BMC Surgery

REVIEW

Open Access

Advances and challenges in the application of donor-derived cell-free DNA for diagnosis and treatment in liver transplantation: a narrative review

Yiwu Zhong[1], Xu Hu[1], Xiaoping Li[2], Yinbiao Qiao[3], Haoyu Li[3], Shijie Zhou[3], Shusen Zheng[3,4*] and Jianhui Li[2,3,4,5*]

Abstract

In the field of liver disease treatment, liver transplantation (LT) has become an effective option for end-stage liver disease. However, issues such as immune rejection and graft damage remain important factors influencing the success rate of liver transplantation and patients' quality of life. In recent years, with the advancement of genetic testing technologies, the study and application of donor-derived cell-free DNA (dd-cfDNA) in LT diagnosis and treatment have gradually gained attention. This review explores the research advancements in dd-cfDNA within liver transplant management, evaluating its potential applications throughout the liver transplantation process, while exploring the challenges faced by current studies and outlining future research directions. As a strategic tool for postoperative monitoring in LT, dd-cfDNA shows promising potential in areas such as immune rejection, graft damage, immunosuppressant adjustment, complication monitoring, and personalized treatment, and is poised to become a reliable biomarker in LT management.

Keywords Donor-derived cell-free DNA, Biomarkers, Liver transplantation, Non-invasive Monitoring, Transplant monitoring

Introduction

With continuous advancements in medical technology, liver transplantation has become the ultimate treatment for end-stage liver disease [1]. However, ischemia–reperfusion injury (IRI), immune rejection, infections, and postoperative complications remain important factors that influence patient outcomes after liver transplantation. In recent years, an increasing number of studies have focused on the use of donor-derived cell-free DNA (dd-cfDNA) as a non-invasive biomarker for monitoring post-transplant immune status and predicting rejection [2–5]. This paper reviews the research progress and clinical applications of dd-cfDNA in liver transplantation and discusses future research directions and challenges.

In the field of organ transplantation, especially liver transplantation, the progress of research on dd-cfDNA

*Correspondence:
Shusen Zheng
shusenzheng@zju.edu.cn
Jianhui Li
surgeonlee@126.com
[1] Zhejiang Chinese Medical University, Hangzhou 310053, China
[2] Key Laboratory of Artificial Organs and Computational Medicine in Zhejiang Province, Shulan International Medical College, Zhejiang Shuren University, Hangzhou, Zhejiang, China
[3] NHC Key Laboratory of Combined Multi-Organ Transplantation, Hangzhou 310003, China
[4] Department of Hepatobiliary and Pancreatic Surgery, Key Laboratory of Artificial Organs and Computational Medicine of Zhejiang Province, Shulan (Hangzhou) Hospital, Shulan International Medical College, Zhejiang Shuren University, Hangzhou 310022, China
[5] The Organ Repair and Regeneration Medicine Institute of Hangzhou, Hangzhou 310003, China

has attracted widespread attention. This DNA fragment, derived from necrotic or apoptotic donor tissue, can be accurately detected and quantified through next-generation sequencing (NGS) and other technologies, providing a non-invasive and sensitive method for clinical monitoring of allograft injury. Firstly, the application of dd-cfDNA detection, especially in kidney transplantation, has been fully demonstrated [6]. By detecting the concentration and absolute quantification of dd-cfDNA in circulating body fluids, signs of graft injury can be detected weeks to months in advance, providing a valuable"time window"for clinical treatment and delaying the loss of graft function. In pancreas-kidney combined transplantation [7], dd-cfDNA also shows its ability to predict acute rejection reactions early, providing a simple and non-invasive method for precise postoperative rejection monitoring. Therefore, dd-cfDNA is expected to become a non-invasive dynamic indicator for detecting graft injury or acute rejection (AR) after organ transplantation. Although the application prospect of dd-cfDNA in organ transplantation is broad, its research in liver transplantation is still in its infancy and faces many difficulties that need to be overcome. This article aims to discuss the research progress of dd-cfDNA in the diagnosis and treatment of liver transplantation and its clinical application.

Biological basis of cell-free DNA

Cell-free DNA (cfDNA) is a marker of cell death [8], and therefore it may be released from necrotic or apoptotic cells in the transplanted organ or from donor-derived hematopoietic cells in the recipient's blood or other organs. Other causes of transplant organ tissue injury can also result in the release of cfDNA into the plasma. For example, infections affecting the graft, such as cytomegalovirus, biliary and vascular complications, and tumor involvement of the transplanted organ [9]. Dd-cfDNA only accounts for a small part of the total cfDNA (recipient + donor origin), and studies on sex-mismatched bone marrow and solid organ transplants have shown that cfDNA in the plasma mainly comes from hematopoietic cells, with non-hematopoietic cell sources accounting for only a small proportion [10]. During LT, the sources of dd-cfDNA mainly include the death of donor liver cells, immune reactions after transplantation, and physiological or pathological changes in the recipient's liver [11]. Especially during AR, ischemia–reperfusion injury (IRI), and early graft dysfunction after liver transplantation, the concentration of dd-cfDNA will significantly increase, providing a theoretical basis for its potential application in liver transplantation.

In 1948, Mandel and Metais first discovered the existence of cfDNA and its release during cell apoptosis or necrosis due to damage [12, 13]. The length of cfDNA segments in the plasma of healthy volunteers is 185 ~ 200 base pairs, while the length of cfDNA segments released by tumor cells exists in the form of nucleoprotein complexes and has higher variability [14]. A study on cfDNA fragment size in monitoring graft injury during living-related LT for inborn errors of metabolism found that the ratio of short to long fragments can reflect the early trend of graft injury [15]. When a large amount of short single-stranded cfDNA (160 ~ 200 base pairs) was injected into mice, it was found that despite its low concentration in the blood, it could still be detected in the glomeruli after 24 h. In contrast, when larger cfDNA fragments (2000 ~ 6000 base pairs) were injected, no cfDNA was observed in the glomeruli. This result indicates that cfDNA in the plasma may be captured in the glomeruli through an unknown mechanism based on its fragment size [16]. The clearance mechanism of cfDNA in the plasma is still unclear. Studies have shown that the liver plays an important role in the clearance of cfDNA, and the reticuloendothelial system may be involved in this process. In addition, the presence of plasma-derived cfDNA in urine suggests that the kidney may also be involved in the clearance of cfDNA. A study on the clearance of fetal cfDNA in the maternal circulation showed that only 0.2% to 19% of fetal cfDNA was cleared through the maternal kidney [17]. This finding indicates that kidney excretion plays a secondary role in the clearance of cfDNA.

Research progress of dd-cfDNA in liver transplantation

Detection techniques of dd-cfDNA

Although the extraction of cfDNA has been standardized in research, the methods used to determine its donor origin remain diverse. Studies have identified that the most straightforward approach involves detecting the sex-determining region Y (SRY) gene or the testis-specific protein Y (TSPY) gene, both of which are located in the repetitive regions of the Y chromosome [18–23]. This method is particularly effective in female recipients, as these genes are absent in their genome. The detection process is relatively simple and does not require donor genetic material for comparison, making it a convenient option. However, this approach is limited in its ability to quantify dd-cfDNA and is applicable only to a small subset of transplant recipients, thus restricting its broader application. Additionally, similar techniques can be utilized for analyzing Rh gene mismatches in cases where the donor and recipient have differing Rh genotypes [24].

