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ORIGINAL REVIEW

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Non-Invasive Surveillance in Kidney Transplantation: A Systematic Review of Donor-Derived Cell-Free DNA as a Biomarker for Transplant Rejection

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Abstract

The ability to monitor kidney allografts is limited by traditional forms of assessment like invasive biopsy, and also delayed biomarkers, such as serum creatinine (Rizvi et al., 2023). This systematic review was performed to assess the clinical usefulness of donor-derived cell-free DNA (dd-cfDNA) as a non-invasive biomarker of allograft injury. A systematic search of the Google Scholar and PubMed databases yielded 1,153 records; following a manual screening of titles and abstracts, 83 studies were selected for inclusion. Results indicate a rapidly maturing evidence base, including an increase in publications and a corresponding increase in sample size over time, reflected most notably in a landmark study on nearly 3,000 patients (Aubert et al., n.d.). The literature generally met a set of curated criteria for non-invasive biomarker studies derived by the author. For example, most studies obtained 3 or more out of 5 criteria: "Measured Blood?", "Analyzes rejection probability?", "Temporal Precedence Explicitly Stated", "Longitudinal Monitoring for Timing", and "Compares to Traditional Biomarkers?". A key finding of the review was the consistent and generally favorable diagnostic accuracy of dd-cfDNA, with most studies indicating an Area Under the Curve (AUC) greater than 0.80 (Mantios et al., 2023). There is, however, a stark geographical disparity in the inclusion of diversified ancestry; the available evidence for Black and Hispanic ancestry is largely based on North American evidence (Bu et al., 2022). This systematic review provides confirmatory evidence that dd-cfDNA is a strong diagnostic biomarker (Kim et al., 2024); however, it did not assess clinical feasibility. Future work must address this gap by focusing on cost-effectiveness and implementation studies, in addition to conducting large, multicenter trials in diverse populations to ensure its equitable and optimal application (Botella et al., 2024).

Keywords: donor-derived cell-free DNA (dd-cfDNA), kidney transplantation, allograft rejection, biomarkers, acute rejection, antibody-mediated rejection (AMR), T-cell mediated rejection (TCMR), diagnostic accuracy, graft monitoring

1. Introduction

Kidney transplantation is a vital therapy for patients with end-stage kidney disease, providing a better overall longevity and improved quality of life compared to long-term dialysis (Dreige et al., 2022), which is the non-surgical alternative to transplantation. Despite its advantages and evidence for superior long-term outcomes, transplantation faces the ongoing challenge of immune-mediated rejection, which remains a prominent and often principal cause of patient graft-loss (Lakhani et al., 2021). The challenge is exacerbated by a poor risk management framework that relies on conventional surveillance practices that typically are complicated and inaccurate to true quantitative values (Wei et al., 2024). This framework is limited by its dependence on invasive and reactive kidney biopsies and the use of non-specific, lagging biomarkers like serum creatinine that often only rise after irreversible graft damage has occurred (Parajuli et al., 2024; Rizvi et al., 2023). Traditionally, markers for the identification and management of rejection have been insufficient due to the slow response of these traditional markers to tissue injury (Rizvi et al., 2023). In short, these significant barriers represent the unmet medical need in transplant medicine, as they do not allow clinicians to intervene prior to true tissue/organ damage (Nie et al., 2025).

In recent years, a new solution has been developed to solve these deficiencies: the measurement of dd-cfDNA (Zhang et al., 2020), small pieces of DNA from the donor that are released from the transplanted organ into the circulation, especially when the organ is activated, or inflamed, or injured through histocompatibility, or rejection (Zhang et al., 2020). When a person has an organ transplant, they also are a recipient of a donor's genetic information at the time of the transplant (Kumar et al., 2023). Quantifying the amount of dd-cfDNA in a blood sample can now provide a direct, non-invasive and organ-specific signal of graft injury (Mayer et al., 2021). This has been depicted as a pioneering biomarker with much potential to detect graft damage early (Botella et al., 2024). The clinical utility of dd-cfDNA stems from its short half-life of about 30 minutes, which allows it to serve as a near real-time indicator of acute allograft injury. When the transplanted kidney is healthy, dd-cfDNA is released at a low, baseline level. However, during an active rejection event, inflammation and cell death in the allograft cause a significant and measurable increase in the amount of dd-cfDNA released into the recipient's bloodstream. By quantifying this increase through techniques like next-generation sequencing (NGS), clinicians can detect rejection far earlier than with traditional markers, creating an opportunity for pre-emptive treatment before the onset of widespread tissue damage.

Though transplant surgeons are currently utilizing dd-cfDNA in clinical practice, the development of the literature is so fast-paced that a comprehensive review of the evidence is needed (Chen et al., 2022). It is no longer sufficient to say that dd-cfDNA is “effective” and the key question to focus on is how effective it is in specific clinical contexts. The high diagnostic accuracy of dd-cfDNA is consistent with previous literature reviews and meta-analyses, which have affirmed the biomarker's potential for non-invasive monitoring. Previous meta-analyses have reported high pooled Area Under the Curve (AUC) values, such as 0.84 for acute rejection, 0.86 for overall rejection, and 0.88 for antibody-mediated rejection. This review builds on that consensus by providing a quantitative synthesis of the field's maturation, documenting the significant increase in study sample sizes and the shift towards more rigorous prospective cohort designs. It also addresses a critical knowledge gap by quantifying the significant geographic and ancestral disparities, highlighting that evidence for Black and Hispanic populations is almost exclusively derived from North American studies. However, a limitation of these existing reviews is that they confirm the biomarker's general utility without systematically analyzing the characteristics of the rapidly evolving evidence base or quantifying the critical knowledge gaps related to its performance in diverse patient populations.

The unique value of this systematic review is that, in addition to synthesizing diagnostic accuracy, it provides the first

quantitative analysis of the field's methodological maturation and its significant demographic and geographic gaps (Sharma et al., 2022); it is guided by the specific hypothesis that donor-derived cell-free DNA (dd-cfDNA) can result in increased diagnostic accuracy in detecting transplant rejection and graft injury versus current standard of care biomarkers, through earlier and non-invasive methods. The focus of this review is to (1) specify and quantify overall diagnostic accuracy of dd-cfDNA for various types of rejection, (2) create and analyze data visualizations to synthesize the characteristics and findings of the included studies, (3) identify trends in performance and address key knowledge gaps, such as the biomarker's utility in diverse, underrepresented patient populations (Benning et al., 2022), and (4) assess the methodological approaches used in the literature to evaluate the timing of rejection.

2. Background

2.1. Kidney Transplantation and Rejection

Kidney transplantation is the most common organ transplantation because its underlying disease, end-stage renal disease (ESRD), is also one of the most common diseases, affecting >800000 persons in the United States (*Kidney Disease Statistics for the United States - NIDDK*, n.d.). The disease carries a significant burden, with a mortality rate of nearly 25% for patients on dialysis, highlighting the critical need for transplantation as a life-saving intervention. Conditions like diabetes and high blood pressure are the leading causes of kidney failure, impacting millions and contributing to the high demand for transplants (*American Kidney Fund*, 2024).

