertensive rats, urinary apolipoprotein A-IV and transthyretin may predict stroke while the serum level of these markers does not correlate with the onset of stroke.[23] Several proteins were excreted in the urine in advance of stroke: transferrin, hemopexin, Gc-globulin, albumin, alpha (2)-HS-glycoprotein, alpha(1)-antitrypsin, kallikrein-binding protein, and transthyretin.[23]

3.1.4. Coagulation disease

Besides, systematic diseases may also induce a significant change in urinary biomarkers. Li et al.[24] suggested that anticoagulant heparin may induce significant protein panel changes in urine but not in plasma. The protein panel involved acute phase response signaling, LXR/RXR activation, coagulation system, intrinsic prothrombin activation pathway, and extrinsic prothrombin activation pathway. The reason may be that urine accumulates changes and could be collected in large volumes.[24]

3.1.5. Trauma

Urinary intestinal fatty acid-binding protein (I-FABP) as a marker of intestinal barrier loss in polytrauma kept at a high level after the advanced trauma life support phase while blood I-FABP level decreased shortly after trauma and hemorrhagic shock.[25]

3.2. Urinary protein biomarkers in chronic illnesses

Chronic illnesses have become a significant burden on global healthcare systems, affecting millions of people worldwide. These conditions often necessitate long-term management, necessitating regular monitoring to assess disease progression, treatment efficacy, and overall patient health. In this context, urinary biomarkers have emerged as invaluable tools in the field of medical research and clinical practice.

3.2.1. Cancers

Matrix metalloproteinases (MMPs) are a kind of zinc-dependent endopeptidase that participates in the destruction of the extracellular matrix and is released and activated outside the cell. MMP overexpression has been verified in the formation and progression of cancers. Moses et al. first reported an increased incidence of urine MMPs in cancer patients.[26] Urinary MMP-2 level revealed the existence of pancreatic cancer.[27] The presence of gastric cancer could be detected by urinary ADAM12 and MMP-9/NGAL complex.[28] The degree and activity of vascular abnormalities were correlated with increased expression of urine MMPs.[29]

The use of MMPs as a possible serum marker for the early detection of colorectal cancer (CRC) has previously been described.[30] Peng et al.[31] evaluated the diagnostic value of urine MMPs 2, 7, and 9 in urine for CRC. Serum MMP9 had a lower diagnostic value for early CRC than urine MMP9, and the link between serum and urine MMP9 warranted more investigation.[31]

Moreover, the discovery of a group of urogenital biomarkers was found to forecast the presence of brain tumors more conveniently than cerebrospinal fluid and tissue specimens by Smith et al.[32] MMP-2, MMP-9, MMP-9/NGAL, and VEGF were all significantly higher in samples from patients with brain tumors compared to controls when a specific panel of urinary biomarkers was evaluated by ELISA.

3.2.2. Systemic Lupus Erythematosus

Systemic Lupus Erythematosus (SLE) may induce the upregulation of urinary angiostatin levels while the serum angiostatin level was unchanged.[33] The AUC of urinary angiostatin/Cr was 0.93 when compared with SLE patients versus health controls. The authors also suggest that urinary angiostatin levels in SLE patients with SLEDAI >2 and active nephritis demonstrate even better sensitivity and specificity compared to the healthy controls (AUC 0.95).[33] Sub-group analysis indicated that the AUC was even higher (0.95) when compared to SLEDAI >2 and with active nephritis patients versus health controls.[33] The molecular mechanisms that underlie the phenomenon were not fully understood. A correlation of urinary angiostatin levels with disease activity/renal pathology was also found. Recently, Kim et al. found that urine SERPINC1 and ORM1 levels were higher in SLE patients with early Lupus nephropathy (LN) compared to SLE patients without LN (ORM1, AUC = 0.886; SERPINC1, AUC = 0.892),[34] both of which were novel biomarkers for early LN.

3.2.3. Respiratory disease

Amino acid biomarker, desmosine, was found to be associated with exacerbation of chronic obstructive pulmonary disease (COPD) but not stable COPD.[35] The specificity of urinary desmosine in indicating COPD exacerbation was better than that in blood.[35]

3.2.4. Digestive disease

Another study by Zhang et al.[36] used tandem mass tagging and two-dimensional liquid chromatography-tandem mass spectrometry to identify a total of 40 statistically significant urine proteins in the thioacetamide liver fibrosis rat model. These distinct proteins were found in the urine before fibrosis was seen and before the serum levels of alanine aminotransferase and aspartate transaminase changed.