Currently, in the field of organ transplantation, the most commonly employed methods for detecting dd-cfDNA include real-time quantitative polymerase chain reaction (qPCR), droplet digital PCR (dd-PCR), and NGS [25, 26]. Each of these techniques has unique

Table 1 The dd-cfDNA assay and its application in liver transplantation

Technology platform	Advantage	Disadvantage	Clinical application	Reference
qPCR	Low cost and easy to operate; Suitable for mass screening	Low sensitivity and specificity; Inability to detect low concentrations of dd-cfDNA	One of the most used tests	[19, 21, 37]
dd-PCR	High sensitivity; Capable of precisely detecting minor variations in dd-cfDNA	The procedure is complex and expensive; Unsuitable for widespread application; Requires high-quality samples	Suitable for precise monitoring of dd-cfDNA; Holds considerable predictive value in the early stages of acute rejection	[38, 39]
NGS	Extremely sensitive; Capable of detecting mutations, methylation, and other alterations in dd-cfDNA	High cost; Complex data processing	Capable of providing a comprehensive analysis of mutations and genomic alterations	[29, 36, 40]
Genome analysis	Epigenetic changes in cfDNA can be detected	The methodology is complex; Data analysis requiring advanced technical support	Genomic methylation analysis can provide important information about immune response and rejection	[41–43]

Abbreviations: *dd-cfDNA* Donor-derived cell-free DNA, *qPCR* Quantitative polymerase chain reaction, *dd-PCR* droplet digital PCR, *NGS* Next-generation sequencing

features, offering effective tools for the quantification and analysis of dd-cfDNA (Table 1). The quantification of dd-cfDNA is typically based on genetic markers, such as single nucleotide polymorphisms (SNPs), which enable differentiation between donor and recipient alleles [27]. As a result, the assessment of dd-cfDNA levels can be performed without the need for prior genetic typing of both the donor and recipient. Clinical detection methods are primarily categorized into random[23, 28]and targeted approaches [29–31]. The random approach typically utilizes adapter ligation technology in combination with NGS, while the targeted method employs dd-PCR[29]or focuses on preselected SNPs within targeted NGS panels [30, 31]. It is important to note that amplification efficiency can be influenced by factors such as the size of the amplicon and the length of cfDNA fragments [26, 32, 33]. Despite these advances, there is currently no standardized protocol for detecting organ transplantation-specific targeted SNPs, and most studies rely on commercially available detection systems, including AlloSure, Prospera, and TRAC, among others [34]. In recent years, a novel approach for human leukocyte antigen (HLA)-based dd-cfDNA detection has emerged, which capitalizes on the incompatibility of the HLA-DRB1 locus between donors and recipients. This method has been optimized using dd-PCR [35]. While these techniques are capable of providing fractional measurements (e.g., % dd-cfDNA), it is worth emphasizing that absolute quantification remains unaffected by variations in recipient cfDNA, such as those induced by infections, and has been validated exclusively for dd-PCR-based methods [6, 36].

Changes in dd-cfDNA concentration during the perioperative period of LT

In liver transplant recipients, levels of dd-cfDNA in plasma increase dramatically immediately following transplantation [9, 44], accounting for up to 90% of the total free DNA present. This proportion decreases rapidly over time, with a half-life ranging from 24 to 48 h, and typically falls below 15% by the 10 th day post-surgery. However, in cases of immune rejection, the proportion of dd-cfDNA on day 10 remains elevated, around 20%, and gradually increases to 55% to 60%. Following reperfusion of the transplanted liver, elevated levels of dd-cfDNA were detected in the recipient's serum. In the absence of postoperative complications, these levels return to baseline within a few days [30, 45]. Similar trends have been observed in kidney transplant recipients, where dd-cfDNA concentrations increase immediately after transplantation but decline rapidly within the first week post-surgery [36, 46]. Studies by Zhang et al. [22, 23] have demonstrated that, in recipients with stable graft function, the plasma concentrations of dd-cfDNA are 0.9% in heart transplant recipients, 1.2% in kidney transplant recipients, and 3.5% in liver transplant recipients. These differences may be attributed to factors such as the size of the transplanted organ and the rate of cellular regeneration. Further investigations into dd-cfDNA kinetics have revealed that [39, 47], in recipients with stable graft function, the average plasma dd-cfDNA concentration ratio decreases to 0.5% ± 0.2% approximately 10 ± 6 days following transplantation. Notably, continuous monitoring of cfDNA levels during the perioperative period of living donor liver transplantation has indicated that the elevated cfDNA levels observed during surgery and the immediate postoperative phase reflect

Table 2 Clinical applications and significance of dd-cfDNA in liver transplantation

Clinical Application	Changes in dd-cfDNA Levels	Significance	Reference
Early Diagnosis of AR	Early in acute rejection, dd-cfDNA shows a significant increase, usually higher than the normal range	Can serve as an early warning signal for acute rejection, providing higher sensitivity and earlier diagnostic capability compared to traditional liver biopsy	[5]
Monitoring of Graft Function	Positively correlated with the recovery or deterioration of liver function, particularly with the levels of ALT and AST	Can be used to monitor liver damage or functional recovery in real time, overcoming the limitations of traditional biochemical tests and biopsies	[3]
Prediction of IRI	Rise rapidly and are correlated with the severity of the damage	Predict the occurrence of IRI and its impact on liver function, offering an opportunity for early intervention	[51]
Infection Monitoring and Complication Diagnosis	In cases of infection or other complications, dd-cfDNA levels often increase significantly	Help identify bacterial or viral infections after transplantation	[52]
Long-term Monitoring and Prognostic Assessment	Are associated with chronic rejection and chronic graft dysfunction	Not only for short-term monitoring, but also for the prediction of long-term post-transplant rejection and chronic liver failure	[11]

Abbreviations AR Acute rejection, *IRI* Ischemia–reperfusion injury, *ALT* Alanine Aminotransferase, *AST* Aspartate Aminotransferase

cellular trauma and inflammation associated with the transplant procedure [48].

Application of dd-cfDNA in liver transplantation

Orthotopic liver transplantation (OLT) is a critical therapeutic approach for end-stage liver diseases. However, postoperative challenges such as immune rejection, organ injury, and functional recovery continue to pose significant difficulties in clinical practice [49]. In recent years, dd-cfDNA has gained increasing attention as a non-invasive biomarker in the field of liver transplantation (Table 2). The dynamic fluctuations in dd-cfDNA levels offer valuable insights into immune responses, organ injury, and the effects of treatment, thereby providing essential support for the clinical management of liver transplant recipients [50].