When an organ is transplanted, it is important to ensure the recipient's immune system will accept it and not recognize it as foreign; otherwise, an organ can be rejected (Nguyen et al., 2025). The most common immune responses are described as acute rejection, which happens shortly after the transplant has taken place, and chronic rejection, which occurs over time (Rizvi et al., 2023). Ultimately, failure associated with acute and chronic rejection can be caused through progressive permanent damage in the transplanted organ with long-term changes in allograft function (Wei et al., 2024). Graft failure is a severe outcome, leading to a return to dialysis where the 5-year survival rate is only around 42%, underscoring the importance of effective allograft monitoring. Allograft rejection is primarily categorized into two distinct pathways: T-cell mediated rejection (TCMR) and antibody-mediated rejection (ABMR). TCMR is a cellular response where the recipient's T-cells directly attack the transplanted organ. ABMR is a humoral response driven by donor-specific antibodies (DSAs) that target the graft, leading to inflammation and tissue damage. Distinguishing between these subtypes is critical for guiding appropriate treatment, as they respond to different immunosuppressive therapies.

Currently, the monitoring of kidney transplant physiology is performed using a combination of clinical and biochemical monitoring protocols (Oellerich et al., 2021). Clinicians often take note of potential clinical signs or symptoms related to possible kidney dysfunction, such as decreased urinary output or developing edema in the legs and ankles from fluid overload (Kim et al., 2024). However, to truly assess graft dysfunction, clinicians must perform invasive procedures to obtain tissue or organ biopsies, which allows for definitive diagnosis of the pertinent histopathological process (Moein et al., 2024).

The current monitoring modalities have multiple problems that can greatly influence patient care (Rizvi et al., 2023). First, serum creatinine is a lagging and suboptimal marker; in fact, serum creatinine will only begin to elevate when potentially irreversible injury to the donated kidney has already occurred (Rizvi et al., 2023).

Both the invasiveness of biopsies and the lagging sensitivity of serum creatinine are limitations in post-transplant management (Aubert et al., 2024). There is a clear need for a noninvasive and improved biomarker for the kidney transplant rejection technique that can provide an early and accurate evaluation of graft health (Akalin et al., 2021).

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2.2. Cell-free DNA use in transplantation

cfDNA, which consists of the short fragments of DNA that are free-circulating in the bloodstream, shows promise as a biomarker in transplantation research (Zhang et al., 2020). Cell-free DNA originates when cells in the human body experience apoptosis, programmed cell death or necrosis, uncontrolled cell death (Oellerich et al., 2021). When cells die and degrade, the genetic information in the cells is released into the biological fluid surrounding the cells, including blood plasma, urine, and cerebrospinal fluid (Nguyen et al., 2025).

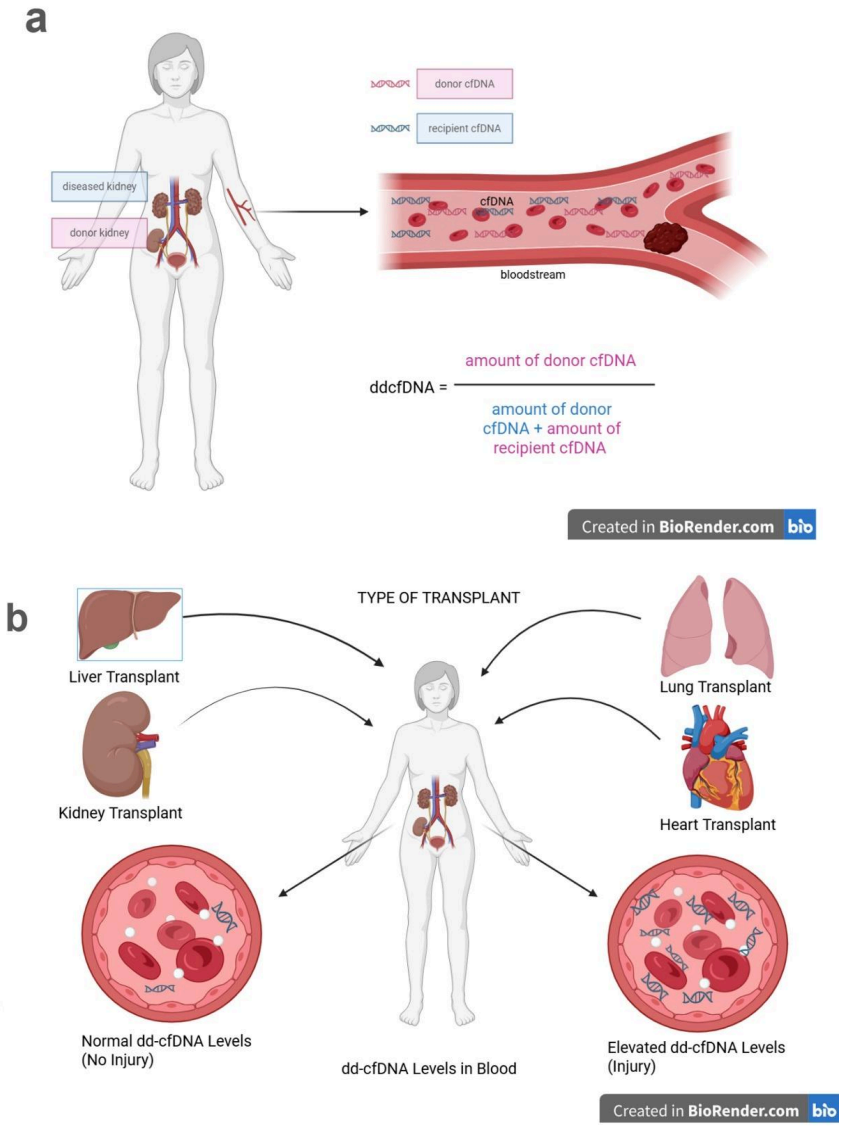

Figure 1: The Biological Principle of Donor-Derived Cell-Free DNA (dd-cfDNA) as a Biomarker.

Note: (a) This diagram illustrates that following a kidney transplant, both recipient and donor cfDNA circulate in the bloodstream. The fractional abundance of dd-cfDNA is calculated as the proportion of donor cfDNA relative to the total amount of cfDNA. This figure was created by the author using BioRender.com. (b) This diagram shows that dd-cfDNA is a general biomarker for all solid organ transplants, including liver, kidney, lung, and heart. In a healthy, uninjured state, dd-cfDNA levels are low; however, during an injury event like rejection, damaged cells from the transplanted organ release a higher amount of dd-cfDNA into the blood. This figure was created by the author using BioRender.com.

cfDNA comes from blood plasma, the fluid part of blood (Wolf-Doty et al., 2021). Several molecular methods are used currently to assess cfDNA quantity (Halloran et al., 2022). Both quantitative polymerase chain reaction (qPCR) and next-generation sequencing (NGS) can be viewed as modern methods for measuring cfDNA (Akalin et al., 2021). qPCR does work similarly to a targeted measure, where specific primers are used to locate and amplify a pre-established and a small number of unique donor sequences (Nie et al., 2025). For NGS, millions of cfDNA fragments can be read concurrently across the genome (Kumar et al., 2023b). In this way, cfDNA can be assessed to identify the donor if they donated an organ (Wei et al., 2024).
Figure 1a provides biological context for utilizing dd-cfDNA as a biomarker. After a solid organ transplant, like a kidney, the recipient's blood will contain their own cfDNA in addition to a small amount of cfDNA from the donor organ (Fig. 1a) (Oellerich et al., 2021). Under normal circumstances and with a healthy transplant, the amount of dd-cfDNA circulating in the blood is in a baseline state of low concentrations (Bu et al., 2022a). However, when an injury event causes a greater rate of cell death in the allograft, such as rejection, inflammation, or other forms of graft stress, the allograft will release dd-cfDNA into the recipient's circulation (Fig. 1b) (Rizvi et al., 2023). These real-time changes in dd-cfDNA concentrations can reflect ongoing allograft injury (Nguyen et al., 2025).

cfDNA is a dynamic biomarker. It is constantly shed into the bloodstream, and the half-life of a single cfDNA molecule is approximately 30 minutes in the bloodstream (Wolf-Doty et al., 2021), meaning that it provides real-time information about tissue degeneration. Changes in specific cfDNA characteristics, most notably an extraordinary increase in levels of cfDNA, are indications of tissue injury, inflammation, or subsequently, a disease such as rejection, which validate the value of cfDNA as a non-invasive biomarker (Rizvi et al., 2023). This indicates that cfDNA is a valid source of non-invasive biomarkers (Wei et al., 2024). The drive and excitement around a real-time multi-level signal for more precise management is the appeal of this biomarker that affirms its usefulness (Aubert et al., 2024). dd-cfDNA has become such a strong force and continues to be a focus of transplant research (Halloran et al., 2022).

