Review

Advances in Clinical Applications of ^{1}H NMR-Based Urine Metabolomics

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Abstract: Urinary ^{1}H Nuclear Magnetic Resonance (NMR)-based metabolomics has emerged as a robust, non-invasive approach for profiling systemic and renal metabolic alterations. This review summarizes recent clinical applications across kidney disease, cardiometabolic disorders, cancer, immune-mediated and infectious diseases, pediatric disorders, and lifestyle or environmental exposures. We first outline methodological aspects specific to urine, including sampling strategies, osmolarity variation, normalization approaches, and spectral analysis. We then discuss disease-focused applications, emphasizing key metabolites, study designs, and diagnostic performance, and we highlight areas of concordance and inconsistency cross-studies. Shared advantages and limitations of ^{1}H NMR-based urine metabolomics are consolidated in a dedicated section on challenges, and future directions are explored with particular attention to standardization, automation, artificial intelligence–assisted spectral interpretation, multi-omics integration, and large-scale validation. A brief comparison with mass spectrometry (MS) illustrates the complementary roles of NMR and MS in clinical metabolomics. Overall, ^{1}H NMR-based urine metabolomics has potential as a scalable platform for non-invasive phenotyping and holds considerable promise for personalized medicine once technical and translational barriers are addressed.

Keywords: urine metabolomics; ^{1}H NMR spectroscopy; biomarkers; non-invasive diagnosis; metabolic syndrome; clinical metabolomics

1. Introduction

Metabolomics aims to comprehensively characterize low-molecular-weight metabolites, typically below 1500 Da, in biological systems. Because metabolites represent the integrated output of genetic variation, environmental exposures, diet, and gut microbiome activity, metabolomics has become a key tool for understanding disease mechanisms and discovering biomarkers. In clinical research, metabolomic profiling has been applied to a growing range of conditions, including cancer, cardiovascular disease, metabolic disorders, and neurological diseases, and has shown utility in diagnosis, prognosis, patient stratification, and therapy monitoring [1].

Two broad analytical strategies are commonly distinguished. Untargeted metabolomics seeks to profile as many detectable metabolites as possible, including unknown features, with the goal of discovering novel biomarkers and perturbations in pathways that might not have been anticipated a priori. Targeted metabolomics focuses on precisely quantifying predefined panels of metabolites, such as amino acids, lipids, or organic acids, often to validate candidates emerging from untargeted screens or to interrogate specific biochemical pathways in more detail. Both approaches have been applied to a variety of biofluids, including blood, urine, cerebrospinal fluid, and saliva, each providing complementary information regarding systemic and organ-specific metabolism.

Among these matrices, urine is especially attractive. It can be collected non-invasively, in relatively large volumes, and repeatedly over time, which facilitates longitudinal and population-based studies. Urinary metabolites reflect not only renal function but also systemic metabolic status, dietary patterns, medication use, environmental exposures, and gut microbiota activity. These characteristics make urine a particularly suitable matrix for large-scale epidemiology and patient-friendly clinical applications. At the same time, its composition is strongly influenced by hydration, diurnal variation, and recent diet, and it is not under tight homeostatic control. These features make normalization strategies and careful study design critical to ensuring reliable quantification and interpretation [2,3].

^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy is a core analytical platform in metabolomics [4]. It exploits the magnetic properties of nuclear spins, such as ^{1}H, placed in a strong magnetic field and irradiated with radiofrequency pulses. The resulting spectrum encodes information about the chemical environment of nuclei and thereby yields structural and quantitative information about metabolites. A single one-dimensional ^{1}H NMR experiment can detect hundreds of metabolites in a complex mixture such as urine. NMR is inherently quantitative, highly reproducible, and non-destructive, allowing samples to be re-analyzed or subjected to complementary assays [5–7]. These attributes make it particularly well suited to untargeted profiling in clinical settings, where robustness and cross-study comparability are crucial [8].

Recent years have witnessed a rapid expansion in clinical applications of ^{1}H NMR-based urine metabolomics. Studies now span nephrology, cardiometabolic disease, oncology, rheumatology, infectious disease, pediatrics, and nutrition. At the same time, cross-cutting methodological issues, such as urine dilution, spectral overlap, and heterogeneous preprocessing pipelines remain important barriers to routine clinical implementation (Figure 1).

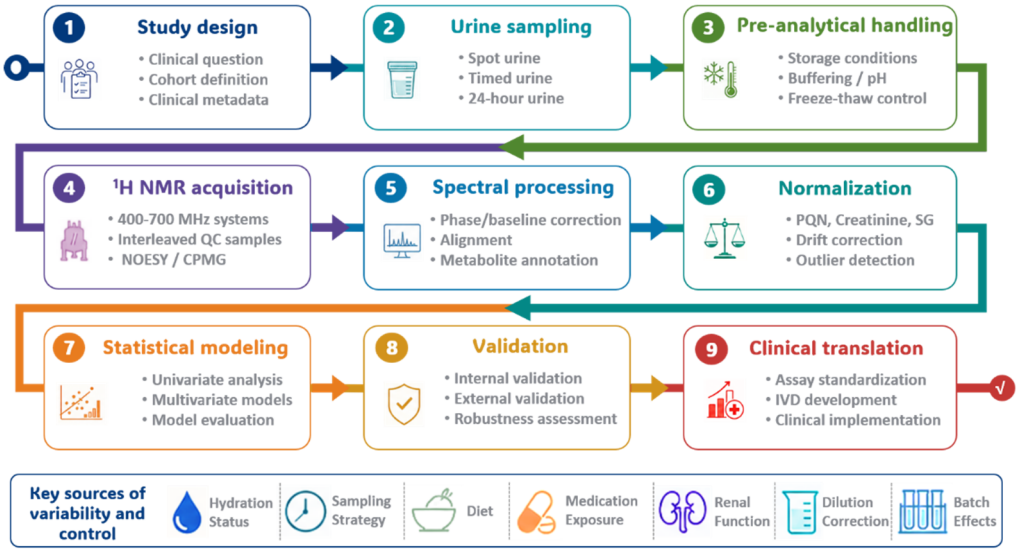

Figure 1. Methodological workflow for ^{1}H NMR-based urine metabolomics in clinical studies. The workflow summarizes key steps from study design and urine sampling to pre-analytical handling, NMR acquisition, spectral processing, normalization, statistical modeling, validation, and clinical translation. NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser effect spectroscopy; CPMG, Carr-Purcell-Meiboom-Gill; PQN, probabilistic quotient normalization; SG, specific gravity; IVD, *in vitro* diagnostic.