4. Urinary gene biomarkers

Urinary gene biomarkers represent a cutting-edge and promising frontier in the field of medical research and personalized medicine. As our understanding of the human genome continues to advance, scientists have discovered that genetic information can be extracted from urine samples, offering a non-invasive and easily accessible means of assessing an individual's health and susceptibility to various diseases. Unlike traditional biomarkers, which are typically proteins or metabolites, urinary gene biomarkers focus on the analysis of specific genes or gene expression patterns found in the cells shed into the urine. These genetic signatures can provide critical insights into an individual's predisposition to certain conditions, disease progression, and therapeutic response. Additionally, urinary gene biomarkers may offer advantages over blood-based biomarkers, as urine collection is less invasive and can be conducted more frequently, enabling longitudinal monitoring and early detection of health issues.

One of the most prevalent malignant tumors is prostate cancer (PCa), which makes up 20 % of all cancers and is the most common in men. Early-stage PCa is typically asymptomatic, and the specificity of the available biomarkers for its identification is limited. Chen et al.[37] assessed circulating tumor DNA (ctDNA) in blood or urine employed as PCa biomarkers. When ctDNA aberration profiles were compared between blood and urine, more changes, such as TP53, AR, MYC, ATM, and SPOP mutations, were found in urine than in blood.[37] In addition to blood, urine ctDNA may be used in clinical settings as a potent and practical non-invasive method in customized treatment for PCa patients.

Besides ctDNA, hypermethylated DNA can be found in urine and plasma from PCa patients and may be a valuable biomarker. Payne et al.[38] collected plasma and urine samples from 142 PCa patients. Four DNA methylation biomarkers were measured by real-time PCR in sodium bisulfite-modified DNA: GSTP1, HIST1H4K, RASSF2, and TFAP2E. The PCa sensitivity and area under the ROC curve (AUC) of the four biomarkers were higher for urine than plasma DNA.

MicroRNAs (miRNAs) are endogenous non-coding short (18–22 nucleotide) RNA molecules. MiRNAs have emerged as novel biomarkers that represent diverse disease states because they are remarkably stable in blood and other body fluids. Following cardiac surgery, severe acute kidney damage (AKI) is linked to poor clinical results. Du et al.[39] examined the possible role of miRNA-21 as a risk factor for developing postoperative AKI and other adverse outcomes. They found that miRNA-21 upregulation in plasma and urine was related to the course of AKI in patients with the condition. However, urine miRNA-21 was found to be a better AKI predictor than serum miRNA-21 with greater (1.5–2.6 fold) odds ratios.[39]

PIWI-interacting RNAs (piRNAs) are a novel family of naturally occurring, short non-coding RNAs involved in sequence-specific chromatin modifications and transposable element silencing.[40] Numerous distinct piRNAs are expressed in physiological fluids like blood or urine in various forms of cancer.[40] Renal cell carcinoma (RCC) is the most prevalent adult kidney tumor, accounting for around 3 % of all malignancies.[41] Patients with RCC had substantially greater levels of piR-823

expression in both serum and urine compared to healthy people (AUC = 0.6264 and 0.7433, respectively).[41] Urine piR-823 has better diagnostic usefulness than serum in RCC patients.

5. Urinary biomarkers of metabolites

Urinary biomarkers of metabolites have emerged as a powerful tool in the field of biomedical research, offering valuable insights into various physiological and pathological processes within the human body.[42] Metabolites are small molecules that act as intermediates or end products of various biochemical pathways, reflecting the dynamic and complex metabolic processes occurring in cells and tissues. The presence and abundance of specific metabolites in urine can provide crucial information about an individual's health status, metabolic state, and response to environmental factors or therapeutic interventions.

According to Bi et al.[21]'s results, there were almost the same amounts of measured metabolites in sera and urine (903 vs. 1033) in COVID-19 patients. However, unlike proteins, 62 % of serum metabolites (557 metabolites) were found in urine. Urine had lots of unique biomarkers of metabolites compared with sera.