The transplantation field is actively looking for new, non-invasive, and accurate ways to monitor graft health, given the many challenges with established surveillance modalities (Rizvi et al., 2023). The gold standard now, kidney biopsy, is invasive, with procedural and other risk and discomfort for the patient. Serum creatinine, the most widely used non-invasive biomarker, is a surrogate, vague and non-specific biomarker. CfDNA refers to all small fragments of DNA found circulating in the bloodstream. At a healthy baseline, all individuals will have a detectable level of cfDNA originating from normal cell death. However, after a transplant, a fraction of this total cfDNA originates from the donated organ; this specific component is known as donor-derived cell-free DNA (dd-cfDNA). While elevated total cfDNA indicates cell death somewhere in the body, the donor-derived fraction (dd-cfDNA) is a more specific biomarker for allograft health because its origin is exclusively the transplanted organ, thereby providing a clearer view of its health. Crucially, this signal reflects the real-time process of cell death as it happens, allowing for the detection of low-level or early-stage allograft injury, whereas conventional markers like serum creatinine only rise after substantial, cumulative, and often irreversible functional damage has already occurred. While biopsy serves as the definitive diagnostic tool, its invasiveness and the lagging nature of conventional biomarkers make them unsuitable for the frequent, proactive monitoring needed to prevent irreversible graft damage.

Recent advancements have uncovered the study of donor-derived cell-free DNA (dd-cfDNA) as an alternative solution that directly counters these flaws by offering a non-invasive and accelerated method for assessing transplant status, potentially transforming post-transplant care (Botella et al., 2024). The following results will synthesize the evidence to quantify the biomarker's accuracy and its timelines for early detection, thereby assessing its potential to become a new standard in post-transplant surveillance (Bu et al., 2022). The clinical utility of dd-cfDNA stems from its short half-life of about 30 minutes, which allows it to serve as a near real-time indicator of acute allograft injury. When the transplanted kidney is

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healthy, dd-cfDNA is released at a low, baseline level. However, during an active rejection event, inflammation, and cell death in the allograft cause a significant and measurable increase in the amount of dd-cfDNA released into the recipient's bloodstream. By quantifying this increase through techniques like next-generation sequencing (NGS), clinicians can detect rejection far earlier than with traditional markers, creating an opportunity for pre-emptive treatment before the onset of widespread tissue damage.

3. Methods

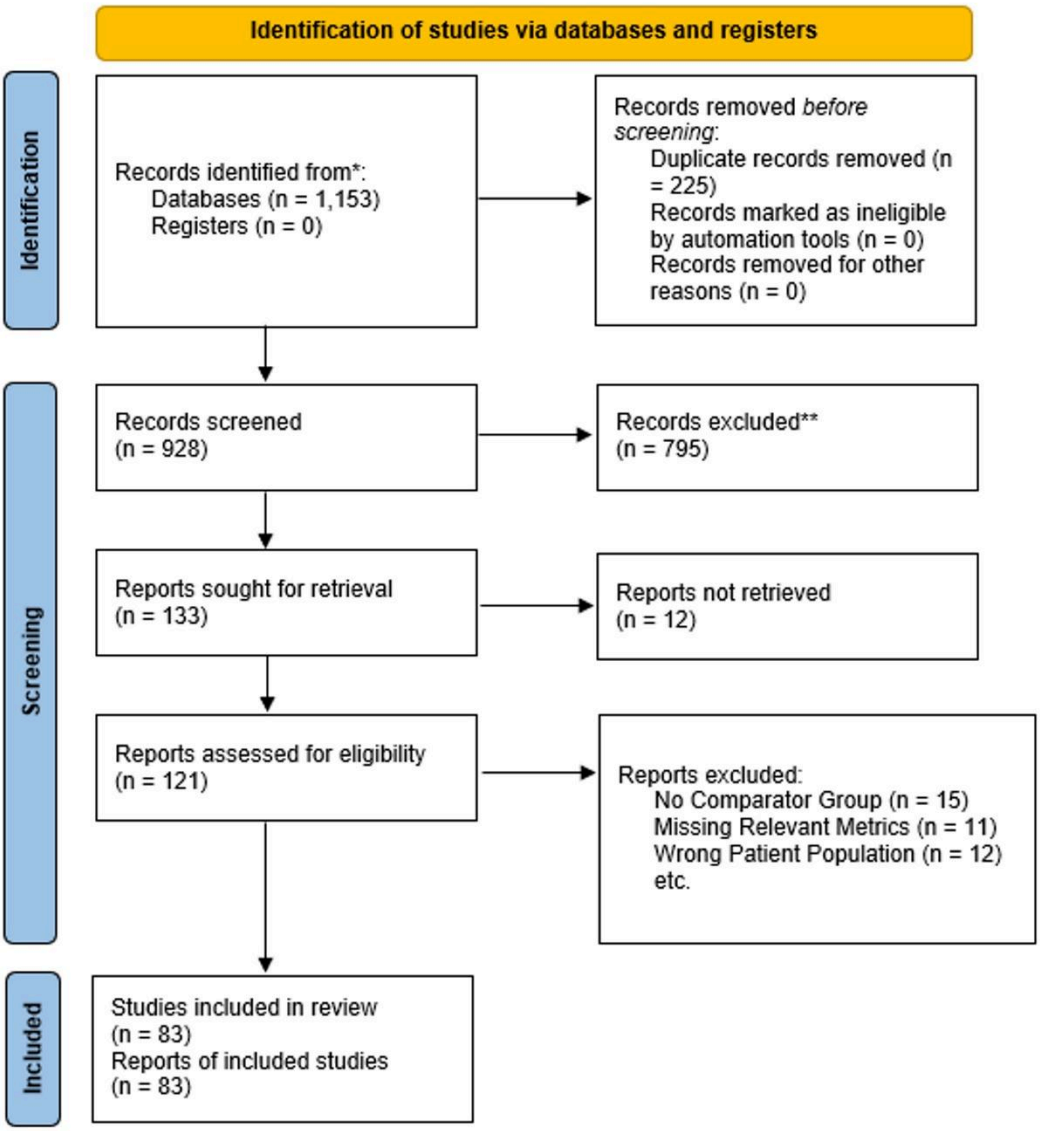

Figure 2: PRISMA 2020 Flow Diagram for Study Selection.

Note: This diagram illustrates the flow of information through the different phases of the systematic review, mapping out the number of records identified, included, and excluded.