Across disease domains, urinary ^{1}H NMR signatures repeatedly converge on a limited set of physiological axes, most notably central carbon/energy metabolism (e.g., glucose handling, ketone bodies, and TCA intermediates), host-microbial co-metabolism (e.g., hippurate and other gut-derived co-metabolites), and kidney-specific tubular handling and acid–base homeostasis that directly shape urinary organic acid excretion. This convergence is mechanistically informative, but it also cautions against over-interpreting single metabolites as disease-specific or causal markers, because urinary readouts integrate biology with substantial pre-analytical variability. In practice, hydration status, spot versus 24-h urine, diet and medication exposure, and renal function can introduce between-study heterogeneity that rivals disease effects, especially for microbiome-linked metabolites, underscoring the need for harmonized reporting of sampling protocols and analytical pipelines, explicit confounder adjustment, and sensitivity analyses. From a translation standpoint, urine ^{1}H NMR is most likely to deliver clinical value where non-invasive sampling enables longitudinal monitoring, biomarker panels demonstrate incremental utility beyond standard-of-care tests, and workflows can be standardized across sites; accordingly, adopting minimum reporting standards and transparent metabolite identification levels is essential to enable external validation and eventual IVD-grade implementation [9,10].

In this review, we focus specifically on clinical applications of ^{1}H NMR-based urine metabolomics. After summarizing methodological considerations, we organize disease-focused sections according to the biological relevance of urine and major organ systems. We begin with kidney diseases, where urine is the primary diagnostic biofluid and most directly reflects organ function, then examine adult metabolic and cardiovascular diseases, oncology, immune-mediated and infectious conditions, and pediatric developmental disorders. We conclude with lifestyle, diet, and environmental applications (Table 1). Finally, we discuss shared challenges and prospects for clinical translation, including a brief comparison with MS-based metabolomics.

Table 1. Representative clinical applications of ^{1}H NMR-based urine metabolomics.

Category	Disease	Subjects/Cohort	Specific Metabolites	Performance	Study Category	Clinical Application	Reference
Urological Cancers	Prostate Cancer vs. Benign Prostatic Hyperplasia	Discovery: 391; Validation: 292	histidine, acetate, lactate, glucose, glycine, oxaloacetate, lipids, N,N-dimethylglycine, sarcosine	Limited multivariate separation (Q2X = 0.15); significant univariate differences	Urine-only ^{1}H NMR	Discovery of non-invasive diagnostic biomarkers	[11]
	Urological Cancer Differentiation	Bladder Cancer (BCa) 29; Prostate Cancer (PCa) 24; Renal Cell Carcinoma (RCC) 12	4-hydroxybenzoate, N-methylhydantoin, creatinine, glutamine, acetate	BCa vs. RCC: AUC 0.778; PCa vs. BCa: AUC 0.898; PCa vs. RCC: AUC 0.951	Urine-only ^{1}H NMR	Non-invasive differentiation between three major urological cancers	[12]
	Bladder Cancer Monitoring	100 BC patients, 100 normal controls	NMR: 4-HPA, hippurate, urea, mannitol, trigonelline; MS: 25 compounds (peptides and lipids)	NMR model AUC 0.879; MS model accuracy 97%	Multi-platform (NMR + LDI-MS)	Non-invasive detection and monitoring of BC stages and grades	[13]
	Renal Cell Carcinoma Staging	82 for tumor size regression, 70 for stage classification	4-guanidinobutanoic acid, 3-hydroxyanthranilic acid, citrate, pyruvate, etc.	AUC 0.95, Accuracy 86%, Sens 80%, Spec 91%	Multi-platform (LC-MS + NMR)	Non-invasive RCC staging and tumor size prediction	[14]
Kidney Diseases	Kidney Allograft Function	55 primary kidney transplant patients	Citrate, acetone, and a multimetabolite combination panel	eGFR prediction correlation: r = 0.79 (24 h urine), r = 0.68 (morning urine)	Combined urine/serum NMR	Supplementary tool for monitoring graft viability and function	[15]
	ADPKD Progression	Baseline: 324; 3-year follow-up: 112	Myoinositol/citrate ratio, betaine, phenylacetylglycine, alanine/citrate ratio	Metabolite model AUC = 0.72; combined with htTKV AUC = 0.75	Urine-only ^{1}H NMR	Identifying fast-progressive patients to guide clinical therapy	[16]
	Incident Kidney Stone Risk	418 first-time symptomatic stone formers, 440 controls	Hippuric acid, trigonelline, 2-furoylglycine, imidazole, citrate, creatine, alanine	Clinical model AUC 0.76; combined with NMR metabolites AUC 0.78	Urine-only ^{1}H NMR	Improving understanding of stone pathogenesis and diagnostic assistance	[17]
Metabolic Diseases	Type 2 Diabetes Screening	10 T2DM patients, 15 healthy controls	Glucose, citrate, creatinine, indoxyl sulfate, and hippurate	60 MHz AUC 0.94; 400 MHz AUC 0.96	Urine-only ^{1}H NMR	Routine POC metabolomics analysis in lab or clinical sites	[18]
	Metabolic Syndrome	11,754 participants (75% training, 25% testing)	glucose, lipids, aromatic amino acids, salicyluric acid, maltitol, TMAO, p-cresol sulfate	AUC 0.83–0.87 Sens 86.7%, Spec 74.6%	Urine-only ^{1}H NMR	Adding molecular dimension to MetS definition to identify high-risk individuals	[19]
	Dapagliflozin Treatment Effects	50 T2DM (treated), 53 healthy controls, 30 insulin controls	ketones, lactate, BCAAs, betaine, myo-inositol, alanine, citrate	OPLS-DA model accuracy: 87% (pre-treatment) to 90% (post-treatment)	Combined urine/serum NMR	Exploring SGLT-2 inhibitor effects on the diabetic urinary signature	[20]

Table 1. *Cont.*

Category	Disease	Subjects/Cohort	Specific Metabolites	Performance	Study Category	Clinical Application	Reference
Cardiovascular Diseases	Chronic Venous Disease	622 participants: 517 symptomatic, 105 asymptomatic	formate, creatinine, glycine, citrate, succinate, pyruvate, 2-hydroxyisobutyrate	OPLS-DA model: R2X = 0.87, Q2Y = 0.32	Combined urine/serum NMR	Identifying markers for disease staging, progression, and recurrence	[21]
	Stroke Risk Identification	73 Low Stroke Risk 87 Moderate Stroke Risk 17 High Stroke Risk	Glycolate, 4-HPA, TMAO, trigonelline, total sugar	Glycolate AUC 0.675; other metabolites AUC 0.55–0.65	Urine-only ^{1}H NMR	Early detection and differentiation of stroke risk stages	[22]
	Stroke Recovery Monitoring	10 patients (longitudinal: acute vs. 6-month chronic phase)	pseudouridine, 4-H-3-MMA, inosine, and homovanillate	AUC 0.929	Urine-only ^{1}H NMR	Identifying biomarkers indicative of stroke severity and recovery success	[23]
Inflammation & Infection	Inflammatory Bowel Disease	137 Crohn's Disease, 202 Ulcerative Colitis, 338 healthy controls	Urine: 4-HPA, citrate, Trp, ketones, succinate; Serum: ketones, amino acids	Combined serum + urine subphenotyping model: R2Y 0.636–0.661	Combined urine/serum NMR	Non-invasive subphenotyping (location and severity) of newly diagnosed IBD	[24]
	Congenital CMV Infection	35 infected newborns, 15 healthy controls	betaine, citrate, 3-HB, lactate, and threonine	AUC 0.998 accuracy 98.0%, Sens 100%, Spec 97.0%,	Urine-only ^{1}H NMR	Non-invasive diagnostic and prognostic tool for newborn CMV management	[25]
	CAP with ARDS Assessing Therapy	43 CAP with ARDS, 45 CAP without ARDS	Serum: ketones, citrate, choline; Urine: 1-methylnicotinamide	Combined serum + urine model: AUC 0.952, Sens 82.4%, Spec 94.1%	Combined urine/serum NMR	Reflecting metabolic alterations and evaluating therapeutic effects	[26]