Yin et al.[43] used Zucker diabetic fatty (ZDF) rats to determine whether changes in urine glucose occurred before rises in blood glucose. The high-fat diet was fed to ZDF rats (fa/fa) and Zucker lean (ZL) rats (fa/+), and their fasting blood glucose and urine glucose levels were measured.[43] The ZL rats' urine glucose levels were normal after 12 weeks of feeding, whereas the ZDF model rats' values rose 10 weeks before their blood glucose levels rose.[43] Therefore, urine glucose levels were disturbed before an increase in blood glucose was noticed in Zucker diabetic fatty rats.

6. Urine electrolyte biomarkers

Urinary biomarkers of electrolytes have emerged as a fascinating area of research in the field of medical science, offering unique insights into the body's ion homeostasis and its implications on health and disease.[44] Ions, crucial components of various physiological processes, play a fundamental role in maintaining cellular functions and organ systems.

Phosphate (P) levels in urine may be elevated due to the large consumption of inorganic P salts from dietary additives.[45] Elevated P levels in the blood have been associated with vascular dysfunction and calcification. The levels of P in both urine and plasma were examined about the incidence of cardiovascular disease (CVD).[45] Donat-Vargas et al. found that urinary P was associated with a significantly increased risk of CVD, while Plasma P was negatively associated with CVD.[45]

Traditional Chinese medicine has long used the root of Achyranthes bidentata Blume (AB) to treat osteoporosis. In comparison to the model group, urinary calcium (Ca) and creatinine (Cr) (U–Ca/Cr) and urinary P/Cr ratios in the 17α-estradiol-treated group were significantly lower, while Serum-Ca and Serum-P levels among other groups did not significantly differ from one another.[46] Urinary Ca and P were more sensitive to predict the treatment effect compared with serum Ca and P[46].

7. Urinary biomarkers of extracellular vesicles

Extracellular vesicles (EVs) are a diverse group of small membranous structures released by cells into their surrounding microenvironment.[47] These tiny vesicles play a fundamental role in intercellular communication by carrying a cargo of proteins, lipids, and nucleic acids that can be transferred between cells and tissues. Among the various types of EVs, exosomes and microvesicles are particularly abundant in bodily fluids, including urine, and have gained significant attention in the field of biomedical research as potential carriers of urinary biomarkers.

Cholangiocarcinoma (CCA) is a diverse category of biliary tumors with a poor prognosis. Lapitz et al. aimed to characterize the transcriptome profile of EVs in serum and urine from healthy people and CCA patients.[48] In Urinary extracellular vesicles (uEVs),s, a total of 11, 323 and 26,066 RNAs were found in healthy persons and CCA patients, accounting for a total of 27,319 transcripts discovered.[48] High diagnostic sensitivity was demonstrated by the discovery of distinct RNA patterns in serum and urine EVs from CCA patients compared to control groups. In urine EVs extracted from patients with CCA, Ras-related GTP binding D (RRAGD), MAP6 domain containing 1 (MAP6D1), and INO80 complex subunit D (INO80D) were found to be the most promising mRNA biomarkers, with AUC values of 1.00[48]. Notably, with AUC values of 0.904, 0.930, and 0.896, respectively, the miR200c, lncRNAs HLA complex group 4 (HCG4), and LOC100134868 also showed significant accuracies for the diagnosis of CCA.[48] Most changed mRNAs are involved in CCA-causing processes. In general, patients with CCA had unique RNA patterns in urine EVs that closely resemble the tumor, creating brand-new, highly promising liquid biopsy indicators.

Diabetic nephropathy (DN) is a frequent systemic microvascular diabetic consequence and the major cause of chronic kidney disease globally. UEVs, which are natural nanoscale vesicles that preserve RNA from destruction, might be used as an invasive diagnostic biomarker for DN. In comparison to 12 T2DM samples, 1684 different mRNAs, 123 circRNAs, 126 lncRNAs, and 66 miRNAs were discovered in uEVs from 12 T2DN samples by Zhao et al.'s study.[49]

