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Metabolomics in Search of Noninvasive Biomarkers for Allograft Rejection in Pediatric Kidney Transplantation

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Abstract

Introduction: Kidney transplantation is recognized as the most effective treatment for children with end-stage renal disease (ESRD), providing significant improvements in quality of life and long-term survival. Traditional methods to detect involve after allograft rejection AR primarily invasive biopsy procedures that, while diagnostic, carry significant risks, especially in pediatric patients. Therefore, there is an urgent need for safer, less invasive, and more patient-friendly methods to monitor graft health. Metabolomics, the comprehensive analysis of small-molecule metabolites within a biological sample, offers a promising solution.

Materials and Methods: This paper is a non-systematic review. PubMed and Scopus-indexed journals were used to collect articles for research. In general, 6 papers were included.

Results: Our findings indicate that specific urinary metabolites can serve as sensitive and specific indicators of AR, offering a safer alternative to biopsies. Metabolomic profiling not only provides real-time insights into graft health, but also supports personalized management strategies to improve patient outcomes. This study contributes to the evolving field of transplant diagnostics, demonstrating how non-invasive methods such as metabolomics could revolutionize the monitoring and treatment of pediatric kidney transplant recipients.

Keywords: Metabolomics, pediatrics, kidney transplantation, rejection, biomarkers.

Introduction

Kidney transplantation is recognized as the most effective treatment for children with end-stage renal disease (ESRD), offering significant improvements in quality of life and long-term survival [1, 2].

Additionally, pediatric kidney transplantation can improve growth and development outcomes, neurocognitive function, learning ability, and quality of life compared to young patients on chronic hemodialysis or peritoneal dialysis [3, 4].

Despite advances in posttransplant management, including immunosuppressive therapy, long-term success is still limited by complications of immunosuppression, rejection, and disease recurrence [5].

In Figure 1, we have plotted some of the possible complications according to their time of onset. It is important to note that some of these are unique to the pediatric population. And the risk of acute graft rejection (AR) remains high in both adults and children and can lead to graft dysfunction and loss if not promptly recognized and treated [6].

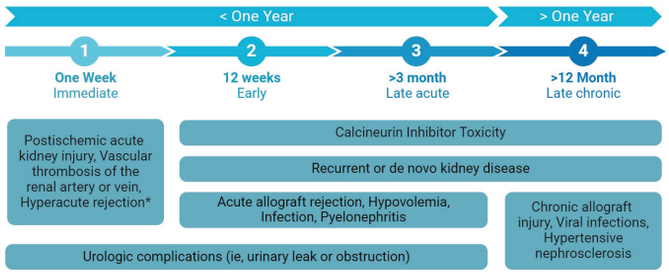

Figure 1 – Possible complications according to their time of onset

Traditional methods of detecting AR mainly involve invasive biopsy procedures that, while diagnostic, carry significant risks, especially in pediatric patients. These risks include bleeding, infection, and the psychological distress associated with invasive testing [7]. Therefore, there is an urgent need for safer, less invasive, and more patient-friendly methods to monitor graft health.

In recent years, attention has focused on identifying noninvasive biomarkers that can reliably predict acute rejection episodes. Non-invasive biomarkers detectable in biological fluids such as blood, urine, or saliva are a promising alternative to biopsy [8]. They have the potential to transform post-transplant care by allowing earlier and more frequent monitoring of graft status without the discomfort and risks associated with tissue biopsy.

Metabolomics, the comprehensive analysis of small molecule metabolites within a biological sample, offers a promising solution [9]. By reflecting the body's dynamic response to biological conditions or disease states, metabolomic profiling has the potential to serve as a sensitive and specific biomarker for the early detection of graft rejection.

The aim of our paper is to explore the use of metabolomics as a noninvasive biomarker of metabolomics profile in children with diverse graft conditions, including non-specified chronic injury after kidney transplantation. By analyzing changes in metabolic pathways and identifying signature metabolites associated with rejection, this study aims to contribute to the development of a safer and more effective approach to posttransplant monitoring that may significantly improve patient management and outcomes.