Q2X: Goodness of prediction/Predictive quality; BCa: Bladder Cancer; PCa: Prostate Cancer; RCC: Renal Cell Carcinoma; AUC: Area Under the Curve; 4-HPA: 4-Hydroxyphenylacetate; MS: Mass Spectrometry; LDI-MS: Laser Desorption/Ionization Mass Spectrometry; LC-MS: Liquid Chromatography-Mass Spectrometry; eGFR: estimated Glomerular Filtration Rate; htTKV: height-adjusted Total Kidney Volume; T2DM: Type 2 Diabetes Mellitus; POC: Point-of-Care; TMAO: Trimethylamine N-oxide; MetS: Metabolic Syndrome; BCAAs: Branched-Chain Amino Acids; OPLS-DA: Orthogonal Projections to Latent Structures Discriminant Analysis; SGLT-2: Sodium-Glucose Co-transporter-2; R2X: Goodness of fit; Q2Y: Goodness of prediction; 4-H-3-MMA: 4-Hydroxy-3-methoxymandelate; Trp: Tryptophan; R2Y: Goodness of fit; CMV: Cytomegalovirus; 3-HB: 3-Hydroxybutyrate; CAP: Community-Acquired Pneumonia; ARDS: Acute Respiratory Distress Syndrome.

2. Methodological Considerations for ^{1}H NMR-Based Urine Metabolomics

Among the biological samples used in clinical metabolomics, urine is particularly attractive because it can be collected non-invasively, in relatively large volumes, and repeatedly over time. Nevertheless, different biological samples, including urine, serum, and other matrices, reflect distinct aspects of metabolism and present different practical advantages and limitations. A brief comparison of these commonly used sample types is provided in Table 2.

Table 2. Advantages and disadvantages of different biological samples in clinical metabolomics.

Biological Sample	Main Information	Advantages	Disadvantages	Typical Applications
Urine	Excreted metabolites; renal and systemic metabolism	Non-invasive; Easy repeated sampling; Large sample volume; Simple preparation	Affected by hydration, diet, circadian rhythm, and renal function; Requires normalization	Kidney disease, metabolic disorders, exposure studies, longitudinal monitoring
Serum/plasma	Circulating systemic metabolites	Clinically familiar; Reflects systemic metabolism; Easy to combine with routine tests	Invasive collection; affected by fasting, hemolysis, anticoagulants, and high protein/lipid content	Cardiometabolic disease, cancer, inflammation, prognosis and risk prediction
Cerebrospinal fluid	Central nervous system metabolism	Closely reflects brain and spinal cord biochemical changes	Invasive collection; limited volume; Unsuitable for repeated screening	Neurological and neurodegenerative diseases
Saliva	Oral and systemic metabolites	Non-invasive; Simple and suitable for repeated sampling	Low metabolite abundance; Affected by oral microbiota, food residues, and pH	Oral disease, stress/endocrine studies, pediatric studies
Feces/stool	Gut microbial and dietary metabolism	Directly reflects gut microbiome activity	Low acceptability; Heterogeneous matrix; Difficult processing and normalization	Gut microbiome-related metabolism, dietary exposure assessment, and inflammatory bowel disease
Tissue/cell extracts	Local organ- or disease-specific metabolism	High biological specificity; useful for mechanism studies	Invasive; limited repeatability; affected by tissue heterogeneity	Cancer metabolism, organ-specific disease, biomarker validation

The design and interpretation of urine metabolomics studies require attention to specific pre-analytical and analytical factors. Urine can be collected as spot samples, timed overnight voids, or complete 24-h urine collections. 24-h urine minimizes short-term fluctuations and is preferred when accurate quantification of excretion is required, but the logistical burden is higher than for fasting morning spot samples, which are more practical in many clinical settings. Regardless of collection scheme, storage temperature, duration before freezing, and freeze–thaw cycles should be standardized to reduce pre-analytical variability.

Because urine concentration varies widely between and within individuals, normalization is essential. Simple approaches such as creatinine normalization are widely used but are influenced by muscle mass and kidney function. Osmolality-based adjustment, probabilistic quotient normalization, and total spectral area normalization attempt to correct for dilution effects more generally, but each method has distinct assumptions and limitations [3]. Comparative work has shown that normalization choices can materially alter associations in epidemiological studies, underscoring the need for careful selection and transparent reporting [27–30].

Methodological choices in urine ^{1}H NMR can materially influence biological interpretation and cross-study comparability. Pre-analytical control of buffering and pH reduces chemical-shift variability and resonance overlap for organic acids and amino acids, while field strength and pulse sequence selection affect detectability and quantification in congested spectral regions, emphasizing the need to anchor mechanistic claims to analytically robust features with clear identification confidence. Among these factors, urine dilution correction is a major driver of heterogeneity: creatinine normalization, although widely used, may be misleading in clinically important settings such as muscle wasting/frailty and advanced renal dysfunction, where creatinine production and/or tubular handling is altered. We therefore recommend that studies justify their dilution-correction strategy, report key determinants of creatinine variability (e.g., renal function and, where feasible, proxies of muscle mass), and perform sensitivity analyses using at least one alternative approach (e.g., PQN and/or specific gravity/osmolality-based correction). For clinical translation, standardization should extend beyond acquisition parameters to include transparent metadata reporting, QC procedures, and reproducible spectral annotation/quantification workflows so that findings can be independently replicated and analytically validated [10,31,32].

Most clinical urine metabolomics studies use 400–700 MHz NMR spectrometers, although higher-field instruments and benchtop low-field systems are also used in selected settings. One-dimensional ^{1}H pulse sequences such as NOESY and Carr–Purcell–Meiboom–Gill are commonly employed for water suppression and macromolecule filtering. Samples are often buffered with phosphate and adjusted to near-neutral pH to minimize

chemical shift variability. Internal standards, such as trimethylsilylpropionic acid (TSP) or formate, are added for chemical shift referencing and, in some protocols, for absolute quantification. Spectral processing involves Fourier transformation, phase and baseline correction, alignment, and either binning or targeted deconvolution. Metabolite identification relies on curated databases and software tools, sometimes supported by two-dimensional experiments to resolve congested regions of the spectrum.