8. Union of urinary and serum biomarkers

The convergence of urinary and serum biomarkers represents an exciting frontier in the realm of medical research, promising a holistic and comprehensive approach to disease diagnosis, monitoring, and personalized medicine. Combining the unique advantages of both urinary and serum biomarkers, this integrated approach offers a powerful toolkit for unraveling complex disease mechanisms and improving patient care. Soliman et al.[50] assessed serum and urine levels of 8 protein markers, including ALCAM, hemopexin, peroxiredoxin 6 (PRDX6), properdin, platelet factor 4 (PF4), TFPI, calpastatin, and VCAM-1 in 36 adult SLE patients and 12 healthy controls. They found that urine: serum fractional excretion ratios for the majority of the studied proteins fared better in diagnosing active renal involvement in SLE than equivalent urine and serum protein measures. Recently, a serum and urine lipidomics study based on ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) revealed the lipid alterations in rheumatoid arthritis (RA) rats, and the AUC of PI(18:1/16:0), PC(22:4/16:0), LacCer (d18:1/12:0) from serum, and 25-hydroxycholesterol from urine were 1.00, 0.94, 1.00, and 1.00, indicating a combination of urine and serum analysis may provide a more reliable assessment of RA.[51]

9. Revolutionizing disease management

The impact of urinary biomarkers on disease management cannot be overstated. Their ability to detect diseases at an early stage opens up new possibilities for intervention and treatment. By identifying diseases before symptoms manifest, clinicians can implement personalized treatment strategies, potentially improving patient outcomes and prognosis. Furthermore, urinary biomarkers offer a means for monitoring disease progression, treatment efficacy, and predicting disease recurrence, enabling a proactive approach to patient care.

10. Challenges

While the potential of urinary biomarkers is immense, several challenges need to be addressed. Standardization of sample collection, processing, and analysis protocols is crucial for widespread adoption. Additionally, further research is needed to expand the repertoire of diseases that can be accurately diagnosed using urinary biomarkers. Collaborative efforts between researchers, clinicians, and industry leaders are vital to overcoming these challenges and fully realizing the transformative potential of urinary biomarkers in clinical practice.

11. Conclusion

In conclusion, this review presents a paradigm shift in disease identification, highlighting the possible superiority of urinary biomarkers over serum biomarkers. With the enhanced sensitivity, specificity, or non-invasive nature, urinary biomarkers may have the potential to revolutionize the field of medical diagnostics. By enabling earlier detection, personalized treatment strategies, and improved disease management, urinary biomarkers or union with serum biomarkers will hold immense promise for improving patient outcomes and transforming the landscape of healthcare, providing hope for a future where diseases can be detected and treated more effectively than ever before.

Author contributions

Study concept and design, writing, and revision of the manuscript: MZ, GY, XC, YB, FL; Data acquisition, data analysis, drafting of manuscript: XC, YB, HF, QJ, ZC.

Funding

Funding from Naval Medical Center of PLA, Second Military Medical University (21M3201, 21M3202) .

Declaration of competing interest

None.

Acknowledgment

We thank Prof. Youhe Gao for his important guidance in this review.