Methods

This review was conducted using peer-reviewed journals indexed in PubMed, Google Scholar, Scopus, and EMBASE. The literature search spanned from inception to 2024, focusing on articles related to metabolomics in pediatric kidney transplantation. The search strategy was designed to identify relevant studies, and the screening process included several steps.

First, all references identified by the database searches were independently reviewed at the abstract level by the lead author. Studies considered potentially relevant were selected for full-text retrieval and further assessment. To be eligible for inclusion, studies had to meet the following criteria 1) Population: Pediatric patients, regardless of body mass, who had undergone renal transplantation; 2) Intervention: Use of metabolomic analysis of urine samples; 3) Outcome: Any reported clinical outcome related to kidney transplantation or rejection; 4) Study design: Case reports, case series, retrospective or prospective studies; 5) General: Studies without identified conflicts of interest and those considered unbiased.

The following medical subject headings (MeSH) were used: 'metabolites'/exp OR metabolites AND 'kidney graft rejection'/exp OR 'kidney graft rejection' AND 'acute graft rejection'/exp OR 'acute graft rejection' AND 'child'/exp OR child; 'metabolites'/exp OR metabolites AND 'kidney graft rejection'/exp OR 'kidney graft rejection' AND ('child'/exp OR child; 'metabolites'/exp OR metabolites AND 'kidney transplantation'/exp OR 'kidney transplantation' AND 'child'/exp OR child AND 'kidney injury'/exp OR 'kidney injury'; 'metabolites'/exp OR metabolites AND 'kidney allograft rejection'/exp OR 'kidney allograft rejection' AND 'child'/exp OR child.

Studies were excluded if their populations overlapped with those of previously included articles or if they focused on adult populations. Following the screening and selection process, data were extracted and analyzed for inclusion in the final review. Only English language publications were included.

The flowchart of this literature search according to the PRISMA guidelines is shown in Figure 2.

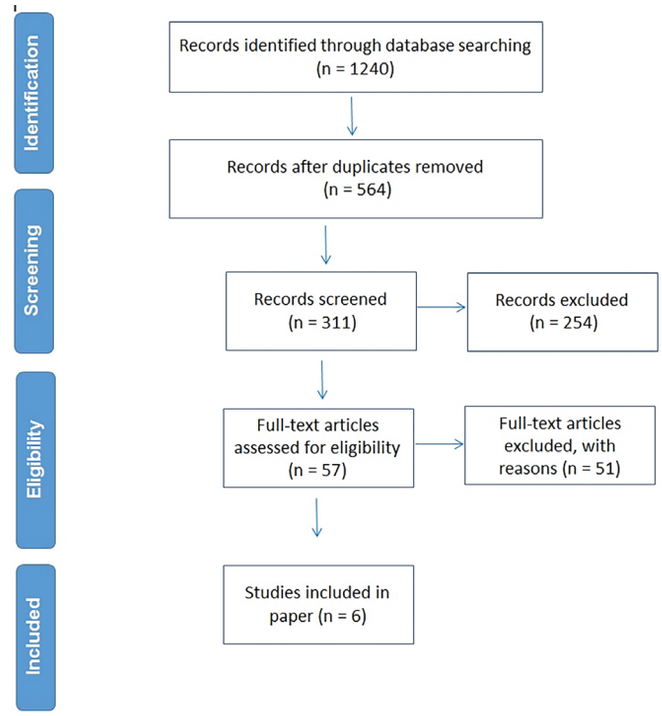

Figure 2 – Flowchart of Study Selection

Results

A total of six clinical research articles were included in the final analysis. These studies primarily presented aggregated clinical and laboratory data from pediatric patients who had undergone renal transplantation. A total of 716 samples were reported, with the number of metabolites reported ranging from 10 to 20 in each study.

All included studies focused on posttransplant patients; however, the objectives of the studies varied. Two studies specifically investigated the metabolite profiles associated with T-cell-mediated rejection, while one study focused on antibody-mediated rejection. In addition, one study each investigated acute rejection, chronic rejection and nonrejection renal injury. A detailed summary of the metabolite data and the study objectives is provided in Table 1.