Monitoring of immune rejection after transplantation

Numerous studies have explored dd-cfDNA in liver transplantation, with variations in the scale, design, detection methods, and outcomes of interest (Table 3). Nevertheless, most findings indicate that dd-cfDNA shows promise in monitoring graft health and detecting injury, especially in AR. Immune rejection is a common complication following liver transplantation, and studies have demonstrated a close association between changes in dd-cfDNA and acute rejection episodes [38, 53, 54]. Schütz et al. [5] observed that cfDNA levels in liver transplant recipients were significantly higher than those in healthy controls, with an increase in dd-cfDNA being positively correlated with the occurrence of AR. Furthermore, elevated dd-cfDNA levels can serve as an early indicator of AR [55, 56], often preceding detectable abnormalities in routine liver function tests. Subsequent research has further validated the potential of dd-cfDNA as a non-invasive tool for immune monitoring [57]. Goh et al. [58] found that persistent increases in dd-cfDNA levels were closely linked to the onset of acute immune rejection. Their study emphasized that regular dd-cfDNA monitoring allows for the timely detection of rejection, helping to prevent adverse outcomes associated with delayed diagnosis. Several studies have also tracked dd-cfDNA levels following successful treatment of acute rejection, showing that dd-cfDNA concentrations typically return to baseline levels in most cases [19, 55, 59–61]. However, the time required for recovery can vary. Bloom et al. highlighted that dd-cfDNA levels may remain elevated for up to one-month post-treatment and only return to baseline after two to three months, which could indicate lingering graft injury. Some studies suggest that by establishing a threshold for dd-cfDNA levels, it is possible to early differentiate between stable patients and those experiencing AR. For instance, a cutoff value of 10% dd-cfDNA threshold has been proposed to assess the stability of liver transplant recipients regarding rejection, showing good specificity and sensitivity (> 90% and > 86%, respectively) [4, 5, 45, 62]. Compared to stable patients, the dd-cfDNA percentage in liver transplant recipients with AR is approximately four times higher. The median dd-cfDNA in AR patients typically ranges from 30 to 40%, while in non-rejection cases, the median is around 11%. In addition, based on the fragment characteristics of cfDNA, the size of dd-cfDNA fragments can also aid in differentiating rejection reactions from other types of damage.

Monitoring graft injury

It is important to note that increases in dd-cfDNA levels are not always indicative of AR but can also occur in response to other causes of acute graft injury [3]. Studies have shown that [51], during ischemia–reperfusion injury (IRI), a common occurrence in liver transplants, extensive cell death results in the release of large amounts of cfDNA, leading to a marked elevation in dd-cfDNA levels in the bloodstream. Thus, dd-cfDNA may serve as a potential biomarker for assessing the extent of graft injury. Furthermore, a prospective observational cohort study found that dd-cfDNA levels serve as a reliable marker of graft injury after liver transplantation, with its increase being inversely correlated with liver function recovery [63]. In other words, patients with slower liver function recovery tend to exhibit higher levels of dd-cfDNA, indicating that dd-cfDNA can function as an early biomarker of liver injury. One of the major advantages of dd-cfDNA monitoring is its ability to assess graft integrity in a non-organ- or disease-specific manner, making it applicable for detecting tissue injury in various allografts, including heart, lung, liver, and kidney [64–67]. Endothelial cells play a significant role in the early immune response, inflammatory response, and microvascular injury following transplantation [68]. A significant portion of dd-cfDNA is derived from the endothelial cells of the allogeneic graft. IRI may be the cause of high dd-cfDNA levels immediately after transplantation, likely associated with endothelial cell damage and dysfunction Long-term monitoring of dd-cfDNA concentrations can provide valuable prognostic information regarding the survival and function of liver grafts [69]. In particular, within the first 2 to 3 months post-transplantation, dynamic changes in dd-cfDNA levels can offer insights

Table 3 Comparison of diagnostic performance of different dd-cfDNA thresholds for detecting liver graft injury

Study design and sample size	Year	Sample types	Assay method	Injury types	Thresholds	ROC-AUC	Sensitivity	Specificity	Reference
Multi-center Prospective ($n = 107$)	2017	Plasma	dd-PCR	AR	10.00%	0.97	90.30%	92.90%	[5]
Single-center Prospective ($n = 40$)	2019	Plasma	dd-PCR	AR	898 cp/mL	0.99	83.30%	100.00%	[58]
Single-center Prospective ($n = 49$)	2021	Plasma	NGS	Pediatric Rejection	28.70%	0.88	72.70%	94.70%	[45]
Single-center Retrospective ($n = 27$)	2022	Plasma	qPCR	AR	13.80%	0.77	85.70%	63.30%	[56]
Single-center Prospective ($n = 51$)	2022	Plasma	dd-PCR	TCMR	33.50%	0.73	NA	97.00%	[42]
Multi-center Prospective ($n = 219$)	2022	Plasma	NGS	AR	5.30%	0.95	87.00%	NA	[2]

Thresholds are assay-dependent and cannot be directly compared across studies due to methodological differences

AR Acute Rejection, *qPCR* Quantitative polymerase chain reaction, *dd-PCR* droplet digital PCR, *NGS* Next-generation sequencing, *TCMR* T-cell-mediated rejection, *NA* not available, *ROC-AUC* Receiver Operating Characteristic—Area Under the Curve

into the immune status and liver function recovery of the recipient.

Guiding individualized immunosuppressive therapy protocols

Immunosuppression (IS) plays a crucial role in preventing rejection after liver transplantation. However, the dosage and effectiveness of immunosuppressants must be precisely monitored to avoid both under- and over-suppression. Studies have shown that dynamic changes in dd-cfDNA levels are closely associated with the effectiveness of immunosuppressive treatment [70, 71]. A sustained increase in dd-cfDNA levels during immunosuppressive therapy may indicate insufficient IS [72], suggesting the need for timely adjustments in the dosage of immunosuppressive agents. Maintaining a high level of IS, particularly with calcineurin inhibitors (CNI), reduces the risk of AR but can increase the incidence of adverse effects such as infections, chronic kidney disease (CKD), malignancies, and cardiovascular diseases [73–79]. Therefore, continuous monitoring of dd-cfDNA levels offers a valuable tool for guiding individualized adjustments in immunosuppressive therapy post-transplant. This approach may help reduce the risk of early graft loss by ensuring optimal immunosuppression. One of the key advantages of dd-cfDNA monitoring is its ability to detect injury before clinical symptoms manifest, allowing for timely intervention in AR and other causes of graft injury, ultimately improving transplant outcomes.

As a tool for early detection of other complications

With the continuous advancement of research, dd-cfDNA has demonstrated significant potential not only as a biomarker for early detection of rejection and graft injury but also as an effective early warning system for a variety of transplant-related complications [52, 80–82]. Studies have increasingly shown that fluctuations in dd-cfDNA levels can serve as reliable predictors of adverse events following transplantation, including infections, hemorrhage, and other postoperative complications. Notably, during the first week after transplantation, dd-cfDNA levels may show a potential correlation with the onset of these complications [48], making it a valuable tool for early intervention before clinical symptoms become apparent. This ability to detect complications early may help clinicians in managing transplant recipients, particularly in the early postoperative period when prompt clinical responses are important. Most studies have focused on the long-term prognostic value of dd-cfDNA in kidney, lung, and heart transplantations [83]. While similar results may be expected in liver transplantation [84], further research is needed to confirm the relationship between dd-cfDNA and long-term results in LT. Changes in dd-cfDNA concentrations over time can offer insights into the survival prospects of liver transplant recipients, particularly within the first 2 to 3 months post-transplant. During this period, the dynamic fluctuations in dd-cfDNA levels are reflective of the recipient's immune status, as well as liver function recovery. Monitoring these changes can guide clinicians in assessing graft health, detecting subclinical rejection, and evaluating the effectiveness of immunosuppressive therapies. In this context, dd-cfDNA becomes an invaluable tool for assessing the progress of graft function and optimizing post-transplant management strategies. Continuous monitoring of dd-cfDNA levels has predictive value for assessing graft survival. Traditional monitoring methods, such as liver biopsy, remain the gold standard