References

1 Gao Y. Urine-an untapped goldmine for biomarker discovery? *Sci China Life Sci*. 2013;56(12):1145–1146. https://doi.org/10.1007/s11427-013-4574-1 [published Online First: 2013/11/26].
2 Gao Y. Now is the time to test early urinary biomarkers in large-scale human samples. *Sci China Life Sci*. 2019;62(6):851–853. https://doi.org/10.1007/s11427-019-9562-y [published Online First: 2019/05/23].
3 Course CW, Lewis PA, Kotecha SJ, et al. Characterizing the urinary proteome of prematurity-associated lung disease in school-aged children. *Respir Res*. 2023;24(1):191. https://doi.org/10.1186/s12931-023-02494-3 [published Online First: 2023/07/21].
4 Balhara N, Devi M, Balda A, et al. Urine; a new promising biological fluid to act as a non-invasive biomarker for different human diseases. *URINE*. 2023;5:40–52. https://doi.org/10.1016/j.urine.2023.06.001.
5 Taccori A, Maksoud R, Eaton-Fitch N, et al. A systematic review and meta-analysis of urinary biomarkers in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). *J Transl Med*. 2023;21(1):440. https://doi.org/10.1186/s12967-023-04295-0 [published Online First: 2023/07/06].
6 Bakoush O, Torffvit O, Rippe B, et al. Renal function in proteinuric glomerular diseases correlates to the changes in urine IgM excretion but not to the changes in the degree of albuminuria. *Clin Nephrol*. 2003;59(5):345–352. https://doi.org/10.5414/cnp59345 [published Online First: 2003/06/05].
7 Wei J, Gao Y. Early disease biomarkers can be found using animal models urine proteomics. *Expert Rev Proteomics*. 2021;18(5):363–378. https://doi.org/10.1080/14789450.2021.1937133 [published Online First: 2021/06/02].
8 Gao Y. On research and translation of urinary biomarkers. *Adv Exp Med Biol*. 2021;1306:101–108. https://doi.org/10.1007/978-3-030-63908-2_7 [published Online First: 2021/05/08].
9 Qin W, Du Z, Gao Y. Collection and preservation of urinary proteins, using a fluff pulp diaper. *Sci China Life Sci*. 2018;61(6):671–674. https://doi.org/10.1007/s11427-016-9060-2 [published Online First: 2018/01/11].
10 Baldan-Martin M, Chaparro M, Gisbert JP. Systematic review: urine biomarker discovery for inflammatory bowel disease diagnosis. *Int J Mol Sci*. 2023;24(12). https://doi.org/10.3390/ijms241210159 [published Online First: 2023/06/28].
11 Zhang Y, Heng Z, Wei J, et al. Changes in the urine proteome in patients with advanced lung cancer after different drug treatments. *URINE*. 2023;5:1–12. https://doi.org/10.1016/j.urine.2023.02.001.
12 Hua Y, Meng W, Wei J, et al. Changes to urinary proteome in high-fat-diet ApoE(-/-) mice. *Biomolecules*. 2022;12(11). https://doi.org/10.3390/biom12111569 [published Online First: 2022/11/12].
13 Shao C, Zhao M, Chen X, et al. Comprehensive analysis of individual variation in the urinary proteome revealed significant gender differences. *Mol Cell Proteomics*. 2019;18(6):1110–1122. https://doi.org/10.1074/mcp.RA119.001343 [published Online First: 2019/03/22].
14 Tang S, Gao Y. Urinary proteome changes during pregnancy in rats. *Biomolecules*. 2022;13(1). https://doi.org/10.3390/biom13010034 [published Online First: 2023/01/22].
15 Coca SG, Yalavarthy R, Concato J, et al. Biomarkers for the diagnosis and risk stratification of acute kidney injury: a systematic review. *Kidney Int*. 2008;73(9):1008–1016. https://doi.org/10.1038/sj.ki.5002729 [published Online First: 2007/12/21].
16 Ueta K, Watanabe M, Iguchi N, et al. Early prediction of acute kidney injury biomarkers after endovascular stent graft repair of aortic aneurysm: a prospective observational study. *J Intensive Care*. 2014;2:45. https://doi.org/10.1186/s40560-014-0045-4 [published Online First: 2014/01/01].
17 Parikh CR, Devarajan P, Zappitelli M, et al. Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery. *J Am Soc Nephrol*. 2011;22(9):1737–1747. https://doi.org/10.1681/ASN.2010111163 [published Online First: 2011/08/13].
18 Tuladhar SM, Puntmann VO, Soni M, et al. Rapid detection of acute kidney injury by plasma and urinary neutrophil gelatinase-associated lipocalin after cardiopulmonary bypass. *J Cardiovasc Pharmacol*. 2009;53(3):261–266. https://doi.org/10.1097/FJC.0b013e31819d6139 [published Online First: 2009/02/28].
19 Liu Y, Guo W, Zhang J, et al. Urinary interleukin 18 for detection of acute kidney injury: a meta-analysis. *Am J Kidney Dis : the official journal of the National Kidney Foundation*. 2013;62(6):1058–1067. https://doi.org/10.1053/j.ajkd.2013.05.014 [published Online First: 2013/07/09].