Table 1 Summary of the studies from 1999-2024 on metabolites as non-invasive biomarkers of allograft injury/ rejection in kidney pediatric transplantation

	Author, year	Sample, n	Goal of the study	Result
1.	Blydt-Hansen et al. 2014 [10]	Urine, n = 57 Pts	T cell–mediated rejection (TCMR) metabolites	10 metabolites classifier, AUC = 0.88
2.	Blydt-Hansen et al. 2017 [11]	Urine, n = 59 Pts	Antibody-mediated rejection metabolites	10 metabolites classifier AUC = 0.806
3.	Mincham et al. 2018 [12]	Urine, n = 40 Pts.	Association b/w TCMR acuity and urine biomarkers	Urine metabolites + CXCL10 are better in assessing TCMR acuity than GFR
4.	Landsberg et al. 2018 [13]	Urine, n = 51 Pts.	Chronic injury metabolites after KTx	20 metabolites classifier, IFTA (AUC = 0.71), percent GS (AUC = 0.81)
5	Archdekin et al. 2019 [14]	Urine, n =199 samples	Non-rejection kidney injury metabolites	20 metabolites classifier, AUC = 0.79
6.	Sigdel et al. 2020 [15]	Urine, n = 310 Pts	Acute rejection, and kidney injury metabolites in KTx	11 metabolites and 9 metabolites

Pts, Participants; KTx kidney transplantation.

This variation in the focus of the study highlights the diversity of metabolic alterations associated with different forms of graft injury in pediatric renal transplantation. Despite differences in the type of rejection studied, all reports provided valuable insight into the potential utility of metabolomics as a biomarker of graft health and injury.

T cell-mediated rejection (TCMR)

Blydt-Hansen et al. established one of the first reports on metabolites associated with T-cell-mediated rejection (TCMR) in the pediatric cohort [10]. The availability of urine samples provided an opportunity to investigate the metabolic signature that directly reflects the catabolic and anabolic pathways inside transplanted kidney tissue. Urine samples of 57 patients were collected and cases with biopsy confirmed TCMR and non-TCMR were analyzed. Metabolics was run in accordance with the provided list of urine metabolites through liquid chromatography and then mass spectrometry [16]. 134 metabolites were identified in each sample. Urine samples were selected only in the presence of biopsy material collected for surveillance in a two-year period. The quantitative amount of metabolites was normilized to urine creatinine. The partial least squares discriminant analysis (PLS-DA) model suggested a threshold for a discriminant score of -0.4 to predict the presence of TCMR in comparison to non TCMR samples. The predictive accuracy of this discriminant score (AUC = 0.892) was shown to be higher compared to the prediction based on creatinine value (AUC = 0.756). Metabolites that were run in this PLS-DA model retained more than 50% significance of the TCMR discriminant value and were shown to be significantly different from the non TCMR group. The metabolites were: proline, PC: aa: C34: 4, kynurenine, sarcosine, methionine.SO, PC: ae: C38: 6, sulfoxide, threonine, glutamine, phenylalanine, alanine. The prediction model based on the selected 10 metabolites comprised AUC = 0.88 which is still higher than AUC = 0.756 based on GFR. Furthermore, the discriminant score for the condition of borderline tubulitis that reflects the extent of kidney injury was placed in the range between non-TCMR and TCMR cases. This supports the notion that tubulitis is in the continuity towards development of TCMR phenotype [10]. This study investigated metabolites that could reflect the state of T cell–mediated rejection in a period of 2 years after transplantation.

Mincham et al. assessed the association between the severity of kidney rejection histology and urinary biomarkers [12]. The study examined a sequential pair of biopsy samples along with urine metabolites and CXCL10. CXCL10 chemokine was selected as a marker of T cell cytotoxicity. The first tissue sample was performed > 2.5 months after kidney transplantation. The second kidney sampling was carried out in a period of 1-3 months after the first biopsy. The urine metabolites and CXCL10 was taken before biopsy. At the same time, GFR measurements were performed at the beginning of the study, every time prior to biopsy, and at a 12-month interval. The material of 40 patients was collected. Metabolite discriminant score (MDS), was obtained according to the same pattern as in the previous studies conducted by Blydt-Hansen et al. following the PLS analysis. The results showed that the change in GFR taken to assess severity between two consequential biopsies did not reflect any significance in TCMR acuity. On the contrary, changes as well as the change in metabolite discriminant scores of two tissue samples, were shown to contain significant association with the severity of T-cell-mediated kidney rejection [12]. This was the first study to implement an assessment of the metabolic change in combination with chemokine CXCL10 to assess the acuity of TCMR in two consecutive histological samples.