for diagnosing graft rejection and injury; however, they are invasive, carry inherent risks, and are not suitable for frequent or routine use [85, 86]. By contrast, dd-cfDNA testing only requires a routine blood sample, making it a far more accessible, non-invasive, and cost-effective alternative. Furthermore, dd-cfDNA monitoring significantly reduces patient discomfort and minimizes the risk of infection associated with more invasive methods. It also helps reduce healthcare costs, making it a more sustainable option for long-term surveillance. The ability of dd-cfDNA to sensitively detect early signs of graft injury, rejection, and other transplant-related complications enables timely intervention and more personalized treatment strategies [11, 87].

Future prospects and challenges

An expanding body of evidence suggests that dd-cfDNA may hold potential in the diagnosis and management of liver transplantation [83, 88]. Firstly, by regularly monitoring dd-cfDNA levels, early signs of IRI or immune rejection can be detected, allowing for timely intervention. Secondly, the adoption of dd-cfDNA-guided personalized immunosuppressive therapy is progressively gaining traction [71]. Adjusting the dosage and regimen of immunosuppressants according to the patient's immune status and dd-cfDNA levels can improve treatment outcomes and reduce the risk of side effects. Research results indicate [63] that dd-cfDNA levels are closely related to graft function and can serve as one of the indicators for predicting graft survival rates. Lastly, with the continuous development of technology, more in-depth research using dd-cfDNA for genetic mutation detection and epigenetic analysis is being gradually conducted [89], providing more comprehensive information for the diagnosis and treatment of liver transplantation. It should be noted that the methodologies and findings of current studies are not entirely consistent. Moreover, the clinical implementation of dd-cfDNA as a diagnostic test in liver transplantation has yet to be fully validated, limiting its widespread application at this stage.

Despite the promising potential of dd-cfDNA as a biomarker for immune rejection in liver transplantation, several challenges persist: 1) Complexity of dd-cfDNA origin [90, 91]. The cfDNA not only originates from the donor liver but may also be derived from other organs or cells in the recipient, particularly under pathological conditions. Accurately distinguishing between dd-cfDNA and recipient-derived cfDNA remains a technical challenge. In clinical practice, distinguishing whether an increase in dd-cfDNA is due to IRI or AR is of significant clinical value. For example, a study by Agbor-Enoh et al. [92] demonstrated that dd-cfDNA fragments (< 120 bp) in antibody-mediated rejection (AMR) are smaller in size compared to those in acute cellular rejection (ACR). Epigenetics may also provide valuable insights. Epigenetic marks regulate chromatin compaction, thereby influencing gene expression. The most extensively studied of these marks are DNA methylation and post-translational modifications of histones. Some studies have utilized tissue-specific methylation patterns to identify the origin of cfDNA and construct methylation profiles specific to different tissues [93, 94]. In the context of transplantation, this approach can be employed to assess the proportion of dd-cfDNA. Epigenetic regulation plays a key role in inflammation-related pathways [95]. Inflammation is associated with global DNA hypomethylation, which can be instrumental in distinguishing IRI. 2) Standardization concerns [96, 97]. The standardization and harmonization of dd-cfDNA detection techniques remain unresolved issues. Variations in methods and protocols across different laboratories may impact the consistency and reliability of results. The donor's health status can impact dd-cfDNA levels and bias the diagnosis. Research has demonstrated that maintaining consistency with a single platform is crucial when conducting dd-cfDNA testing and creating comparative data in clinical practice [98]. We believe that the broad adoption of dd-cfDNA monitoring in LT necessitates further validation through comprehensive clinical randomized controlled trials to support its implementation. It is expected that the accuracy and precision of dd-cfDNA will continue to improve as research progresses. Another important issue in the field of liver transplantation is the potential difference in dd-cfDNA levels between split liver transplantation and whole liver transplantation, as well as between first-time and multiple transplantations. This requires further study for confirmation. 3) Barriers to clinical translation**.** Dd-cfDNA, as a biomarker for graft injury in solid organ transplantation, requires more prospective studies to establish its clinical utility [69]. Widespread clinical adoption is also hindered by practical challenges, including cost and specialized equipment requirements. Nevertheless, with ongoing advancements in technology, the clustered regularly interspaced short palindromic repeats-associated proteins (CRISPR–Cas) system-based assay [99, 100], known for their high sensitivity, specificity, low cost, and ease of use, are emerging as promising alternatives for dd-cfDNA detection. A cohort study utilizing the CRISPR-Cas system-based to detect Mycobacterium tuberculosis cfDNA has demonstrated the feasibility of this CRISPR-Cas system -based diagnostic technology in clinical practice [101].

In recent years, the value of dd-cfDNA in early diagnosis and prediction of rejection reactions after liver transplantation, as well as its role in assessing graft injury and monitoring treatment outcomes, still requires further

research and discussion, and it has not yet been widely applied in clinical practice. Additionally, how to combine dd-cfDNA with other immunological monitoring techniques and indicators to develop more reliable and precise biomarkers for optimal diagnostic and treatment strategies is also an important direction for future research. With the continuous development of liver transplantation technology, machine perfusion (MP) has gradually become an indispensable part of the liver transplantation process [102, 103]. Notably, a study by Kanou et al. [104] found that detecting cfDNA in ex vivo lung perfusion fluid can help assess the injury to the donor lung before transplantation and has a predictive role in the risk of primary graft dysfunction in lung transplantation. By leveraging the mechanical perfusion platform to move the detection of liver graft injury to the pre-transplant mechanical perfusion period, focusing on the role of dd-cfDNA in the perfusion fluid in indicating graft injury has significant clinical value. However, research on the application of dd-cfDNA in liver transplantation machine perfusion is still in the exploratory phase, and there is a lack of large-scale clinical data to support its use. Therefore, future studies should focus on further investigating the combined detection of dd-cfDNA with other biomarkers in the perfusion fluid, such as enzymes and cytokines associated with graft damage. This research direction has the potential to enhance the diagnostic value of dd-cfDNA in the liver transplantation machine perfusion process, thereby providing more precise monitoring tools for clinical practice. The dynamic monitoring of dd-cfDNA in conjunction with MP technology can provide important information for assessing cold ischemic injury to liver grafts, optimizing perfusion schemes, and improving transplant success rates. It is expected to become a standardized monitoring tool in the mechanical perfusion process of liver transplantation, further advancing liver transplantation technology and improving patient prognosis and quality of life.