20 Vanmassenhove J, Vanholder R, Nagler E, et al. Urinary and serum biomarkers for the diagnosis of acute kidney injury: an in-depth review of the literature. *Nephrol Dial Transplant : official publication of the European Dialysis and Transplant Association - European Renal Association*. 2013;28(2):254–273. https://doi.org/10.1093/ndt/gfs380 [published Online First: 2012/11/02].
21 Bi X, Liu W, Ding X, et al. Proteomic and metabolomic profiling of urine uncovers immune responses in patients with COVID-19. *Cell Rep*. 2022;38(3), 110271. https://doi.org/10.1016/j.celrep.2021.110271 [published Online First: 2022/01/14].
22 Batra R, Uni R, Akchurin OM, et al. Urine-based multi-omic comparative analysis of COVID-19 and bacterial sepsis-induced ARDS. *Mol Med*. 2023;29(1):13. https://doi.org/10.1186/s10020-023-00609-6 [published Online First: 2023/01/27].
23 Sironi L, Tremoli E, Miller I, et al. Acute-phase proteins before cerebral ischemia in stroke-prone rats: identification by proteomics. *Stroke; a journal of cerebral circulation*. 2001;32(3):753–760. https://doi.org/10.1161/01.str.32.3.753 [published Online First: 2001/03/10].
24 Li M, Zhao M, Gao Y. Changes of proteins induced by anticoagulants can be more sensitively detected in urine than in plasma. *Sci China Life Sci*. 2014;57(7):649–656. https://doi.org/10.1007/s11427-014-4661-y [published Online First: 2014/06/09].
25 Vollrath JT, Klingebiel F, Blasius F, et al. I-FABP as a potential marker for intestinal barrier loss in porcine polytrauma. *J Clin Med*. 2022;11(15). https://doi.org/10.3390/jcm11154599 [published Online First: 2022/08/13].
26 Moses MA, Wiederschain D, Loughlin KR, et al. Increased incidence of matrix metalloproteinases in urine of cancer patients. *Cancer Res*. 1998;58(7):1395–1399 [published Online First: 1998/04/16].
27 Roy R, Zurakowski D, Wischhusen J, et al. Urinary TIMP-1 and MMP-2 levels detect the presence of pancreatic malignancies. *Br J Cancer*. 2014;111(9):1772–1779. https://doi.org/10.1038/bjc.2014.462 [published Online First: 2014/08/20].
28 Shimura T, Dagher A, Sachdev M, et al. Urinary ADAM12 and MMP-9/NGAL complex detect the presence of gastric cancer. *Cancer Prev Res*. 2015;8(3):240–248. https://doi.org/10.1158/1940-6207.CAPR-14-0229 [published Online First: 2015/01/17].
29 Marler JJ, Fishman SJ, Kilroy SM, et al. Increased expression of urinary matrix metalloproteinases parallels the extent and activity of vascular anomalies. *Pediatrics*. 2005;116(1):38–45. https://doi.org/10.1542/peds.2004-1518 [published Online First: 2005/07/05].
30 Bockelman C, Beilmann-Lehtonen I, Kaprio T, et al. Serum MMP-8 and TIMP-1 predict prognosis in colorectal cancer. *BMC Cancer*. 2018;18(1):679. https://doi.org/10.1186/s12885-018-4589-x [published Online First: 2018/06/23].
31 Peng L, Zhang X, Zhang ML, et al. Diagnostic value of matrix metalloproteinases 2, 7 and 9 in urine for early detection of colorectal cancer. *World J Gastrointest Surg*. 2023;15(5):931–939. https://doi.org/10.4240/wjgs.v15.i5.931 [published Online First: 2023/06/21].
32 Smith ER, Zurakowski D, Saad A, et al. Urinary biomarkers predict brain tumor presence and response to therapy. *Clin Cancer Res*. 2008;14(8):2378–2386. https://doi.org/10.1158/1078-0432.CCR-07-1253 [published Online First: 2008/04/17].
33 Wu T, Du Y, Han J, et al. Urinary angiostatin–a novel putative marker of renal pathology chronicity in lupus nephritis. *Mol Cell Proteomics*. 2013;12(5):1170–1179. https://doi.org/10.1074/mcp.M112.021667 [published Online First: 2013/01/25].
34 Kim YE, Lee EJ, Kim K, et al. Urine SERPINC1/ORM1 as biomarkers for early detection of lupus nephritis in MRL-lpr mice. *Front Immunol*. 2023;14, 1148574. https://doi.org/10.3389/fimmu.2023.1148574 [published Online First: 2023/09/25].
35 Huang JT, Chaudhuri R, Albarbarawi O, et al. Clinical validity of plasma and urinary desmosine as biomarkers for chronic obstructive pulmonary disease. *Thorax*. 2012;67(6):502–508. https://doi.org/10.1136/thoraxjnl-2011-200279 [published Online First: 2012/01/18].
36 Zhang F, Ni Y, Yuan Y, et al. Early urinary candidate biomarker discovery in a rat thioacetamide-induced liver fibrosis model. *Sci China Life Sci*. 2018;61(11):1369–1381. https://doi.org/10.1007/s11427-017-9268-y [published Online First: 2018/07/02].