Statistical analysis spans unsupervised methods such as principal component analysis and supervised approaches such as partial least-squares and orthogonal partial least-squares discriminant analysis, complemented by univariate hypothesis testing. An increasing number of studies use machine-learning techniques to develop diagnostic or risk-prediction models from NMR-derived metabolite sets. Performance is usually reported using metrics such as area under the receiver operating characteristic (ROC) curve or Harrell's C-index, and integration with clinical covariates and gut microbiome data is becoming more common.

3. Kidney Diseases

Given the central role of the kidney in urine production, renal pathophysiology is naturally reflected in urinary metabolite profiles. Recent ^{1}H NMR studies have explored disease progression in autosomal dominant polycystic kidney disease (ADPKD), risk factors for nephrolithiasis, and markers of kidney allograft function [15–17].

In ADPKD, Dekker et al. analyzed spot urine samples from 324 patients at baseline and at three-year follow-up using ^{1}H NMR spectroscopy [16]. Fifty-five metabolites and ratios were quantified, and patients were categorized as fast or slow progressors according to annual decline in estimated glomerular filtration rate. A model based on urinary metabolites alone achieved prognostic performance comparable to that of height-adjusted total kidney volume, a widely used imaging-based marker, and a combined model improved discrimination further. Longitudinal analysis revealed that the myo-inositol/citrate ratio increased in fast progressors but remained stable in slow progressors, suggesting that this ratio may serve as an indicator of progressive renal function loss.

Kidney stone disease has also been investigated using urine metabolomics. Thongprayoon et al. compared 24-h urine from 418 first-time symptomatic stone formers with those from 440 controls [17]. Conventional chemistries showed expected differences in oxalate, calcium, phosphate, potassium, and creatinine. NMR-quantified metabolites further distinguished cases from controls, with stone formers showing lower hippurate, trigonelline, 2-furoylglycine, imidazole, and citrate, and higher creatine and alanine. Although adding NMR-derived metabolites did not substantially improve classification beyond standard chemistries, the findings highlight the relevance of gut microbiota–derived metabolites and organic acids in stone pathogenesis.

After kidney transplantation, monitoring allograft function is crucial. In a cohort of 55 primary transplant recipients, Baranovicova et al. analyzed both morning and 24-h urine samples by ^{1}H NMR [15]. They found that urinary creatinine levels were relatively independent of serum creatinine and estimated glomerular filtration rate, whereas citrate and acetone concentrations tracked allograft function more closely. Multimetabolite models combining several urinary metabolites showed promising predictive value, and the good agreement between morning and 24-h urine profiles suggested that carefully normalized spot samples may suffice for metabolomics-based monitoring.

Taken together, these studies indicate that ^{1}H NMR-based urine metabolomics can complement imaging and routine chemistry in nephrology. Consistent changes in citrate and related organic acids point to shared pathways in ADPKD progression, stone formation, and allograft dysfunction [33,34]. Urinary citrate is mechanistically compelling because it lies at the interface of cellular energy metabolism and renal tubular acid–base physiology. Citrate is a TCA-cycle intermediate that is freely filtered and predominantly reabsorbed in the proximal tubule; critically, citrate reabsorption and excretion are strongly influenced by acid–base status, with acid loading and lower tubular pH promoting citrate reabsorption and thereby reducing urinary citrate (hypocitraturia). This pH-dependent tubular handling provides a plausible physiological explanation for the repeated observation of altered citrate excretion across nephrology-related indications (e.g., nephrolithiasis, CKD phenotypes, and post-transplant states) and highlights why citrate should rarely be interpreted in isolation. Differences cross-studies may reflect variability in systemic bicarbonate/acid retention, potassium balance, dietary alkali load, and medication use (e.g., alkali therapy), as well as heterogeneity in kidney function and sampling strategy. Accordingly, when citrate is proposed as a candidate marker, interpretation and model development should incorporate renal function measures and acid–base indices, and—where feasible—evaluate longitudinal responsiveness (e.g., to alkali therapy or disease progression) to strengthen biological plausibility and translational relevance [35–37].

In kidney diseases, urinary NMR profiles are particularly informative because urine captures both systemic metabolic alterations and kidney-specific handling of metabolites, making it well suited to longitudinal monitoring and risk stratification rather than one-time classification. Cross-study inconsistency is frequently attributable to

renal function distribution, comorbidities, medication and diet effects, and heterogeneous sampling protocols, underscoring the need for stratified analyses and harmonized pre-analytics. Future validation should prioritize multi-center prospective cohorts and explicitly quantify incremental value over established clinical markers and imaging [38,39].

4. Adult Metabolic and Cardiovascular Diseases

4.1. Metabolic Syndrome, NAFLD, and Type 2 Diabetes

Metabolic syndrome (MetS) represents a cluster of abdominal obesity, dyslipidemia, hypertension, and insulin resistance that increases the risk of cardiovascular disease and type 2 diabetes mellitus (T2DM). In a large European cohort of 11,754 adults, Bruzzone et al. used ^{1}H NMR-based urine metabolomics to identify a molecular signature of MetS [19]. Seventeen metabolites, including glucose, aromatic amino acids, trimethylamine N-oxide, p-cresol sulfate, salicyluric acid, and maltitol, showed monotonic changes with increasing MetS severity. Models based on these metabolites achieved robust discrimination between MetS and non-MetS individuals, suggesting that urinary metabolomics can provide a molecular dimension to the clinical definition of MetS. Recent serum ^{1}H NMR metabolomics further showed that diabetic patients with chronic stable angina and myocardial infarction display distinct metabolic signatures, supporting the role of NMR profiling in cardiometabolic risk stratification [40].

Serum-based NMR metabolomics has also been used to construct risk scores for MetS. Gil-Redondo et al. developed MetSCORE in a cohort of more than 20,000 donors by combining lipoprotein and metabolite information [41]. MetSCORE demonstrated excellent discrimination for MetS defined by World Health Organization criteria and could rank individuals according to future risk, indicating that NMR-derived signatures may be useful for early intervention strategies.

The relationship between MetS, T2DM, and non-alcoholic fatty liver disease has been examined in animal models that integrate microbiome profiling. Qin et al. established a rat model of T2DM with non-alcoholic fatty liver disease (NAFLD) using a high-fat diet and streptozotocin, and combined 16S rRNA gene sequencing with ^{1}H NMR-based urine metabolomics [42]. They reported reduced gut microbial diversity, altered abundances of several genera, and changes in urinary metabolites involved in ketone body metabolism, the tricarboxylic acid cycle, and butanoate metabolism. Correlation analyses linked specific bacterial taxa to these metabolites, providing mechanistic clues to the interplay between gut dysbiosis and MetS-related metabolic alterations [43,44].