Conclusion

In conclusion, dd-cfDNA has emerged as a promising non-invasive biomarker with significant biological relevance in the context of allogeneic liver transplantation. Beyond its established role in monitoring immune rejection and graft injury, dd-cfDNA offers valuable potential for evaluating the efficacy of immunosuppressive therapies, detecting early signs of complications, and supporting personalized treatment strategies. The clinical application of dd-cfDNA faces several challenges, including its complex origin, standardizing detection methods, and its clinical translation. Establishing critical thresholds for dd-cfDNA, analyzing dd-cfDNA fragment sizes, and incorporating epigenetic approaches may help address these challenges. A CRISPR-Cas-based detection technology may also offer potential solutions to these challenges in the future. Moreover, MP not only improves the quality of donor livers but also provides an early"Window"for detecting graft injury by shifting the monitoring of graft damage to the perfusion phase. This approach focuses on biomarkers in the perfusion fluid, with dd-cfDNA being a promising candidate. Although dd-cfDNA has shown promising potential as a biomarker in the field of liver transplantation, further research is needed to fully realize its clinical application value.

Abbreviations

LT	Liver transplantation
DNA	Deoxyribonucleic acid
Dd-cfDNA	Donor-derived cell-free DNA
NGS	Next-generation sequencing
AR	Acute rejection
IRI	Ischemia-reperfusion injury
SRY	Sex-determining region Y
TSPY	Testis-specific protein Y
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
dd-PCR	Droplet digital PCR
SNPs	Single nucleotide polymorphisms
HLA	Human leukocyte antigen
OLT	Orthotopic liver transplantation
IS	Immunosuppression
CNI	Calcineurin inhibitors
CKD	Chronic kidney disease
CRISPR-Cas	Clustered regularly interspaced short palindromic repeats-associated proteins
MP	Machine perfusion
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ACR	Acute cellular rejection
AMR	Antibody-mediated rejection

Authors' contributions

Yiwu Zhong and Xiaoping Li: Framework construction, article writing, project administration, and literature collection. Yinbiao Qiao and Haoyu Li: Review & editing. Xu Hu and Shijie Zhou: Literature search and framework construction. Shusen Zheng and Jianhui Li: Review & editing, resource, funding.

Funding

This review was supported by the Spring City Plan: the High-level Talent Promotion and Training Project of Kunming (2022SCP002), Research Unit Project of Chinese Academy of Medical Sciences (2019-I2M-5-030), National Natural Science Foundation of China (81721091), National S&T Major Project (No. 2017ZX10203205). The funder did not participate in the designing, performing or reporting in the current study.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