3.1. Search Strategy

A comprehensive literature search was conducted using the Publish or Perish application (*Publish or Perish*, n.d.) to identify relevant articles. Searches were performed using the Google Scholar and PubMed databases. The search focused on publications related to donor-derived cell-free DNA (dd-cfDNA) and transplantation, utilizing the following keywords: "dd-cfDNA," "donor-derived cell-free DNA," "cell-free DNA renal transplant," "allograft surveillance biomarkers," "kidney transplant rejection," "biomarker acute rejection," "Kidney Transplantation" (MeSH), "Graft Rejection" (MeSH), "Cell-Free Nucleic Acids" (MeSH), "Biomarkers" (MeSH), "Sensitivity and Specificity" (MeSH), "Comparative Effectiveness Research" (MeSH), "Diagnostic Imaging" (MeSH), "Creatinine/blood" (MeSH), "Monitoring, Immunologic" (MeSH), "diagnostic tool," "diagnostic performance," "surveillance method," "human subjects," "adult," "renal", "renal transplant," "kidney allograft," "diagnosis," "diagnostic," "AUC," "predictive value," "rejection," "sensitivity," "specificity," "performance," "comparison," "receiver operating characteristic curve," "serum creatinine," "biopsy," "imaging," "donor-specific antibodies," "DSA," "Racial Groups" (MeSH), "Ethnic Groups" (MeSH), "rejection monitoring," "allograft," and "kidney."

These keywords were categorized to capture key aspects of the research question:

- **Population:** Keywords of "adult," "human subjects," "kidney transplant," "renal transplant," and "Kidney Transplantation" (MeSH) (including MeSH terms) were used to specify the patient group. "Racial Groups" (MeSH) and "Ethnic Groups" (MeSH) were included to address the "diverse ancestries" element.

- **Intervention:** Phrases such as "dd-cfDNA," "donor-derived cell-free DNA," "cell-free DNA renal transplant," and "Cell-Free Nucleic Acids" (MeSH) specifically targeted the primary diagnostic tool of interest.

- **Outcome:** Terms like "acute rejection," "graft rejection," "allograft rejection," and "Graft Rejection" (MeSH) directly identified the clinical event under investigation.

- **Comparison:** To encompass "standard biochemical and imaging surveillance," keywords like "serum creatinine," "biopsy," "imaging," "donor-specific antibodies," and related MeSH terms were utilized.

- **Diagnostic Role/Timing:** Phrases such as "diagnostic tool," "diagnostic performance," "surveillance method," "sensitivity," "specificity," "AUC," and "predictive value" were crucial for identifying studies assessing the diagnostic utility, accuracy, and particularly the comparative timing of dd-cfDNA.

The search process was restricted to articles published between January 1st, 2020, and May 31st, 2025.

Inclusion criteria. Inclusion criteria were designed to bring in articles directly aligned with the research question:

- English-language studies;
- Human subjects, specifically adult kidney transplant recipients (age ≥18 years);
- Use of donor-derived cell-free DNA (dd-cfDNA) as a primary or comparative diagnostic tool for acute rejection;
- Includes comparison to at least one standard surveillance method (e.g., serum creatinine, biopsy, imaging, donor-specific antibodies);
- Studies assessing the diagnostic role of dd-cfDNA in acute rejection, ideally reporting metrics (e.g., sensitivity, AUC) or qualitatively describing performance;
- Studies involving patients from diverse racial/ethnic backgrounds (where available);
- Studies published between January 1st, 2000 and May 31st, 2025; and

- Original research articles only (literature reviews, systematic reviews, and meta-analyses were excluded).

Articles not meeting the inclusion criteria were excluded.

3.2. Selection Process

The resulting set of articles was exported into a Google Sheets document, and the complete selection process is detailed in the PRISMA flow diagram (Figure 2). Following this, a preliminary screening of manual reading of abstracts and titles was conducted to decide whether to include or exclude. The screening included: Reasoning as to why the article was chosen, Full Text Accessibility (only for texts included), Number of Citations, Authors, Title of Article, Year Published, Source, Publisher, Article URL, Cites URL, GS Rank, Query Date, Type of Article, DOI, ISSN, Citation URL, Volume, Issue, Start Page, End Page, ECC, Cites Per Year, Cites Per Author, Author Count, Age, and Abstract.

Table 1: Characteristics of Selected Included Studies.

First Author	Year	Journal	Sample Size	Study Design	AUC for Rejection
Aubert, O.	2024	Nature Medicine	2,957	Prospective Cohort	0.87 (ABMR)
Bu, L.	2024	Kixty International	1,277	Prospective Cohort	0.81 (Any Rejection)
Halloran, P.	2022	Transplantation	385	Prospective Cohort	0.93 (ABMR)
Bromberg, J.	2024	Transplantation	191	Prospective Cohort	0.84 (Subclinical AR)
Stites, E.	2020	Am. Journal of Transplant.	231	Retrospective Cohort	0.82 (TCMR)
Kim, H.D.	2024	Frontiers in Immunology	206	Retrospective Cohort	0.91 (Clinical ABMR)
Parajuli, S.	2024	Clinical Transplantation	148	Retrospective Cohort	Not Reported
Bennings, L.	2023	Transplant International	104	Prospective, Single-Center	0.78 (Any Rejection)
Lim, H.	2023	Frontiers in Immunology	129	Observational	0.94 (ABMR)
Akilova, A.	2025	Nephrology Dialysis Transplantation	184	Randomized Trial	0.84 (ABMR)
Chen, X.T.	2022	Clinical Chemistry	102	Prospective Cohort	0.89 (Any Rejection)
Gäcin, H.	2025	Am. Journal of Transplant.	45 studies	Systematic Review	N/A

Yang, S.	2024	Bosnian Journal of Basic Medical Sciences	21 studies	Meta-Analysis	0.88 (ABMR)
Shen, J.	2024	Clinical Transplantation	68	Prospective Cohort	0.83 (Acute Rejection)
Mayer, K.A.	2024	Transplant International	115	Prospective Cohort	0.81 (ABMR)
Many, 2025	(Multiple)	N/A	N/A	(Multiple)	Median=0.83, Range=0.58–0.94

Note: This table summarizes key information for a representative sample of the 83 studies included in the systematic review. The complete dataset used for this review is available in a Google Sheets document, which can be found in the "Code and Data Availability" section.

3.3. Data Extraction and Synthesis

The initial data extraction included standard article metadata such as GS Rank, Query Date, DOI, ISSN, Citations, Age of publication, and Author Count. For included articles, specific variables relevant to the research question were gathered. Gemini (Google LLC, 2025) was used to assist in extracting these variables, including: Title, Journal, Publication Year, Country, Study (single or multicenter), Study Type, Sample Size, Average Age, Proportion Male, Patient Race/Ethnicity, Type of Rejection, Type of cf-DNA, and AUC.

3.4. Study Scoring and Relevance Assessment

A scoring system was curated from the literature and specifically tailored to this review's research goals to assess the direct relevance of each included article. Articles were awarded one point (1) if the following criteria were met, and zero points (0) if not. This included: Measured Blood? Analyzes Rejection Probability? Temporal Precedence Explicitly Stated? Longitudinal Monitoring for Timing? Compared to Traditional Biomarkers?