For T2DM risk prediction, Xie et al. integrated NMR-derived metabolites into a 10-year risk model in the UK Biobank and externally validated it in the German ESTHER cohort [45]. Eleven metabolites related to glycolysis, ketone bodies, amino acids, and lipids improved the performance of a traditional clinical score, increasing the C-index in both internal and external validation. This work illustrates how NMR-based metabolomics can add predictive value beyond conventional risk factors.

The technological spectrum of NMR platforms has been explored in diabetes as well. Edgar et al. assessed low-field (60 MHz) benchtop ^{1}H NMR for urinary metabolomics in patients with T2DM and healthy controls [46]. Despite lower spectral resolution and greater peak overlap, key biomarkers such as glucose and ketone bodies could still be detected and quantified, and patients were readily distinguished from controls. These findings suggest that compact, lower-cost NMR instruments could support point-of-care metabolic monitoring when accompanied by appropriate standardization [18].

Drug effects on the urinary metabolome have been evaluated in patients treated with sodium–glucose co-transporter-2 inhibitors. Bletsa et al. used ^{1}H NMR to profile urine from 50 patients with T2DM before and after three months of dapagliflozin therapy [20]. In addition to the expected increase in urinary glucose, they observed higher levels of ketone bodies, lactate, branched-chain amino acids, and other metabolites, indicating a broader reconfiguration of systemic energy metabolism that may underlie the cardio-renal benefits of this drug class.

In gestational diabetes mellitus (GDM), Yu et al. reviewed studies using metabolomics and proteomics to identify urinary biomarkers [47]. Amino acids, lipids, and purine derivatives were among the candidate markers, but the review emphasized substantial heterogeneity in sampling (spot versus 24-h urine), normalization strategies, and analytical platforms, complicating comparisons and highlighting the need for standardized protocols before urine-based biomarkers can be adopted clinically.

4.2. Stroke and Chronic Venous Disease

Cardiovascular and cerebrovascular diseases have also been examined with urine metabolomics. Oliveira et al. used ^{1}H NMR to interrogate urinary metabolites associated with stroke risk in older adults categorized by Framingham Stroke Risk Score [22]. Elevated trimethylamine N-oxide and reduced glycolate and trigonelline were

associated with higher stroke risk, with glycolate emerging as a particularly discriminative marker. Although confirmatory studies are needed, these results suggest that urinary metabolites may help refine stroke risk stratification.

To understand metabolic recovery after stroke, Petersson et al. performed a longitudinal urine metabolomics study using 700 MHz ^{1}H NMR [23]. They observed changes in phenylalanine, tyrosine, tryptophan, purine, and glycerophospholipid metabolism between acute and chronic phases, and identified pseudouridine as a metabolite associated with motor recovery. These findings indicate that metabolic profiling can provide insights into post-stroke cellular processes and may eventually contribute to prognostication or therapeutic targeting.

In chronic venous disease, Onida et al. combined serum and urine ^{1}H NMR metabolomics to compare symptomatic patients with asymptomatic volunteers and to explore associations with disease stage [21]. Serum aromatic amino acids showed positive correlations with severity, while several urinary metabolites, including citrate, succinate, formate, glycine, pyruvate, and 2-hydroxyisobutyrate, decreased as disease progressed. These patterns implicate alterations in tricarboxylic acid cycle activity and hypoxia-inducible pathways in venous pathology.

Across cardiometabolic phenotypes, urinary metabolite patterns frequently map onto insulin resistance–related fuel switching (glucose handling and ketone bodies), altered amino acid metabolism, and gut-derived co-metabolites that couple diet and microbial ecology to host cardiometabolic risk, suggesting that a pathway-centric synthesis is more informative than isolated metabolite listing when integrating evidence across MetS, diabetes, and vascular conditions. Nevertheless, heterogeneity in dietary standardization, medication exposure, obesity distribution, and sampling design (spot versus timed urine) can substantially confound associations, with microbiome-linked metabolites being especially sensitive to recent diet and antibiotic/probiotic use and therefore prone to reduced cross-cohort reproducibility unless exposure assessment and dilution correction are robust. From a translation perspective, the most defensible near-term applications are risk prediction and longitudinal monitoring in large cohorts where standardized pipelines and external validation are feasible, while low-field/benchtop NMR may enable targeted point-of-care workflows in selected scenarios [48] (e.g., metabolic monitoring) provided analytical performance, cross-platform harmonization, and clinical utility endpoints are rigorously established against existing standards of care [19,46,49,50]. Overall, studies in adult metabolic and cardiovascular disease consistently highlight perturbations in glucose metabolism, ketone bodies, tricarboxylic acid cycle intermediates, and microbiota-derived co-metabolites. Large-scale cohorts demonstrate that NMR-derived signatures can meaningfully improve risk prediction for MetS and T2DM, whereas smaller stroke and venous disease cohorts point to potential non-invasive markers of risk and disease severity that warrant further validation.

5. Oncology

5.1. Urological Cancers

Urological malignancies are among the most suitable targets for urine metabolomics because of their close anatomical relationship with the urinary tract. In a multi-platform study combining LC-MS and ^{1}H NMR, Bifarin et al. identified a 24-metabolite urinary panel associated with renal cell carcinoma (RCC) stage, suggesting potential utility for disease stratification [14]. Bladder cancer has also been investigated using combined ^{1}H NMR and mass spectrometry approaches. Ossoliński et al. reported significant urinary metabolic differences between 100 patients and 100 controls, achieving an area under the curve (AUC) greater than 0.87 for cancer detection [13]. In prostate cancer, Bruzzone et al. observed alterations in metabolites related to nitrogen and carbon metabolism, although multivariate models did not achieve clear separation from benign prostatic hyperplasia [11]. Urinary ^{1}H NMR metabolomics has additionally been explored for differential diagnosis among bladder cancer, prostate cancer, and RCC, with reported AUC values above 0.70 in a small cohort [12].

5.2. Non-Urological Cancers

Urinary metabolomics has also been applied to several non-urological malignancies, although urinary signatures in these settings are more likely to reflect systemic metabolic alterations than tumor-proximal effects. Studies have reported urinary metabolic signatures associated with papillary thyroid microcarcinoma, neuroendocrine neoplasms, pancreatic ductal adenocarcinoma, and early-stage non-small cell lung cancer [51–54]. In pancreatic cancer, a six-metabolite urinary panel was proposed for disease detection. In addition, serum-based ^{1}H NMR studies have identified metabolic signatures associated with metastatic melanoma [55]. Although serum-only studies fall outside the primary scope of this review, they provide complementary evidence supporting broader oncological applications of NMR metabolomics.