Received: 3 December 2024 Accepted: 11 April 2025
Published online: 13 May 2025

References

1. European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu. EASL clinical practice guidelines: liver transplantation. J Hepatol. 2016;64:433–85.
2. Levitsky J, Kandpal M, Guo K, Kleiboeker S, Sinha R, Abecassis M. Donor-derived cell-free DNA levels predict graft injury in liver transplant recipients. Am J Transplant. 2022;22:532–40.
3. Knight SR, Thorne A, Lo Faro ML. Donor-specific Cell-free DNA as a Biomarker in Solid Organ Transplantation. A Systematic Review Transplantation. 2019;103:273.
4. Baumann AK, Beck J, Kirchner T, Hartleben B, Schütz E, Oellerich M, et al. Elevated fractional donor-derived cell-free DNA during subclinical graft injury after liver transplantation. Liver Transpl. 2022;28:1911–9.
5. Schütz E, Fischer A, Beck J, Harden M, Koch M, Wuensch T, et al. Graft-derived cell-free DNA, a noninvasive early rejection and graft damage marker in liver transplantation: A prospective, observational, multicenter cohort study. PLoS Med. 2017;14: e1002286.
6. Whitlam JB, Ling L, Skene A, Kanellis J, Ierino FL, Slater HR, et al. Diagnostic application of kidney allograft-derived absolute cell-free DNA levels during transplant dysfunction. Am J Transplant. 2019;19:1037–49.
7. Gadi VK, Nelson JL, Boespflug ND, Guthrie KA, Kuhr CS. Soluble Donor DNA Concentrations in Recipient Serum Correlate with Pancreas-Kidney Rejection. Clin Chem. 2006;52:379–82.
8. Fournié GJ, Martres F, Pourrat JP, Alary C, Rumeau M. Plasma DNA as cell death marker in elderly patients. Gerontology. 1993;39:215–21.
9. Lo YM, Tein MS, Pang CC, Yeung CK, Tong K-L, Hjelm NM. Presence of donor-specific DNA in plasma of kidney and liver-transplant recipients. The Lancet. 1998;351:1329–30.
10. Lui YY, Chik K-W, Chiu RW, Ho C-Y, Lam CW, Lo YD. Predominant Hematopoietic Origin of Cell-free DNA in Plasma and Serum after Sex-mismatched Bone Marrow Transplantation. Clin Chem. 2002;48:421–7.
11. Oellerich M, Christenson RH, Beck J, Schütz E, Sherwood K, Price CP, et al. Donor-Derived Cell-Free DNA Testing in Solid Organ Transplantation: A Value Proposition. J Appl Lab Med. 2020;5:993–1004.
12. Hu Z, Chen H, Long Y, Li P, Gu Y. The main sources of circulating cell-free DNA: Apoptosis, necrosis and active secretion. Crit Rev Oncol Hematol. 2021;157: 103166.
13. Mandel P, Metais P. Les acides nucléiques du plasma sanguin chez l'homme [Nuclear Acids In Human Blood Plasma]. C R Seances Soc Biol Fil. 1948;142:241–3.
14. Schwarzenbach H, Hoon DSB, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer. 2011;11:426–37.
15. Ng HI, Zhu X, Xuan L, Long Y, Mao Y, Shi Y, et al. Analysis of fragment size distribution of cell-free DNA: A potential non-invasive marker to monitor graft damage in living-related liver transplantation for inborn errors of metabolism. Mol Genet Metab. 2019;127:45–50.
16. Celec P, Vlková B, Lauková L, Bábíčková J, Boor P. Cell-free DNA: the role in pathophysiology and as a biomarker in kidney diseases. Expert Rev Mol Med. 2018;20: e1.
17. Yu SC, Lee SW, Jiang P, Leung TY, Chan KA, Chiu RW, et al. High-Resolution Profiling of Fetal DNA Clearance from Maternal Plasma by Massively Parallel Sequencing. Clin Chem. 2013;59:1228–37.
18. Sigdel TK, Vitalone MJ, Tran TQ, Dai H, Hsieh S, Salvatierra O, et al. A Rapid Noninvasive Assay for the Detection of Renal Transplant Injury. Transplantation. 2013;96:97.
19. García Moreira V, Prieto García B, Baltar Martín JM, Ortega Suárez F, Alvarez FV. Cell-Free DNA as a Noninvasive Acute Rejection Marker in Renal Transplantation. Clin Chem. 2009;55:1958–66.
20. Woodward R, Grskovic M, Dedrick R. Increased Plasma Levels of Graft-Derived Cell-Free DNA Correlate With Rejection in Solid Organ Transplant Recipients.: Abstract# 594. Transplantation. 2014;98:222.
21. Macher HC, Suárez-Artacho G, Guerrero JM, Gómez-Bravo MA, Álvarez-Gómez S, Bernal-Bellido C, et al. Monitoring of Transplanted Liver Health by Quantification of Organ-Specific Genomic Marker in Circulating DNA from Receptor. PLoS ONE. 2014;9: e113987.
22. Zhang J, Tong K-L, Li PK, Chan AY, Yeung C-K, Pang CC, et al. Presence of Donor- and Recipient-derived DNA in Cell-free Urine Samples of Renal Transplantation Recipients: Urinary DNA Chimerism. Clin Chem. 1999;45:1741–6.
23. Snyder TM, Khush KK, Valantine HA, Quake SR. Universal noninvasive detection of solid organ transplant rejection. Proc Natl Acad Sci. 2011;108:6229–34.
24. Macher HC, Suárez-Artacho G, Jiménez-Arriscado P, Álvarez-Gómez S, García-Fernández N, Guerrero JM, et al. Evaluation of the State of Transplanted Liver Health by Monitoring of Organ-Specific Genomic Marker in Circulating DNA from Receptor. Adv Exp Med Biol. 2016;924:113–6.
25. Filippone EJ, Farber JL. The Monitoring of Donor-derived Cell-free DNA in Kidney Transplantation. Transplantation. 2021;105:509–16.
26. Oellerich M, Sherwood K, Keown P, Schütz E, Beck J, Stegbauer J, et al. Liquid biopsies: donor-derived cell-free DNA for the detection of kidney allograft injury. Nat Rev Nephrol. 2021;17:591–603.
27. Gordon PMK, Khan A, Sajid U, Chang N, Suresh V, Dimnik L, et al. An Algorithm Measuring Donor Cell-Free DNA in Plasma of Cellular and Solid Organ Transplant Recipients That Does Not Require Donor or Recipient Genotyping. Front Cardiovasc Med. 2016;3:33.
28. Sharon E, Shi H, Kharbanda S, Koh W, Martin LR, Khush KK, et al. Quantification of transplant-derived circulating cell-free DNA in absence of a donor genotype. PLoS Comput Biol. 2017;13: e1005629.
29. Beck J, Bierau S, Balzer S, Andag R, Kanzow P, Schmitz J, et al. Digital Droplet PCR for Rapid Quantification of Donor DNA in the Circulation of Transplant Recipients as a Potential Universal Biomarker of Graft Injury. Clin Chem. 2013;59:1732–41.
30. Grskovic M, Hiller DJ, Eubank LA, Sninsky JJ, Christopherson C, Collins JP, et al. Validation of a Clinical-Grade Assay to Measure Donor-Derived Cell-Free DNA in Solid Organ Transplant Recipients. J Mol Diagn. 2016;18:890–902.
31. Sigdel TK, Archila FA, Constantin T, Prins SA, Liberto J, Damm I, et al. Optimizing Detection of Kidney Transplant Injury by Assessment of Donor-Derived Cell-Free DNA via Massively Multiplex PCR. J Clin Med. 2018;8:19.
32. Dauber E-M, Kollmann D, Kozakowski N, Rasoul-Rockenschaub S, Soliman T, Berlakovich GA, et al. Quantitative PCR of INDELs to measure donor-derived cell-free DNA—a potential method to detect acute rejection in kidney transplantation: a pilot study. Transpl Int. 2019;33:298.
33. Zhu X, Ng HI, Xuan L, Long Y, Mao Y, Shi Y, et al. Sequencing data of cell-free DNA fragments in living-related liver transplantation for inborn errors of metabolism. Data Brief. 2020;29: 105183.
34. Garg N, Mandelbrot DA, Parajuli S, Aziz F, Astor BC, Chandraker A, et al. The clinical value of donor-derived cell-free DNA measurements in kidney transplantation. Transplant Rev. 2021;35: 100649.
35. Sorbini M, Togliatto GM, Simonato E, Boffini M, Cappuccio M, Gambella A, et al. *HLA-DRB1* mismatch-based identification of donor-derived cell free DNA (dd-cfDNA) as a marker of rejection in heart transplant recipients: A single-institution pilot study. J Heart Lung Transplant. 2021;40:794–804.
36. Oellerich M, Shipkova M, Asendorf T, Walson PD, Schauerte V, Mettenmeyer N, et al. Absolute quantification of donor-derived cell-free DNA as a marker of rejection and graft injury in kidney transplantation: Results from a prospective observational study. Am J Transplant. 2019;19:3087–99.
37. Lui YYN, Woo K-S, Wang AYM, Yeung C-K, Li PKT, Chau E, et al. Origin of Plasma Cell-free DNA after Solid Organ Transplantation. Clin Chem. 2003;49:495–6.
38. De Vlaminck I, Martin L, Kertesz M, Patel K, Kowarsky M, Strehl C, et al. Noninvasive monitoring of infection and rejection after lung transplantation. Proc Natl Acad Sci U S A. 2015;112:13336–41.
39. Gielis EM, Ledeganck KJ, Dendooven A, Meysman P, Beirnaert C, Laukens K, et al. The use of plasma donor-derived, cell-free DNA to monitor acute rejection after kidney transplantation. Nephrol Dial Transplant. 2020;35:714–21.
40. Puttarajappa CM, Mehta RB, Hariharan S. Author response to comments on economic analysis of subclinical rejection screening in kidney transplantation. Am J Transplant. 2021;21:1348–9.
41. Lehmann-Werman R, Magenheim J, Moss J, Neiman D, Abraham O, Piyanzin S, et al. Monitoring liver damage using hepatocyte-specific methylation markers in cell-free circulating DNA. JCI Insight. 2018;3: e120687.
42. Cox DRA, Low N, Goh SK, Lee E, Vago A, Jackett L, et al. Low Levels of Hepatocyte-Specific Methylation in Cell-Free DNA Are a Strong