These scoring criteria were curated by the author to assess each study's direct relevance in answering the research question. “Measured Blood?” confirmed the appropriate sample for dd-cfDNA, as blood plasma is the standard medium for this analysis (Wolf-Doty et al., 2021). “Analyzes Rejection Probability?” ensured focus on the primary clinical outcome of rejection (Bu et al., 2022). “Temporal Precedence Explicitly Stated?” and “Longitudinal Monitoring for Timing?” were crucial for evaluating the “earlier identification” aspect of the hypothesis, a key feature of dd-cfDNA's utility (Bromberg et al., 2024). Finally, “Compared to Traditional Biomarkers?” verified that the study included the necessary comparative analysis against standard surveillance methods like serum creatinine to establish the biomarker's superior performance (Mantios et al., 2023). Data extracted from the included articles included study type and design, specifically identifying if the research was a prospective cohort, retrospective, or observational study, along with other standard article metadata.

3.5. Data Synthesis

Articles receiving the high scores (3-5 points) were selected for in-depth analysis. Preliminary conclusions were written from these high-scoring articles after reading them entirely and compiled into a summary document with a brief summary and overview of each article for future data synthesis and reference.

3.6. Data Analysis

Data analysis involved a narrative synthesis of findings from high-scoring articles. Extracted data on diagnostic timing, performance metrics, such as AUC and sensitivity, and patient demographics (including ancestry) were noted for future reference and figures. No formal statistical meta-analysis was performed due to the heterogeneity of the study populations and assays. Instead, comparisons between papers were made through a narrative synthesis of their findings, supported by data visualizations (e.g., box plots, scatter plots) that compare reported performance metrics and trends across different study contexts, such as geography and patient demographics.

The primary measure used to evaluate dd-cfDNA's diagnostic performance is the Area Under the Receiver Operating Characteristic (AUROC) curve. The AUROC is a valuable statistical measure that summarizes a test's ability to perform binary classification of patients with and without a condition across all possible thresholds; a value of 1.0 represents a perfect test, while a value of 0.5 represents a test with no discriminatory ability.

The Receiver Operating Characteristic (ROC) curve is a representation of a diagnostic test's overall performance, as described by one number, the Area Under the Curve (AUC). The AUC takes a value between 0.5 (no better than chance) and 1.0 (perfect test). Values between 0.80 and 0.90 may be considered “good.” For the purposes of this systematic review, the AUC indicates the extent to which dd-cfDNA separates patients with biopsy-proven rejection (the cases) from patients without biopsy proven rejection (the controls); therefore, a high AUC indicates that the biomarker is quite accurate in identifying patients that are actively rejecting their organ.

3.7. Data Visualization

All figures presented in the Results section were generated using Google Colab, a cloud-based computational environment. Data extracted from the included studies were compiled into a structured dataset and imported into the Colab environment for analysis. The Python programming language, along with the data manipulation library Pandas and the data visualization libraries Matplotlib and Seaborn, were used to create all plots, including histograms, box plots, and scatter plots, to visually synthesize the findings of this systematic review.

4. Results

In order to fulfill the objectives of this systematic review, the results were synthesized by firstly, describing the studies included, and then quantifying and gaining a general idea about the diagnostic accuracy of dd-cfDNA, and lastly, identifying some important performance trends and knowledge gaps.

4.1. Comparison to Serum Creatinine

A key objective of this review was to compare the diagnostic accuracy of dd-cfDNA to the current standard of care, serum creatinine. Across the studies that conducted a direct comparison, dd-cfDNA consistently demonstrated superior performance in detecting allograft rejection. The reported AUC for dd-cfDNA was consistently high, generally above 0.80, whereas the AUC for serum creatinine in the same cohorts was significantly lower, often ranging from 0.55 to 0.65. This indicates that serum creatinine has poor to fair discriminatory ability for rejection, while dd-cfDNA has good to excellent discriminatory ability, supporting the hypothesis that it is a more accurate biomarker.

4.2. Identification of Clinically Relevant Subtypes

Other publications were focused on building models to differentiate between AMBR and TCMR. The key finding was that dd-cfDNA was a strong potential biomarker for ABMR in patients who are clinically stable and have normal renal function (as measured by serum creatinine) (Botella et al., 2024). This is a huge advantage, since rejection at the subclinical level of ABMR can cause silent cumulative damage to the graft. Another published study described two patients who developed biopsy-proven ABMR within 60 days of a significantly elevated dd-cfDNA, despite both patients demonstrating normal renal function (Rizvi et al., 2023).

Dd-cfDNA is particularly useful in certain clinical settings and high-risk populations. The data suggests that dd-cfDNA is superior to creatinine for quantifying which patients with dnDSA (de novo donor-specific antibodies)—which are harmful immune proteins developed by the recipient that specifically attack the new organ—were definitively rejecting and needs to be considered for treatment decisions and guidance especially 15 days after transplantation (Botella et al., 2024).

4.3. Early Detection with dd-cfDNA

Additional research focused on the ability of dd-cfDNA to detect graft injury significantly earlier than clinical presentation (Bromberg et al., 2024) or elevations in standard biochemical markers like serum creatinine and proteinuria, which are known to be lagging (Kim et al., 2024). Dd-cfDNA's 30-minute half-life allows for the dynamic, consistent monitoring of ongoing damage and recovery (Wolf-Doty et al., 2021).

4.4. Monitoring Frequency in Included Studies

A review of the included studies revealed significant heterogeneity in the dd-cfDNA monitoring protocols used. The frequency of testing varied widely, from daily monitoring in the immediate post-transplant period in some studies, to weekly, monthly, and quarterly testing in others focused on long-term surveillance. This lack of a standardized approach makes it difficult to draw firm conclusions about the optimal monitoring schedule and highlights a key area for future research.

4.5. Characteristics of Included Studies and Publication Trends

First, to synthesize the characteristics and findings of the included studies (Goal 2), the compiled list of 83 studies that met the inclusion criteria was examined. Followed by examining the distribution of studies per publication year (Fig. 3a). It was found that publications have been steadily increasing over time, peaking in 2024 with approximately 22 studies. A linear regression analysis confirmed a significant positive trend between publication year and the number of studies (linear regression, slope = 3.5 studies/year, $p<0.05$), which suggests a rapid maturation of the evidence base. Furthermore, the relationship of how sample size changed with time in the field of kidney transplant dd-cfDNA research was interesting (Fig. 3b). In contrast to the number of publications, an analysis of variance (ANOVA) showed that while the median sample size appears to trend upwards visually, this increase was not statistically significant ($p > 0.05$). This is likely due to high variability and the presence of several large-scale outliers, potentially impacted by the interruption of research during the Covid-19 pandemic. This indicates that while landmark studies are getting larger, the sample size for a typical study in the field has not significantly changed over this period. The evidence is predominantly composed of prospective cohort designs, with these studies forming the largest single category (Fig. 3e). These studies, which follow participants forward in time, minimize bias and provide a strong evidence base.

To address the third goal of identifying trends and knowledge gaps, particularly regarding the biomarker's utility in diverse,

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underrepresented patient populations, the geographic and demographic distribution of the studies was analyzed. An examination of participant characteristics confirmed that the study focuses on a middle-aged cohort, with an average age of 52 years (Fig. 3c). This is both a useful finding and a commonly observed finding in clinical practice since the average age of transplantation is about 50 years. The analysis also highlighted that study cohorts tended to be predominantly male with a prominent peak, showing a proportion of male participants up to a significant 0.63 (Fig. 3d). To gain a better understanding of the generalizability and nature of the evidence in a global context, a breakdown of the study types by geographic region was conducted. An analysis of the geographic distribution of study types revealed that North American research contributed the most retrospective and observational studies, whereas Europe and Asia contributed a higher proportion of prospective cohort designs (Fig. 3f). A chi-square test of independence confirmed that this difference in the distribution of study types across continents is statistically significant ($\chi 2(6) = 18.98$, $p = 0.004$).