5.3. Treatment Response and Toxicity Monitoring

Beyond tumor detection, metabolomics may also assist in monitoring treatment-related metabolic changes. Ferreira et al. performed longitudinal metabolomic profiling of urine, plasma, and stool samples from patients receiving pelvic lymph node radiotherapy for high-risk prostate cancer [56]. Persistent metabolic alterations were observed across multiple biological matrices, including metabolites associated with cardiovascular risk and gut microbiota function. Similarly, metabolic changes following surgical treatment of early-stage non-small cell lung cancer have been reported, suggesting that metabolomic profiling may provide insights into treatment response and physiological recovery [55].

5.4. Translational Limitations

Despite promising findings, most oncological applications of urinary ^{1}H NMR metabolomics remain exploratory. Many studies involve relatively small cohorts, single-center designs, heterogeneous sampling protocols, or multi-platform analytical workflows, and external validation is often lacking. Furthermore, urinary metabolic profiles may reflect a combination of tumor biology, host systemic responses, treatment effects, diet, microbiome activity, and renal handling of metabolites, which can limit disease specificity. Consequently, urinary ^{1}H NMR metabolomics may contribute to biomarker discovery, disease stratification, and treatment monitoring after further validation, but it should not yet be considered an established diagnostic approach. Future studies should prioritize standardized methodologies, prospective multi-center validation, and demonstration of incremental clinical value beyond existing diagnostic and monitoring tools.

6. Immune-Mediated and Infectious Diseases

Immune-mediated diseases and infections involve complex interactions between host metabolism, inflammation, and the gut microbiome. Urine metabolomics offers a non-invasive means of capturing these systemic changes.

In rheumatoid arthritis, Oliveira et al. used ^{1}H NMR-based urine metabolomics in 90 patients to search for markers of skeletal muscle wasting [57]. They identified 15 metabolites with high variable importance scores, among which dimethylglycine, oxoisovalerate, and isobutyric acid correlated with appendicular lean mass index. These biomarkers reflected altered energy and amino acid metabolism and may help identify patients at risk of sarcopenia. Jutley et al. examined newly presenting rheumatoid arthritis and demonstrated strong relationships between C-reactive protein levels and both serum and urine metabolomes, including evidence of increased glycolysis, disrupted citrate cycle activity, oxidative stress, and enhanced protein catabolism [58]. Their findings emphasize that metabolomics can quantitatively reflect systemic inflammatory states.

In inflammatory bowel disease, Aldars-García et al. conducted serum and urine ^{1}H NMR metabolomics in a large multi-center cohort of treatment-naïve patients with Crohn's disease and ulcerative colitis [24]. They identified distinct metabolic signatures for each subtype, with Crohn's disease showing more pronounced perturbations in energy and amino acid metabolism than ulcerative colitis. These profiles may support disease stratification and contribute to understanding pathogenic mechanisms.

Infections, particularly those affecting neonates and critically ill patients, have also been studied. Spadavecchia et al. profiled urinary metabolites in newborns with congenital human cytomegalovirus infection and found elevated levels of several organic acids, including citrate, 3-hydroxybutyrate, and acetoacetate, and reduced levels of creatine, creatinine, and taurine compared with healthy controls [25]. These patterns suggest pronounced alterations in energy metabolism and may be useful for early diagnosis or prognosis. Yan et al. analyzed serum and urine from patients with community-acquired pneumonia who either did or did not develop acute respiratory distress syndrome [26]. Patients with ARDS showed marked changes in energy and amino acid metabolism, and models based on combined serum and urine metabolites achieved excellent performance in evaluating therapeutic responses.

The metabolic consequences of severe viral infection and recovery were explored by Rosolanka et al. in patients hospitalized with COVID-19 [59]. Urinary acetone and carnitine increased during the acute phase and normalized over time, whereas hippurate levels remained low one month after discharge, consistent with persistent alterations in gut microbiota–derived metabolism. As in other settings, these results point to the importance of host–microbe interactions and energy reprogramming in the aftermath of severe infection.

Immune-mediated and infectious conditions often produce urinary metabolic changes consistent with systemic inflammation, oxidative stress, altered energy utilization, and perturbations in host–microbial co-metabolism, which provides a mechanistic explanation for why some metabolites recur across apparently distinct inflammatory states and should be interpreted as shared upstream physiology rather than disease-specific

fingerprints. However, heterogeneity is amplified by variable timing of sampling relative to symptom onset, differences in disease severity, and medication regimens (notably antibiotics and immunomodulators), while dietary intake and hydration can fluctuate substantially during acute illness, making dilution correction and standardized sampling particularly critical; longitudinal sampling with consistent clinical metadata is needed to disentangle disease trajectories from transient exposure effects [59–62]. Consequently, the most tractable translational direction is often prognostic enrichment and monitoring (e.g., identifying high-risk trajectories or treatment response) rather than stand-alone diagnosis, and future studies should emphasize longitudinal designs, harmonized pre-analytics and QC, integration with routine clinical covariates and inflammatory markers, and external validation across sites and care settings.

7. Pediatric Developmental and Neurodevelopmental Disorders

The diagnostic value of urine metabolomics is well established for certain inborn errors of metabolism, where characteristic metabolic abnormalities directly reflect specific enzymatic defects [2,63,64]. ^{1}H NMR-based screening can detect broad patterns of amino acids, organic acids, and purine/pyrimidine metabolites in a single experiment and thereby complement tandem MS–based newborn screening. Characteristic findings include elevated phenylalanine-to-tyrosine ratios in phenylketonuria, accumulation of branched-chain keto acids in maple syrup urine disease, and specific intermediates in purine and pyrimidine metabolic disorders. Because urine sampling is non-invasive and repeatable, it lends itself particularly well to dynamic monitoring in pediatric patients.

Beyond classical inborn errors, NMR-based urine metabolomics has been applied to congenital anomalies and neurodevelopmental disorders. In neonates with prenatally diagnosed renal pelvis dilatation, Scalabre et al. showed that urinary metabolic profiles could differentiate ureteropelvic junction obstruction requiring surgery from transient dilatation and from healthy controls, implicating amino acid and betaine metabolism in obstructive pathology [65]. In 5q spinal muscular atrophy, Saffari et al. analyzed multiple biofluids and demonstrated that metabolic profiling could distinguish patients from controls with high sensitivity and specificity and could differentiate early- from late-onset disease, suggesting potential applications in diagnosis and severity stratification [66].

Neurodevelopmental conditions such as attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder have also been investigated. Swann et al. combined ^{1}H NMR and LC-MS to characterize urinary and fecal metabolomes in twins with and without ADHD, identifying sex-specific metabolic phenotypes and increased urinary hippurate as a marker of microbial–host co-metabolism in male patients [67]. Sotelo-Orozco et al. examined urine collected during the first year of life in children who later developed autism spectrum disorder or other non-typical neurodevelopment [68]. Subtle alterations in one-carbon metabolism, microbial co-metabolism, and neurotransmitter precursors preceded clinical diagnosis, raising the possibility that early metabolic changes may foreshadow later neurodevelopmental outcomes.