Negative Predictor for Acute T Cell-Mediated Rejection Requiring Treatment Following Liver Transplantation. Liver Transpl. 2022;28:1024–38.
43. Kohut TJ, Barandiaran JF, Keating BJ. Genomics and Liver Transplantation: Genomic Biomarkers for the Diagnosis of Acute Cellular Rejection. Liver Transpl. 2020;26:1337–50.
44. Kanamori H, Yamada Y, Ito Y, Shirosaki K, Yamagishi S, Maeda Y, et al. Noninvasive graft monitoring using donor-derived cell-free DNA in Japanese liver transplantation. Hepatol Res. 2024;54:300–14.
45. Zhao D, Zhou T, Luo Y, Wu C, Xu D, Zhong C, et al. Preliminary clinical experience applying donor-derived cell-free DNA to discern rejection in pediatric liver transplant recipients. Sci Rep. 2021;11:1138.
46. Shen J, Zhou Y, Chen Y, Li X, Lei W, Ge J, et al. Dynamics of early postoperative plasma ddcfDNA levels in kidney transplantation: a single-center pilot study. Transpl Int. 2019;32:184–92.
47. Gielis EM, Beirnaert C, Dendooven A, Meysman P, Laukens K, Schrijver JD, et al. Plasma donor-derived cell-free DNA kinetics after kidney transplantation using a single tube multiplex PCR assay. PLoS ONE. 2018;13: e0208207.
48. Prakash K, Aggarwal S, Bhardwaj S, Ramakrishna G, Pandey CK. Serial perioperative cell-free DNA levels in donors and recipients undergoing living donor liver transplantation. Acta Anaesthesiol Scand. 2017;61:1084–94.
49. Li JH, Xu X, Wang YF, Xie HY, Chen JY, Dong NG, et al. Chinese expert consensus on organ protection of transplantation (2022 edition). Hepatobiliary Pancreat Dis Int. 2022;21:516–26.
50. Raszeja-Wyszomirska J, Macech M, Kolanowska M, Krawczyk M, Nazarewski S, Wójcicka A, et al. Free-Circulating Nucleic Acids as Biomarkers in Patients After Solid Organ Transplantation. Ann Transplant. 2023;28: e939750.
51. Yoshino O, Wong BKL, Cox DRA, Lee E, Hepworth G, Christophi C, et al. Elevated levels of circulating mitochondrial DNA predict early allograft dysfunction in patients following liver transplantation. J Gastroenterol Hepatol. 2021;36:3500–7.
52. Oellerich M, Budde K, Osmanodja B, Bornemann-Kolatzki K, Beck J, Schütz E, et al. Donor-derived cell-free DNA as a diagnostic tool in transplantation. Front Genet. 2022;13:1031894.
53. De Vlaminck I, Valantine HA, Snyder TM, Strehl C, Cohen G, Luikart H, et al. Circulating cell-free DNA enables noninvasive diagnosis of heart transplant rejection. Sci Transl Med. 2014;6:241ra77.
54. Bloom RD, Bromberg JS, Poggio ED, Bunnapradist S, Langone AJ, Sood P, et al. Cell-Free DNA and Active Rejection in Kidney Allografts. J Am Soc Nephrol JASN. 2017;28:2221.
55. Krenzien F, Keshi E, Splith K, Griesel S, Kamali K, Sauer IM, et al. Diagnostic Biomarkers to Diagnose Acute Allograft Rejection After Liver Transplantation: Systematic Review and Meta-Analysis of Diagnostic Accuracy Studies. Front Immunol. 2019;10:758.
56. Fernández-Galán E, Badenas C, Fondevila C, Jiménez W, Navasa M, Puig-Butillé JA, et al. Monitoring of Donor-Derived Cell-Free DNA by Short Tandem Repeats: Concentration of Total Cell-Free DNA and Fragment Size for Acute Rejection Risk Assessment in Liver Transplantation. Liver Transpl. 2022;28:257–68.
57. Thongprayoon C, Vaitla P, Craici IM, Leeaphorn N, Hansrivijit P, Salim SA, et al. The Use of Donor-Derived Cell-Free DNA for Assessment of Allograft Rejection and Injury Status. J Clin Med. 2020;9:1480.
58. Goh SK, Do H, Testro A, Pavlovic J, Vago A, Lokan J, et al. The Measurement of Donor-Specific Cell-Free DNA Identifies Recipients With Biopsy-Proven Acute Rejection Requiring Treatment After Liver Transplantation. Transplant Direct. 2019;5: e462.
59. Kanzow P, Kollmar O, Schütz E, Oellerich M, Schmitz J, Beck J, et al. Graft-Derived Cell-Free DNA as an Early Organ Integrity Biomarker After Transplantation of a Marginal HELLP Syndrome Donor Liver. Transplantation. 2014;98:e43-45.
60. Beck J, Oellerich M, Schulz U, Schauerte V, Reinhard L, Fuchs U, et al. Donor-Derived Cell-Free DNA Is a Novel Universal Biomarker for Allograft Rejection in Solid Organ Transplantation. Transplant Proc. 2015;47:2400–3.
61. Lewis D, Glehn-Ponsirenas R, Gulbahce N, Hooey LJ, Chaffin JM, Miles J, et al. High levels of donor-derived cell-free DNA in a case of graft-versus-host-disease following liver transplantation. Am J Transplant. 2022;22:973–6.
62. Sebastian A, Silvy M, Coiffard B, Reynaud-Gaubert M, Magdinier F, Chiaroni J, et al. A review of cell-free DNA and epigenetics for non-invasive diagnosis in solid organ transplantation. Front Transplant. 2024;3:1474920.
63. Sorbini M, Carradori T, Patrono D, Togliatto G, Caorsi C, Vaisitti T, et al. Circulating cell-free DNA in liver transplantation: A pre- and post-transplant biomarker of graft dysfunction. Artif Organs. 2025;49:649-662.
64. Khush KK, Patel J, Pinney S, Kao A, Alharethi R, DePasquale E, et al. Non-invasive detection of graft injury after heart transplant using donor-derived cell-free DNA: A prospective multicenter study. Am J Transplant. 2019;19:2889–99.
65. Agbor-Enoh S, Wang Y, Tunc I, Jang MK, Davis A, Vlaminck ID, et al. Donor-derived cell-free DNA predicts allograft failure and mortality after lung transplantation. EBioMedicine. 2019;40:541–53.
66. Oellerich M, Kanzow P, Beck J, Schmitz J, Kollmar O, Walson P, et al. Graft-Derived Cell-Free DNA (GcfDNA) as a Sensitive Measure of Individual Graft Integrity After Liver Transplantation.: Abstract# A7. Transplantation. 2014;98:874.
67. Kroneisl M, Spraakman NA, Koomen JV, Hijazi Z, Hoogstra-Berends FH, Leuvenink HGD, et al. Peri-Operative Kinetics of Plasma Mitochondrial DNA Levels during Living Donor Kidney Transplantation. Int J Mol Sci. 2023;24:13579.
68. Bruneau S, Woda CB, Daly KP, Boneschansker L, Jain NG, Kochupurakkal N, et al. Key Features of the Intragraft Microenvironment that Determine Long-Term Survival Following Transplantation. Front Immunol. 2012;3:54.
69. Sherwood K, Weimer ET. Characteristics, properties, and potential applications of circulating cell-free dna in clinical diagnostics: a focus on transplantation. J Immunol Methods. 2018;463:27–38.
70. Charlton M, Levitsky J, Aqel B, O'Grady J, Hemibach J, Rinella M, et al. International Liver Transplantation Society Consensus Statement on Immunosuppression in Liver Transplant Recipients. Transplantation. 2018;102:727–43.
71. Starzl TE. Immunosuppressive Therapy and Tolerance of Organ Allografts. N Engl J Med. 2008;358:407–11.
72. Oellerich M, Schütz E, Kanzow P, Schmitz J, Beck J, Kollmar O, et al. Use of Graft-Derived Cell-Free DNA as an Organ Integrity Biomarker to Reexamine Effective Tacrolimus Trough Concentrations After Liver Transplantation. Ther Drug Monit. 2014;36:136–40.
73. VanWagner LB, Serper M, Kang R, Levitsky J, Hohmann S, Abecassis M, et al. Factors Associated With Major Adverse Cardiovascular Events After Liver Transplantation Among a National Sample. Am J Transplant. 2016;16:2684–94.
74. VanWagner LB, Lapin B, Levitsky J, Wilkins JT, Abecassis MM, Skaro AI, et al. High early cardiovascular mortality following liver transplantation. Liver Transplant Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc. 2014;20:1306–16.
75. VanWagner LB, Bhave M, Te HS, Feinglass J, Alvarez L, Rinella ME. Patients transplanted for nonalcoholic steatohepatitis are at increased risk for postoperative cardiovascular events. Hepatology. 2012;56:1741–50.
76. Watt KD, Pedersen RA, Kremers WK, Heimbach JK, Charlton MR. Evolution of Causes and Risk Factors for Mortality Post-Liver Transplant: Results of the NIDDK Long-Term Follow-Up Study. Am J Transplant. 2010;10:1420–7.
77. Golestaneh L, Alvarez PJ, Reaven NL, Funk SE, McGaughey KJ, Romero A, et al. All-cause costs increase exponentially with increased chronic kidney disease stage. Am J Manag Care. 2017;23(10 Suppl):S163–72.
78. Gonwa TA, Mai ML, Melton LB, et al. End-stage renal disease (ESRD) after orthotopic liver transplantation (OLTX) using calcineurin-based immunotherapy: risk of development and treatment. Transplantation. 2001;72:1934–9.
79. O'Leary JG, Levitsky J, Wong F, Nadim MK, Charlton M, Kim WR. Protecting the Kidney in Liver Transplant Candidates: Practice-Based Recommendations From the American Society of Transplantation Liver and Intestine Community of Practice. Am J Transplant. 2016;16:2516–31.
80. Manzi J, Hoff CO, Ferreira R, Glehn-Ponsirenas R, Selvaggi G, Tekin A, et al. Cell-Free DNA as a Surveillance Tool for Hepatocellular Carcinoma Patients after Liver Transplant. Cancers. 2023;15:3165.
81. Jana K, Rammohan A, Ramani A, Gunasekaran B, Vij M, Ramamoorthi M, et al. Role of Donor-derived Cell-free DNA In Predicting Short-term