Antibody-mediated rejection

Blydt-Hansen et al. team also investigated the urine metabolomic signature in the antibody mediated rejection (AMR) after kidney transplantation [11]. The study included 59 patients. AMR was selected in comparison to the non-AMR group on histopathological presence of antibodies that interact with kidney endothelium and presence of donor-specific HLA antibodies (DSA) in plasma. Biopsies were collected in the first month and then every 3-6-12 months after transplantation. Liquid chromatography along with mass spectrometry analysis identified 133 metabolites in each urine sample. Partial least squares (PLS) analysis was applied to determine AMR discriminant score. The mean AMR score was 0.28 ± 0.14 with the threshold level for AMR prediction 0.23, while the mean score for non-AMR was 0.10 ± 0.13. Interestingly, it was shown that other inflammatory conditions such as UTI have an AMR score of 0.07 ± 0.08 which is similar to the non-AMR group. It shows that AMR differentiates between rejection and inflammation. The AMR model highlighted the contribution of 10 metabolites with AUC = 0.806: proline, citrulline, phosphatidylcholine aa.C34.4, C10.2, tetradecanoylcarnitine, lysine, methionine sulfoxide, hexose, threonine, acetylornithine. It is noteworthy that the first five metabolites were statistically significant compared to the AMR and non-AMR groups. Interestingly, the AMR group also demonstrated some clinical differences. Posttransplantation time in the AMR group was almost two times higher versus non-AMR group. Although proteinuria was the same in both groups, the AMR group showed a 25% decrease in eGFR compared to non-AMR samples. When the AMR classifier model was compared to the previous TCMR model, common metabolites such as PC.aa.C34.4, proline, citrulline, methionine sulfoxide and threonine. were noticed [11].

This supports the common pathophysiology in the mechanism of allograft rejection. Therefore, the study demonstrated the development of an antibody-mediated rejection classifier with 10 distinct metabolites that share common composition with T-cell-mediated kidney allograft rejection.

Chronic injury metabolites after KTx

The next study highlighted the search of metabolites specificity for post transplantation chronic kidney injury [13]. The study model was based on the previously elaborated PLS discriminant analysis, resulting in the elucidation of the discriminant score, i.e., Dscore. The Dscore was obtained as a solution to the discriminant equation, where the relative weight of the 133 urine metabolites was considered during model training towards a specific clinical condition. In this study, the dscore was calculated for glomerulosclerosis (GS) and interstitial fibrosis and tubular atrophy (IFTA) that were assumed to be chronic damage processes that accumulate after allograft transplantation [13]. 51 participants were included and sampling time was 28.9 ± 30.3 months after allograft transplantation. As a result, 20 most important metabolites for IFTA (AUC = 0.71), percent GS (AUC = 0.81) were identified. IFTA metabolites consisted of hexose, ornithine, leucine, c8, arginine, SM.OH.C22.2, histamine, C5.1, PC.aa.C30.2, histidine, lysine, C4.1, SM.C16.0, C6.1, C5.OH. C3.DC.M, SM.C20.2, PC.aa.C32.3, C5.M.DC, C3.DC.C4.OH, PC.ae.C38.2. While GS metabolites were arginine, C8, Met. SO, C5.OH.C3.DC.M, histidine, threonine, C10.2, kynurenine, glutamine, SM.OH.C22.2, proline, SM.C16.0, PC.aa.C30.2, ornithine, SM.C26.1, C4.1, leucine, SDMA, SM.C20.2, histamine. The classifiers share 12 metabolites in common, representing the intertwined nature of two processes in the formation of a chronic kidney injury condition. Therefore, the study was the first to define the metabolome composition of urea for such chronic processes such as IFTA and GS.