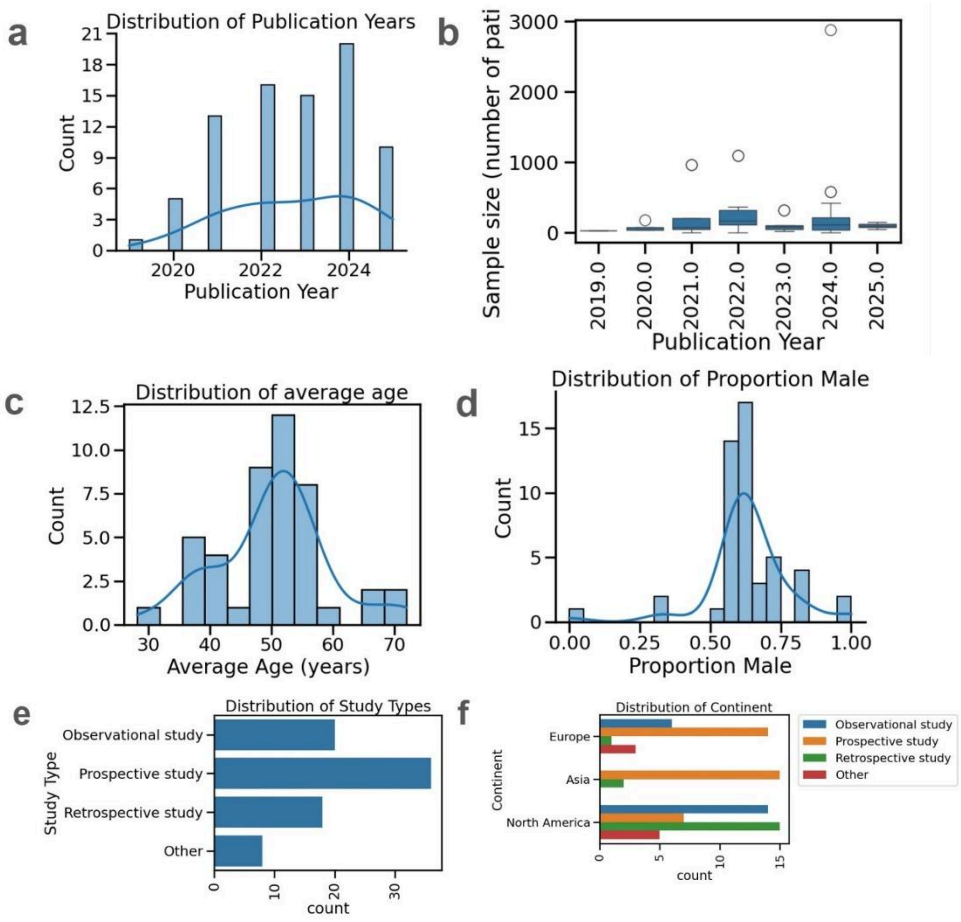


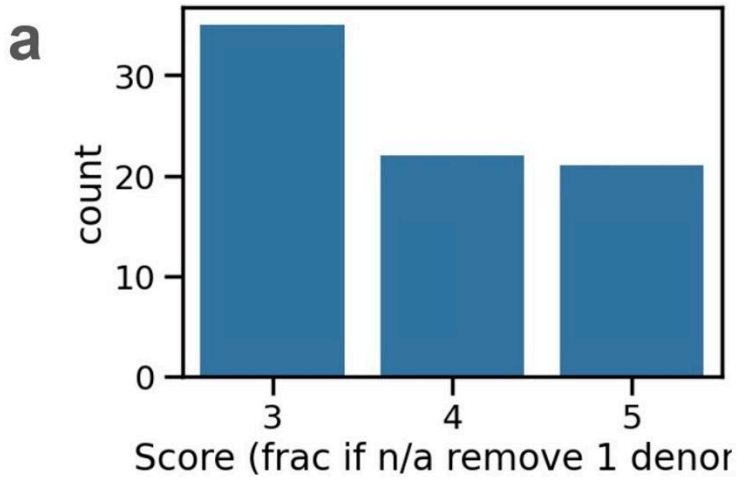
Figure 3: Publication Trends, Demographics, and Methodological Characteristics of Included Studies.

Note: (a) A histogram showing the number of included studies published per year, illustrating a rising trend in research volume peaking in 2024. (b) A box plot visualizing the distribution of study sample sizes by publication year, highlighting an increase in the scale of research and several large-scale outliers. (c) A histogram displaying the distribution of the average age of participants, showing a concentration in the middle-aged demographic. (d) A histogram of the proportion of male participants in study cohorts, indicating a predominance of male-majority studies. (e) A horizontal bar chart quantifying the included studies by their primary methodological design, with prospective studies being the most common. (f) A grouped horizontal bar chart illustrating the distribution of study types across the primary geographic regions of North America, Asia, and Europe.

4.6. Methodological Quality of the Evidence Base

To assess the methodological soundness of the literature used in the study, a 5-point quality scoring system was enacted. The 5-point quality scoring system was created to determine which sources most fit the hypothesis. Each study achieved one point for fulfilling each of the criteria: (1) Measured Blood?, (2) Analyzes probability of rejection?, (3) Temporal Precedence Explicitly Stated?, (4) longitudinal monitoring for time?, (5) compares to traditional biomarkers?. These scoring criteria were chosen to evaluate the relevance of each study to the specific research hypothesis. The criterion "Measured Blood?" evaluated whether the study utilized the appropriate sample type to analyze dd-cfDNA (Sawinski et al., 2021). The criterion "Analyzes rejection probability?" ensured that the study focused on the primary clinical outcome, i.e. rejection (Bu et al., 2022b). "Temporal Precedence Explicitly Stated?" and "Longitudinal Monitoring for Timing?" allowed for the evaluation of the hypothesis, specifically the earlier detection factor, which required identifying studies that monitored the biomarker over time (Parajuli et al., 2024). Finally, "Compares to Traditional Biomarkers?" validated that the study conducted the comparative clinical analysis of the complete framework, thereby determining the "superior performance" of dd-cfDNA analysis compared to traditional clinical surveillance methods such as serum creatinine (Mantios et al., 2023). The distribution of studies per publication year (Fig. 4a) indicates a rapid maturation of the evidence base, with publications steadily increasing over time and peaking in 2024. Most studies reporting their scores are clustered in the upper range of the scale (e.g. 35 studies scored a 3; 22 studies scored a 4; and 21 studies scored a 5).

To add more specificity to the study characteristics that were contributing to high quality evidence, which criteria were most frequently met across the studies were assessed (Fig. 4b). The breakdown shows that foundational criteria were met across studies, particularly utilizing blood as a sample source (n=79) and comparing dd-cfDNA to traditional biomarkers (n≈78).