Pediatric applications span two mechanistically distinct regimes: in inborn errors of metabolism, urinary profiles can capture relatively proximal biochemical consequences of enzymatic defects and therefore support clearer causal interpretation, whereas in neurodevelopmental phenotypes urinary signatures are more likely to reflect broader systemic physiology and host–microbial co-metabolism and should be synthesized at the pathway level (e.g., one-carbon metabolism, neurotransmitter precursors, microbiome-derived co-metabolites) with cautious language. Age-dependent metabolic baselines, growth-related changes in muscle mass and renal handling, and variable diet/medication exposures can strongly affect urinary metabolite levels, undermining cross-study comparability unless age stratification, standardized sampling windows, and robust dilution correction are implemented; microbiome-linked metabolites may be particularly unstable in children due to rapid dietary transitions and frequent antibiotic exposure. From a translational standpoint, urine ^{1}H NMR is well suited for longitudinal monitoring because of non-invasive repeat sampling, but meaningful clinical adoption requires age-specific reference ranges, harmonized protocols, and validation in adequately powered cohorts, with complex neurodevelopmental outcomes most plausibly targeted for stratification and monitoring frameworks rather than diagnostic claims unless supported by prospective evidence and clear incremental value over existing clinical assessments [63,69,70]. Complementary serum-based ^{1}H NMR evidence has also extended metabolic profiling to adult neuropsychiatric stratification, with a pilot study identifying distinct circulating signatures between treatment-resistant and non-treatment-resistant schizophrenia, mainly involving amino acid and bioenergetic metabolism [71].

8. Lifestyle, Dietary, and Environmental Exposures

Diet and lifestyle are major determinants of the urinary metabolome, and ^{1}H NMR-based profiling has been used extensively to identify objective biomarkers of dietary intake and lifestyle behaviors.

Several large studies have linked urinary metabolites to dietary patterns in adults and children. Stratakis et al. investigated European schoolchildren from multiple cohorts and used ^{1}H NMR to identify hippurate, N-methylnicotinic acid, urea, and sucrose as markers of Mediterranean diet adherence and ultra-processed food consumption [72]. These metabolites discriminated against diet quality better than sociodemographic variables and correlated with C-peptide levels, providing a link between diet quality and metabolic health. Using 24-h urine collections in adults from the INTERMAP study, Oude Griep et al. conducted a metabolome-wide association analysis of fruit and vegetable intake [73]. They developed an 11-metabolite score, driven largely by citrate, that was inversely associated with systolic blood pressure and body mass index, suggesting that urinary profiles can objectively capture cardioprotective dietary patterns.

Intervention studies have examined how the urinary metabolome responds to prescribed diets. Clarke et al. followed overweight and obese individuals assigned to a high fruit and vegetable diet for weight loss and quantified 123 urinary metabolites at baseline, two weeks, and ten weeks [74]. Twenty-one metabolites changed significantly from baseline to week ten, and machine-learning models based on these metabolites accurately distinguished baseline from post-intervention samples, indicating that urinary profiles can serve as biomarkers of dietary adherence. Correia et al. compared high- versus low-protein breakfasts in overweight women and found that baseline urinary metabolites such as trigonelline, N,N-dimethylglycine, and trimethylamine were higher in those who experienced greater weight loss with the high-protein breakfast, suggesting that metabolic phenotypes may influence response to dietary interventions [75].

Controlled feeding studies have further clarified the relationship between dietary macronutrient intake and metabolomes. In a trial embedded in the Women's Health Initiative, Zheng et al. used multiple platforms, including NMR, to measure serum and urine metabolites and demonstrated that selected panels could predict daily energy intake from protein and carbohydrate with moderate accuracy [76]. Rowley et al. examined pregnant women with high versus low adherence to a Mediterranean diet, based on validated questionnaires, and identified distinct serum and urine metabolic phenotypes—along with lower inflammatory markers in the high-adherence group—that may mediate dietary influences on maternal and fetal health [77].

The role of the gut microbiome in diet–metabolism interactions is exemplified by studies on hippurate. Brial et al. combined urinary ^{1}H NMR metabolomics with shotgun metagenomics in middle-aged adults and identified strong associations between hippurate excretion, microbial gene richness, and benzoate biosynthetic pathways [78]. In high-fat-diet–fed obese mice, chronic hippurate supplementation improved glucose tolerance and insulin secretion, suggesting that this co-metabolite is not only a marker but also a mediator of metabolic health.

Infant nutrition provides another arena where urine metabolomics has been informative. He et al. and Falaina et al. compared breast-fed infants with those receiving formula diets of varying protein content and composition [79,80]. Serum, urine, and fecal metabolomes differed significantly between groups, and reduced-protein formulas produced metabolic profiles more similar to those of breast-fed infants, although differences persisted, indicating that formula modifications alone cannot fully replicate the metabolic effects of breast milk.

Lifestyle and environmental factors beyond diet also influence urinary metabolomes. Kistner et al. used a multi-platform metabolomics approach to examine acute effects of moderate and vigorous endurance exercise in young, healthy men [81]. Vigorous exercise induced more pronounced changes in urinary metabolites related to glycolysis, the tricarboxylic acid cycle, purine breakdown, and amino acid mobilization, reflecting heightened energy turnover. West et al. studied pregnant women with previous bariatric surgery and reported substantial alterations in maternal serum and urine metabolites, including lower branched-chain amino acids and higher microbial putrefaction products, which were associated with improved insulin sensitivity but also with changes in birth weight [82]. These observations highlight both beneficial and potentially adverse metabolic consequences of malabsorptive procedures.

Environmental exposures can leave a metabolic imprint as well. Sanchez-Rodriguez et al. assessed traffic density around participants' residences and its association with plasma metabolome profiles and urinary oxidative stress biomarkers [83]. Higher traffic density was linked to specific metabolite changes and increased oxidative stress, implicating lipid and microbial co-metabolism pathways in traffic-related health effects.

In exposure research, urinary metabolomics often provides biologically integrated readouts of diet, activity, and environmental inputs through host metabolic processing and microbial transformation, such that mechanistic interpretation should emphasize exposure-to-metabolite pathways and the "integrator" role of urine rather than disease-specific inference; metabolites such as hippurate can function as composite indicators of dietary patterns and microbiome-related processing, while organic acids and nitrogen metabolites may reflect energy turnover and protein metabolism in response to lifestyle interventions [84–88]. Yet exposure-related signatures are highly sensitive to spot sampling variability, recent meals, hydration, and day-to-day behavioral fluctuations, and without standardized timing, repeated measures, and robust dilution correction, associations may be attenuated or

inconsistent across cohorts; combining repeated sampling with appropriate normalization and careful metadata (dietary recalls, medication logs, physical activity measures) can substantially improve reproducibility. Translation is most immediate in epidemiology and preventive medicine, where objective biochemical proxies can complement self-report data to reduce misclassification and to monitor intervention adherence, but clinical implementation should quantify added value over questionnaires and develop standardized reporting that enables cross-cohort calibration and external validation.