Non-rejection kidney injury after KTx

Another study searched for metabolites during nonrejection kidney injury (NRKI) emerged [14]. The authors assumed that NRKI may be one of the pathophysiological mechanisms as a result of adult kidney transplantation to a child that cannot meet the perfusion demands of an organ. During selection, all tissue that contained signs or rejection such as TCMR, AMR were excluded from NRKI group, thus reflecting the cohort with the clinical and histological traits of kidney injury phenotype. The study applied the PLS-DA method to measure the contribution of the 133 metabolites found in each urine sample that best describes the clinical condition of interest [14]. 199 urine samples were run through the machine learning protocol to identify NRKI and non-NRKI metabolites. Thus, 20 most significantly contributing the the phenotype were selected (AUC = 0.79): Orn, Met.SO, Leu, Hexose, Ac.Orn, PC.aa.C34.4, Pro, C5.1, C4, C3.OH, PC.ae.C44.5, PC.aa.C30.2, ADMA, Histamine, C9, Met, C2, C5.M.DC, C5.OH.C3.DC.M. Furthermore, the model was able to distinguish NRKI from clinical rejection with AUC = 0.81. The current investigation was the first in the direction of metabolite identification to differentiate between patterns of kidney injury and kidney rejection.

Acute rejection and kidney injury in KTx

One of the recent studies identified the panel of metabolites that differentiate kidney injury and rejection based on the larger number of participants (n=310) compared to previous studies [15]. Gas chromatography-mass spectrometry analysis identified 266 metabolites present in the urine samples of participants. After applying VSURF methodology, the team identified 9 metabolites (Glycine, N-methylalanine, Adipic acid, Glutaric acid, Inulobiose, Threitol, Isothreitol, Taurine, Sorbitol, Isothreonic acid) that characterized acute and chronic injury after transplantation in comparison to stable allograft phenotype (AUC = 0.950). Applying the same method, they identified 11 metabolites (Glycine, Glutaric acid, Adipic acid, Inulobiose, Threose, Sulfuric. Taurine, acid N-methylalanine, Asparagine, 5-aminovaleric acid lactam, Myo-inositol) differentiating the acute rejection phenotype from the stable allograft (AUC = 0.985). Thus, the study investigated a panel for the the metabolic signature for phenotypes of kidney injury and acute rejection compared to stable kidney transplant [15].

Discussion

Although a few studies were conducted in search of metabolomic profile for detection of allograft rejection, some conclusions could be drawn. Notably, five out six studies described in this review were conducted by the Blydt-Hansen et al. team. The team applied the partial least squares discriminant analysis (PLS-DA) model, highlighting the most frequent metabolites that are converted into a classifier. In every study, gas chromatography and mass spectrometry analysis detected only 133-134 metabolites, in comparison with the last study conducted by Sigdel TK et al., where the team detected 266 metabolites. Although the spectrum of urine metabolites includes 2651 identified compounds [16], only 5 % of the total identified urine metabolites was mentioned in the first five studies and 10 % in the last study. Both teams applied different bioinformatics methodologies to identify the most frequently attractive metabolites. It is interesting to know whether the classifiers composition changes if the number of detected metabolites increases and the bioinformatics methodology is unified. It should also be mentioned that only last study elaborated on the metabolic pathways that were involved based on the metabolite composition. Separation between acute and chronic rejection would also be beneficial for unifying common metabolic panels for acute and chronic states. However, in order to elucidate similar patterns of metabolites as biomarkers predicting allograft rejection, the ideal scenario includes patients with the same meal plan, drug consumption, enzyme liver activity and similar microbiome patterns, since urine contains the waste products of all reactions in the body. Therefore, to generalize the metabolic panel predicting kidney allograft rejection, further studies should be implemented, starting with the description of the metabolic pathways involved and stratification of the patient with respect to the drugs consumed, food preferences, and microbiome characterization.

Traditional methods and their limitations

Traditional methods for detecting renal allograft rejection in pediatric patients include serum creatinine, proteinuria measurement, and renal biopsy. Although these methods are fundamental to transplant monitoring, they have significant limitations that can affect clinical decision-making and patient outcomes.