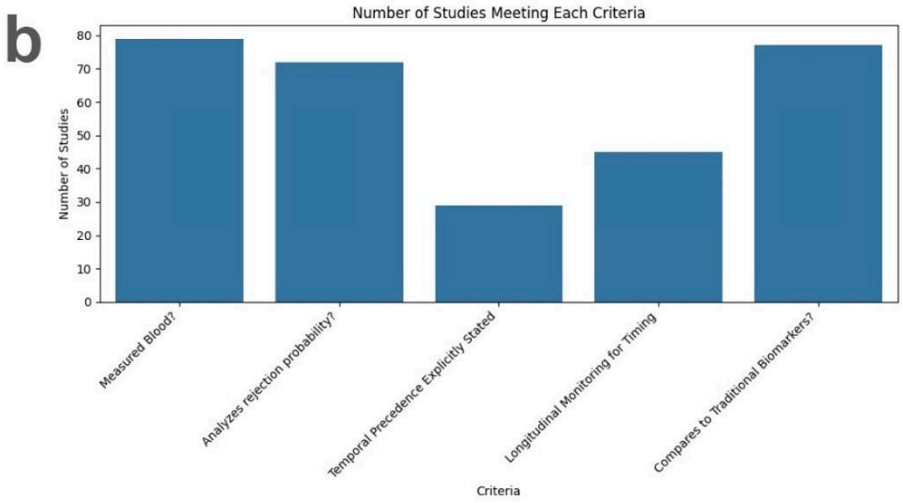


Figure 4: Distribution of Study Quality Scores and Fulfillment of Methodological Criteria.

Note: (a) A histogram showing the distribution of the overall 5-point quality scores across all included studies. (b) A bar chart quantifying the number of studies that met each of the five specific methodological quality criteria.

4.7. Geographic and Ancestral Distribution of the Evidence Base

In order to understand the efficacy of dd-cfDNA in multiple ancestries, which is an important goal for comprehensive utility of dd-cfDNA in kidney transplant settings, the inclusion of diverse racial and ethnic groups across the primary research continents was examined. To this end, the number of studies covering each self-reported race/ancestry group were examined, which were defined as "Asian," "Black," "White," and "Hispanic/Latino." The analysis reveals that North American research provides the most diverse evidence base. North American studies were the exclusive source of data for the inclusion of Black participants (n≈24) (Fig. 5a) and Hispanic/Latino participants (n≈18) (Fig. 5c). Furthermore, North American research also contributed the largest number of studies that include Asian participants (n≈17) (Fig. 5b) and White participants (n≈22) (Fig. 5d).

In contrast, the European studies included in this review, while numerous, did not explicitly report the inclusion of Black, Asian, or Hispanic participants, with many not reporting race altogether, though a small number did report including White patients (n≈3). The Asian studies, as expected, contributed evidence on Asian populations (n≈4) but did not report inclusion of other groups. This distribution highlights a significant gap in the global evidence, suggesting that the generalizability of findings from Europe and Asia to diverse populations is limited.

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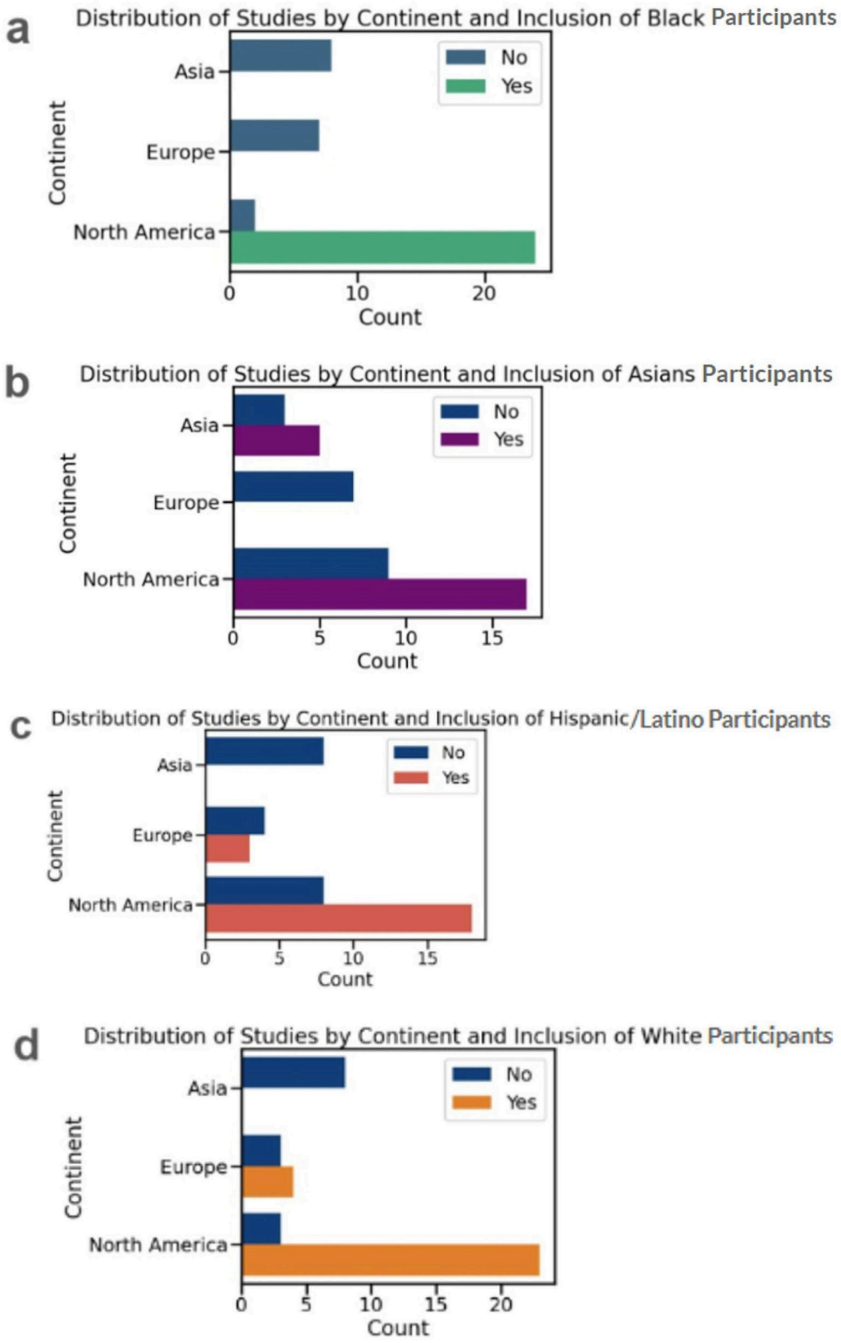

Figure 5: Geographic Distribution of Studies by Patient Ancestry.

Note: A series of horizontal bar charts showing the number of studies from North America, Europe, and Asia that did ("Yes") or did not ("No") report the inclusion of specific patient ancestries. Studies that did not report the race/ethnicity of their participants are not included. (a) Inclusion of Black participants. (b) Inclusion of Asian participants. (c) Inclusion of Hispanic/Latino participants. (d) Inclusion of White participants.

4.8. Diagnostic Accuracy of dd-cfDNA

A major study goal was to determine how effective dd-cfDNA was at identifying cases of kidney transplant rejection; therefore, AUC values were extracted from studies and analyzed. The diagnostic performance of dd-cfDNA, as measured by AUC, was found to be high across the included studies, as summarized in the histogram of Area Under the Curve (AUC) values (Fig. 6a). The data reveals a consensus towards models using dd-cfDNA having strong performance in the prediction of rejection, with a bimodal distribution showing two primary peaks where the evidence is concentrated. The largest cluster of studies reported an Area Under the Curve (AUC) with a peak centered approximately around AUC=0.80, while a second prominent peak centered even higher around 0.90. It is important to put this in perspective; for a standalone clinical diagnostic test, an AUC of 0.80 is generally considered poor as this indicates significant false positive and false negative rates that may cause indeterminate clinical decisions. An interesting finding is that studies rarely report mediocre performance; if this is influenced by publication bias where negative findings are underreported, it would suggest that the true diagnostic accuracy of dd-cfDNA may be overestimated in the current literature.