9. Cross-Cutting Challenges and Methodological Limitations

Despite the breadth of applications reviewed, several recurring limitations constrain the clinical translation of ^{1}H NMR-based urine metabolomics. The first concerns analytical sensitivity and spectral complexity. NMR is inherently less sensitive than MS by approximately two to three orders of magnitude, which restricts detection of low-abundance metabolites such as many hormones and signaling molecules [4]. In addition, key spectral regions, particularly between 3 and 4 ppm, are often congested with overlapping peaks, complicating peak assignment and quantitative analysis, especially at lower field strengths.

A second issue is the intrinsic variability of urine. Hydration status, diurnal patterns, dietary intake, and physical activity all influence urine volume and metabolite concentrations. Although a range of normalization techniques is available, each has limitations, and there is no universally accepted standard [3]. Inconsistent application of normalization methods cross-studies makes it difficult to compare results and to aggregate data across cohorts.

Heterogeneity in sampling and preprocessing protocols represents a third challenge. Studies differ in whether they use spot or 24-h urine, whether samples are collected in fasting or non-fasting states, the choice of buffer and pH adjustment, and the specific pulse sequences employed. These differences can introduce systematic biases that affect both qualitative and quantitative aspects of the data. Transparent reporting and movement toward harmonized protocols will be crucial for reproducibility.

Fourth, urinary metabolites are strongly influenced by diet, medication, and gut microbiome. Many disease-focused studies adjust for age and sex, but do not fully account for dietary patterns, medication use, or probiotic and antibiotic exposure. As a result, it can be difficult to disentangle disease-related metabolic changes from those driven by lifestyle and treatment.

Another important limitation is the interpretation of metabolite changes. As recently emphasized, metabolites are not equivalent to genes, and pathway analysis developed for transcriptomic data should be applied to metabolomics with caution [89]. Because metabolites can be produced, consumed, transported, and excreted, their concentrations do not directly indicate pathway activation or causal involvement in disease. This is especially relevant for urine metabolomics, where metabolite levels may also reflect diet, gut microbial activity, medication exposure, renal filtration, and tubular handling. Therefore, mechanistic interpretation of urinary NMR findings should be conservative and supported by reliable metabolite annotation, clinical context, and, where possible, multi-omics or experimental validation.

A representative example is hippurate. Hippurate is a prototypical host–microbial co-metabolite and provides a useful lens through which to interpret cross-disease recurrence of urinary metabolites while acknowledging the specificity challenge in urine metabolomics. Biochemically, hippurate largely reflects a multi-step cascade linking dietary substrates (e.g., phenolics and aromatic compounds), microbial conversion to benzoate-related intermediates, hepatic glycine conjugation, and subsequent renal excretion; therefore, hippurate may vary across seemingly disparate conditions not because it is disease-specific, but because multiple upstream determinants (dietary pattern, microbial ecology and functional capacity, hepatic conjugation, and kidney function) are perturbed. This framework helps reconcile why hippurate has been reported to change across neurodevelopmental disorders, dietary interventions, cardiometabolic phenotypes, and infectious/inflammatory states, and it underscores an important translational implication: without careful control (or measurement) of diet, medication exposure (especially antibiotics/probiotics), and renal function, hippurate-centered signatures risk limited diagnostic specificity. In reviews, hippurate is therefore best discussed as an informative readout of the gut–liver–kidney co-metabolic axis and as a marker whose interpretability depends strongly on rigorous phenotyping and harmonized pre-analytics [78,90,91].

Finally, many of the disease-specific studies reviewed here involve modest sample sizes and lack independent validation. Although large population-based cohorts exist for some indications, such as MetS and T2DM, most oncology, pediatric, and infectious disease applications remain limited to single-center proof-of-concept studies. Incomplete metabolite annotation and inconsistent reporting of quality-control procedures further complicate external replication [92].

Addressing these cross-cutting challenges will require coordinated efforts among laboratories and disciplines, including the development of shared protocols, reference materials, and data standards, as well as the design of adequately powered, multi-center validation studies.

10. Future Prospects

Future development of ^{1}H NMR-based urine metabolomics will rely on improved sensitivity and resolution, harmonized protocols for urine collection, storage, buffering, pH adjustment, and normalization, and automated workflows for spectral processing and metabolite quantification [4,93,94]. Integration with artificial intelligence, clinical metadata, and multi-omics data, particularly microbiome profiling, may further improve metabolite annotation, predictive modeling, and pathway-level interpretation [81]. For clinical translation, candidate urinary biomarker panels must be validated in independent cohorts and demonstrate incremental value beyond standard clinical tests, together with analytical validity, clinical utility, and cost-effectiveness [95,96].

Compared with MS-based urine metabolomics, NMR has lower analytical sensitivity and narrower metabolite coverage, especially for low-abundance metabolites and complex lipids. However, NMR offers high reproducibility, robust quantification, minimal sample preparation, and strong suitability for large-scale epidemiological studies and longitudinal monitoring. In contrast, MS provides broader coverage and higher sensitivity but is more susceptible to matrix effects, batch variability, and more complex sample preparation. Therefore, NMR and MS should be regarded as complementary rather than competing platforms: NMR can provide a stable quantitative backbone for abundant urinary metabolites, whereas MS can expand metabolite coverage and support deeper biomarker discovery.

11. Conclusions

^{1}H NMR-based urine metabolomics has emerged as a robust, reproducible, and non-invasive approach for metabolic phenotyping across a broad range of clinical applications, including kidney diseases, metabolic and cardiovascular disorders, cancers, immune-mediated diseases, pediatric conditions, and lifestyle-related exposures. Its minimal sample preparation requirements, quantitative capability, and suitability for repeated sampling make it particularly attractive for longitudinal monitoring and large-scale clinical studies.

Current evidence demonstrates considerable promise for biomarker discovery, disease stratification, prognosis assessment, and treatment monitoring. However, most published studies remain exploratory, often involving relatively small cohorts, single-center designs, heterogeneous methodologies, and limited external validation. Consequently, the clinical utility of many reported metabolic signatures has yet to be firmly established.

Future clinical translation will depend on greater standardization of urine collection, sample processing, normalization strategies, metabolite annotation, and data reporting. Equally important are large prospective multi-center studies, independent validation cohorts, and rigorous assessment of analytical validity and clinical utility. Importantly, future investigations should demonstrate incremental value beyond existing diagnostic and prognostic tools. With continued advances in standardization, automation, and multi-omics integration, ^{1}H NMR-based urine metabolomics has strong potential to contribute to precision and preventive medicine.

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