The measurement of creatinine and its derivatives, although inexpensive and accessible, has low specificity and sensitivity. In pediatric practice, subclinical rejection confirmed histologically by biopsy is often found in the absence of any change in creatinine levels [17].

The study by Naesens and colleagues shown that, despite its relatively high specificity for transplant glomerulopathy, microcirculatory inflammation, and glomerular disease, proteinuria has a low sensitivity for intragraft injury [18]. Thus, proteinuria can be >1.0 g/24 h, and significant injury also confirmed histologically.

Although tissue biopsy is the gold standard for assessing graft status in transplantation [19], its use, especially in pediatric patients, is associated with complications, including the risk of adverse events such as bleeding and arteriovenous fistula, variability in interpretation, and is usually limited to the early post-transplant period [7, 20].

There continues to be a debate about the role of protocol biopsies in altering long-term allograft survival due to variability in immunosuppressive regimens and treatment of subclinical rejection. Studies suggest that pre-emptive treatment based on subclinical signs may improve graft survival, but stable patients sometimes show no adverse effects due to lack of treatment despite biopsies indicating potential problems [21, 22]. Another challenge in the routine use of biopsies is the variability in interpretation. Interpretation of biopsy results can vary widely depending on the pathologist's experience and the quality of the specimen [23]. This variability can lead to inconsistent diagnoses that affect treatment decisions. However, biopsy is currently the validation method for the development of new noninvasive markers.

Search for noninvasive biomarkers

The need for non-invasive monitoring is underscored by the results of studies such as the Canadian PROBE study, which suggest that traditional functional monitoring cannot adequately resolve or accurately assess the treatment of rejection episodes [24]. This underscores the growing interest in the need for improved non-invasive monitoring techniques that can provide a continuous and reliable assessment of graft status and help to better tailor personalized treatment strategies.

Urine biomarkers are the most promising way to noninvasively monitor graft status in pediatric kidney transplant patients. Unlike tissue biopsy, urine biomarkers offer a safe, reproducible, and stress-free alternative for ongoing assessment [25]. This is particularly important in children, where avoiding invasive procedures is a priority due to their smaller anatomical size, higher risk of procedural complications and the psychological impact of repeated procedures. Biomarkers in urine can be collected non-invasively and frequently, allowing real-time monitoring of graft function and detection of early rejection without the need for hospital visits or anesthesia [26].

The studies presented in this review offer significant potential for noninvasive monitoring of kidney transplant status in the pediatric population using urinary metabolomic biomarkers. These results highlight the potential of these biomarkers to improve the detection, differentiation, and management of renal allograft injury and rejection, thus improving patient care and reducing the reliance on invasive biopsy procedures.

Metabolomics play an important role in the early detection of AKI in kidney transplant recipients and in the differentiation between NRKI and rejection in children. Thus, the Archdekin study highlights the potential of metabolomics as a powerful tool for the noninvasive diagnosis and differentiation of NRKI from acute graft rejection (AR) in pediatric kidney transplant recipients [15]. The development of a urinary metabolite signature to accurately differentiate NRKI from AR represents a significant step forward in the post-transplant management of pediatric patients. The results of this study are particularly relevant in clinical settings where the distinction between NRKI and rejection is critical to determine the appropriate intervention. Current methods, based primarily on invasive biopsies and serum creatinine measurement, do not adequately detect NRKI at an early stage, often resulting in delayed or inadequate treatment. The introduction of a metabolomic approach could significantly change the approach by providing a rapid, non-invasive, and reliable method to assess renal function and identify lesion types.

Our analysis also highlights the potential of metabolomics to generate highly sensitive and specific biomarkers of acute rejection and BK-viral nephritis (BKVN) [16]. Additionally, the ability to differentiate BKVN from acute rejection using a separate set of four metabolites underscores the individualized approach to metabolomics. BKVN, which is often difficult to diagnose and treat, can have a significant impact on patient management. The ability to distinguish between different types of kidney damage using noninvasive urine tests represents a significant advance in transplantation, especially in pediatric populations who are often more susceptible to the risks associated with invasive procedures.