37 Chen G, Jia G, Chao F, et al. Urine- and blood-based molecular profiling of human prostate cancer. *Front Oncol*. 2022;12, 759791. https://doi.org/10.3389/fonc.2022.759791 [published Online First: 2022/04/12].

38 Payne SR, Serth J, Schostak M, et al. DNA methylation biomarkers of prostate cancer: confirmation of candidates and evidence urine is the most sensitive body fluid for non-invasive detection. *Prostate*. 2009;69(12):1257–1269. https://doi.org/10.1002/pros.20967 [published Online First: 2009/05/22].

39 Du J, Cao X, Zou L, et al. MicroRNA-21 and risk of severe acute kidney injury and poor outcomes after adult cardiac surgery. *PLoS One*. 2013;8(5), e63390. https://doi.org/10.1371/journal.pone.0063390 [published Online First: 2013/05/30].

40 Limanowka P, Ochman B, Swietochowska E. PiRNA obtained through liquid biopsy as a possible cancer biomarker. *Diagnostics*. 2023;13(11). https://doi.org/10.3390/diagnostics13111895 [published Online First: 2023/06/10].

41 Iliev R, Fedorko M, Machackova T, et al. Expression levels of PIWI-interacting RNA, piR-823, are deregulated in tumor tissue, blood serum and urine of patients with renal cell carcinoma. *Anticancer Res*. 2016;36(12):6419–6423. https://doi.org/10.21873/anticanres.11239 [published Online First: 2016/12/07].

42 Ong ES. Urine metabolites and bioactive compounds from functional food: applications of liquid chromatography mass spectrometry. *Crit Rev Anal Chem*. 2023:1–16. https://doi.org/10.1080/10408347.2023.2235442 [published Online First: 2023/07/16].

43 Yin W, Qin W, Gao Y. Urine glucose levels are disordered before blood glucose level increase was observed in Zucker diabetic fatty rats. *Sci China Life Sci*. 2018;61(7):844–848. https://doi.org/10.1007/s11427-017-9134-6 [published Online First: 2017/09/02].

44 Yang C, Cai G-Y, Duan Z-Y, et al. The potential of spot and overnight urine in the evaluating of proteinuria and electrolyte. *URINE*. 2021;3:7–13. https://doi.org/10.1016/j.urine.2021.07.001.

45 Donat-Vargas C, Guallar-Castillon P, Nystrom J, et al. Urinary phosphate is associated with cardiovascular disease incidence. *J Intern Med*. 2023. https://doi.org/10.1111/joim.13686 [published Online First: 2023/06/18].

46 Yin Y, Zhu F, Pan M, et al. A multi-omics analysis reveals anti-osteoporosis mechanism of four components from crude and salt-processed Achyranthes bidentata Blume in ovariectomized rats. *Molecules*. 2022;27(15). https://doi.org/10.3390/molecules27155012 [published Online First: 2022/08/13].

47 Yu Z, Teng Y, Yang J, et al. The role of exosomes in adult neurogenesis: implications for neurodegenerative diseases. Neural Regen Res 2024;19(2):282-288. doi: 10.4103/1673-5374.379036 [published Online First: 2023/07/25].

48 Lapitz A, Arbelaiz A, O'Rourke CJ, et al. Patients with cholangiocarcinoma present specific RNA profiles in serum and urine extracellular vesicles mirroring the tumor expression: novel liquid biopsy biomarkers for disease diagnosis. *Cells*. 2020;9(3). https://doi.org/10.3390/cells9030721 [published Online First: 2020/03/19].

49 Zhao Z, Yan Q, Fang L, et al. Identification of urinary extracellular vesicles differentially expressed RNAs in diabetic nephropathy via whole-transcriptome integrated analysis. *Comput Biol Med*. 2023;166, 107480. https://doi.org/10.1016/j.compbiomed.2023.107480 [published Online First: 2023/09/23].

50 Soliman SA, Stanley S, Vanarsa K, et al. Exploring urine:serum fractional excretion ratios as potential biomarkers for lupus nephritis. *Front Immunol*. 2022;13, 910993. https://doi.org/10.3389/fimmu.2022.910993 [published Online First: 2022/09/13].

51 Shi W, Han Y. An untargeted serum and urine lipidomics research based on UPLC-MS revealed the lipid alterations on adjuvant-induced arthritis rats. *Biomed Chromatogr*. 2023, e5736. https://doi.org/10.1002/bmc.5736 [published Online First: 2023/09/05].