Allograft Health In Liver Transplant Recipients. J Clin Exp Hepatol. 2024;14: 101477.
82. Sabagh AE, Mohamed IB, Aloor FZ, Abdelwahab A, Hassan MM, Jalal PK. Current Status of Biomarkers and Molecular Diagnostic Tools for Rejection in Liver Transplantation: Light at the End of the Tunnel? J Clin Exp Hepatol. 2022;13:139–48.
83. Zhang W, Liu B, Jia D, Wang R, Cao H, Wu H, et al. Application of graft-derived cell-free DNA for solid organ transplantation. Front Immunol. 2024;15:1461480.
84. McClure T, Goh SK, Cox D, Muralidharan V, Dobrovic A, Testro AG. Donor-specific cell-free DNA as a biomarker in liver transplantation: A review. World J Transplant. 2020;10:307–19.
85. Saunders EA, Engel B, Höfer A, Hartleben B, Vondran FWR, Richter N, et al. Outcome and safety of a surveillance biopsy guided personalized immunosuppression program after liver transplantation. Am J Transplant. 2022;22:519–31.
86. Thung SN, Gerber MA. Histological features of liver allograft rejection: Do you see what I see? Hepatology. 1991;14:949–51.
87. Price CP, John AS, Christenson R, Scharnhorst V, Oellerich M, Jones P, et al. Leveraging the real value of laboratory medicine with the value proposition. Clin Chim Acta. 2016;462:183–6.
88. Shanmugam NP, Vasudevan AK, Jana K, Rammohan A, Rela M, Gunasekaran B, et al. 343.1: Clinical utility of Donor-Derived cell-free DNA (ddcfDNA) in non-invasive monitoring of Liver transplant recipients. Transplantation. 2024;108(9S). https://doi.org/10.1097/01.tp.0001065644.89927.8b.
89. Vasco M, Benincasa G, Fiorito C, Faenza M, De Rosa P, Maiello C, et al. Clinical epigenetics and acute/chronic rejection in solid organ transplantation: An update. Transplant Rev. 2021;35: 100609.
90. Szilágyi M, Pös O, Márton É, Buglyó G, Soltész B, Keserű J, et al. Circulating Cell-Free Nucleic Acids: Main Characteristics and Clinical Application. Int J Mol Sci. 2020;21:6827.
91. Edwards RL, Menteer J, Lestz RM, Baxter-Lowe LA. Cell-Free Dna As A Solid-Organ Transplant Biomarker: Technologies and Approaches. Biomark Med. 2022;16:401–15.
92. Agbor-Enoh S, Shah P, Tunc I, Hsu S, Russell S, Feller E, et al. Cell-Free DNA to Detect Heart Allograft Acute Rejection. Circulation. 2021;143:1184–97.
93. Consortium RE, Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, et al. Integrative analysis of 111 reference human epigenomes. Nature. 2015;518:317–30.
94. Sun K, Jiang P, Chan KCA, Wong J, Cheng YKY, Liang RHS, et al. Plasma DNA tissue mapping by genome-wide methylation sequencing for noninvasive prenatal, cancer, and transplantation assessments. Proc Natl Acad Sci U S A. 2015;112:E5503-5512.
95. Gonzalez-Jaramillo V, Portilla-Fernandez E, Glisic M, Voortman T, Ghanbari M, Bramer W, et al. Epigenetics and Inflammatory Markers: A Systematic Review of the Current Evidence. Int J Inflamm. 2019;2019:6273680.
96. Sorber L, Zwaenepoel K, Deschoolmeester V, Roeyen G, Lardon F, Rolfo C, et al. A Comparison of Cell-Free DNA Isolation Kits: Isolation and Quantification of Cell-Free DNA in Plasma. J Mol Diagn. 2017;19:162–8.
97. Markus H, Contente-Cuomo T, Farooq M, Liang WS, Borad MJ, Sivakumar S, et al. Evaluation of pre-analytical factors affecting plasma DNA analysis. Sci Rep. 2018;8:7375.
98. Melancon JK, Khalil A, Lerman MJ. Donor-Derived Cell Free DNA: Is It All the Same? Kidney360. 2020;1:1118–23.
99. Li X, Zhong Y, Qiao Y, Li H, Hu X, Imani S, et al. Advances and Challenges in Cytomegalovirus Detection Methods for Liver Transplant Donors. Diagnostics. 2023;13:3310.
100. Aman R, Mahas A, Mahfouz M. Nucleic Acid Detection Using CRISPR/Cas Biosensing Technologies. ACS Synth Biol. 2020;9:1226–33.
101. Huang Z, LaCourse SM, Kay AW, Stern J, Escudero JN, Youngquist BM, et al. CRISPR detection of circulating cell-free Mycobacterium tuberculosis DNA in adults and children, including children with HIV: a molecular diagnostics study. Lancet Microbe. 2022;3:e482–92.
102. Jia J, Li J, Zhang S, Xie H, Zhou L, Zheng S. A promising ex vivo liver protection strategy: machine perfusion and repair. Hepatobiliary Surg Nutr. 2019;8:142–3.
103. Staubli SM, Ceresa CDL, Pollok JM. The Current Role and Future Applications of Machine Perfusion in Liver Transplantation. Bioengineering. 2023;10:593.
104. Kanou T, Nakahira K, Choi AM, Yeung JC, Cypel M, Liu M, et al. Cell-free DNA in human ex vivo lung perfusate as a potential biomarker to predict the risk of primary graft dysfunction in lung transplantation. J Thorac Cardiovasc Surg. 2021;162:490-499.e2.

Publisher's Note