Advantages of Metabolomics

The use of metabolomics has the distinct advantage of providing a real-time metabolic snapshot of the organ. This is very important in transplantation, where early intervention can dramatically affect patient outcome. The ability to detect acute and borderline TCMR with high accuracy may help physicians more effectively tailor immunosuppressive therapy, potentially prolonging graft survival and improving patient quality of life [10, 13]. The incorporation of urine metabolomics into routine posttransplant monitoring may change current practice by reducing the frequency and need for invasive biopsies, which carry a risk of complications and are particularly challenging in the pediatric population.

Studies have shown that certain metabolomic profiles can predict long-term renal function and graft survival. For example, the Metabolite Discriminant Score (MDS) correlates with changes in kidney graft health over time and has been shown to predict mid- to long-term functional outcomes [13, 22]. This predictive ability allows the development of more personalized management strategies and the adjustment of immunosuppressive therapy prior to the onset of clinical symptoms or irreversible damage.

Considering the potential of urinary metabolomics as the least invasive, other studies are noteworthy. For example, Wang et al. conducted a study in adults showing that the intestinal metabolic profile of patients with AMR was significantly different from that of patients with ESRD, while it was not clearly different from that of recipients with stable renal function [27].

Additionally, metabolomic studies can be performed in different biological media from the same patient, potentially increasing the diagnostic relevance. Iwamoto and colleagues used CE-MS to analyze the metabolomic profiles of saliva, plasma, and urine collected from kidney transplant recipients and donors. Clear differences in metabolomic profiles were demonstrated between recipients with impaired and stable renal function [28].

Challenges and barriers to metabolomics implementation

At the same time, we should understand some challenges and barriers to the implementation of metabolomics [29]. One of the major challenges in using metabolomics for clinical diagnosis, such as the detection of acute transplant rejection, is achieving high sensitivity and specificity. Metabolic changes associated with rejection can be subtle and masked by metabolic fluctuations caused by other physiological or pathological conditions. The identification of metabolites that are consistently and uniquely altered during graft rejection requires comprehensive and controlled studies [30]. The reproducibility of metabolomic analyzes can be affected by variations in sample

collection, processing, and storage, as well as differences in analytical techniques and equipment. Standardizing these aspects is crucial to ensure that metabolomic profiles are reliable and comparable across different settings and time points [31]. For metabolomics to be applicable in clinical settings, the methods used must be compatible with the routine workflow of medical laboratories. This includes aspects such as cost, analysis time, and the need for specialized equipment and trained personnel [32]. Collaboration between researchers, clinicians, and industry is needed to overcome these challenges. The development of robust, standardized, and validated protocols and advanced computational tools for data analysis will increase the reliability and clinical utility of metabolomics.

The limitations of our study are that we only considered urinary metabolomics and only in pediatric practice in the post-transplant period. However, other omics can be considered as potential and diagnostically relevant. For example, consider metabolomics in the diagnosis of other acute and chronic diseases.

Conclusion

Our study highlights the promising potential of metabolomics as a noninvasive biomarker for the detection of graft rejection in pediatric kidney transplant recipients. By identifying specific metabolic signatures in urine, our study provides an important tool that can significantly improve post-transplant monitoring by offering a reliable, safe, and patient-friendly alternative to invasive biopsies. The results highlight the sensitivity and specificity of urinary metabolites in reflecting the status of the graft, which can allow earlier and more accurate interventions to prevent graft loss and improve long-term results.

Furthermore, the use of metabolomics represents a shift toward more personalized medicine, where treatments can be tailored based on individual metabolic changes. This may lead to a more nuanced and effective management of immunosuppression, reducing the incidence of rejection and other complications associated with pediatric kidney transplantation. Future research should focus on large-scale multicenter studies to validate these findings and facilitate the development of standardized guidelines for the use of urinary biomarkers and metabolomics in clinical practice. In addition, the combination of metabolomics with other "omics" technologies, such as genomics and proteomics, may lead to a more comprehensive understanding of graft health and rejection mechanisms. This integrated approach may pave the way for truly personalized medicine in kidney transplantation.

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