A linear regression analysis was then performed to model the relationship of year of publication and AUC analyses in order to assess whether reported performance of the biomarker has improved as studies have accrued (a primary goal of this review). This does demonstrate an increasing association over time (slope ≈ +0.01 per year of AUC) (Fig. 6b). This positive slope suggests that more 'recent' studies, on average, have reported greater AUC than 'earlier' studies. This potential trend may in part reflect field maturation, perhaps including improved laboratory assays (i.e., could be absolute quantification vs. fractional abundance) and more sophisticated study design (years and capabilities are reflected in the increasing median sample sizes). The evidence for dd-cfDNA has not weakened over time; instead, it seems to have grown and solidified. An alternative explanation might include a growing publication pressure to report findings that best represent previous literature. This study's upward trend in AUC is accompanied by an upward trend in the sample sizes of studies over time.

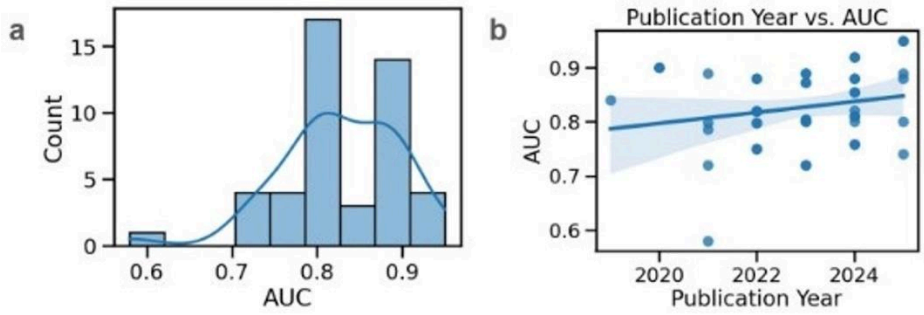

Figure 6: Summary of Diagnostic Accuracy and Performance Trends.

Note: (a) A histogram showing the bimodal distribution of reported Area Under the Curve (AUC) values from all included studies. (b) A regression plot illustrating the positive correlation between the publication year of the studies and their reported AUC values.

4.9. Performance Across Patient Ancestries

To further assess the generalizability of dd-cfDNA's performance, the AUC values were stratified by studies that reported the inclusion of different racial and ethnic groups. For this analysis, studies that did not report AUC or did not specify the racial demographics of their cohort were excluded, resulting in a subgroup of 45 studies. Overall, the data suggests that dd-cfDNA

consistently has accuracy in identifying potential rejection across studies with different cohort demographics. The median AUC of studies that specifically included Black participants (N=24) was 0.81 (Fig. 7a). This is an important observation supporting the biomarker's utility in this patient population, which has historically experienced higher rates of adverse transplant outcomes due to a combination of social and clinical factors.

It is critical to clarify that the available literature does not permit an analysis of dd-cfDNA performance exclusively within the Hispanic/Latino population. Instead, this review analyzed the group of studies that reported the inclusion of Hispanic/Latino participants within their larger, mixed-ancestry cohorts. For this collection of studies, the median AUC was 0.81, showing a narrow and consistent interquartile range (Fig. 7b). Therefore, while this result indicates that the biomarker performs well in diverse studies that include Hispanic patients, it is impossible to draw conclusions from this data about its specific performance in Hispanic populations alone.

The studies measuring the performance of dd-cfDNA that reported Asian participation strengthen the evidence for applicability of dd-cfDNA across racial and ethnic groups. Interestingly, the analysis suggests that studies including Asian participants demonstrate a trend towards higher and more consistent accuracy, with a median Area Under the Curve (AUC) of approximately 0.82 and a notably narrow interquartile range (Fig. 7c). This enhanced consistency may be attributed to factors like more homogeneous patient cohorts or the use of optimized, population-specific assays, highlighting the potential for dd-cfDNA's performance to be further refined in specific clinical and demographic contexts.

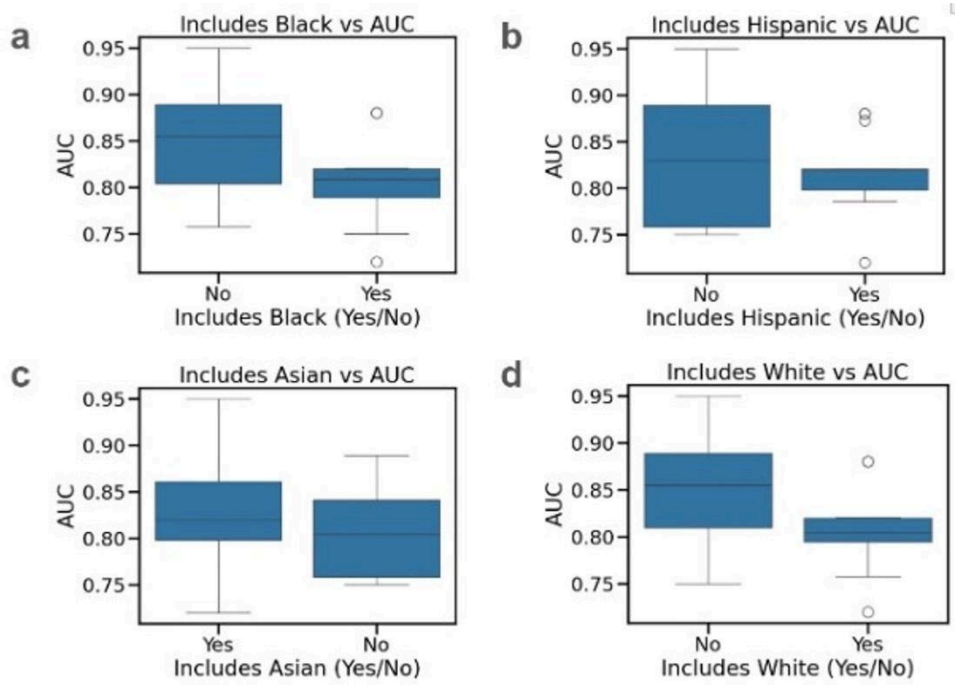

Figure 7: Diagnostic Accuracy (AUC) by Patient Ancestry Inclusion.

Note: A series of box plots showing the distribution of reported Area Under the Curve (AUC) values, comparing studies that did ("Yes") or did not ("No") report the inclusion of specific patient ancestries. (a) Distribution of AUC by inclusion of Black participants. (b Distribution of AUC by inclusion of Hispanic participants. (c) Distribution of AUC by inclusion of Asian participants. (d) Distribution of AUC by inclusion of White participants.

Finally, the analysis of studies including White participants provides further evidence of the biomarker's performance (Fig. 7d). The apparent better performance of studies that did not explicitly report including White participants is likely due to those studies being a heterogeneous mix of smaller or more homogeneous international cohorts where ethnicity is often not specified, whereas the “Yes” group consists primarily of large, diverse North American trials whose real-world complexity results in a more consistent, albeit slightly lower, median AUC.

While there are slight variations in the median AUC across studies that included participants of different self-reported ancestries, an analysis of variance (ANOVA) confirmed that these differences are not statistically significant ($p > 0.05$). This is consistent with the visual interpretation of the overlapping confidence intervals in the box plots. Therefore, while the available data suggests that dd-cfDNA performs with comparable diagnostic accuracy across cohorts with different ancestral compositions, this conclusion is preliminary. More robust data, such as dedicated studies on Hispanic populations and a broader geographic representation for Asian populations, is needed to definitively confirm these findings and account for potential regional biases.

4.10. Performance Across Different Study Contexts

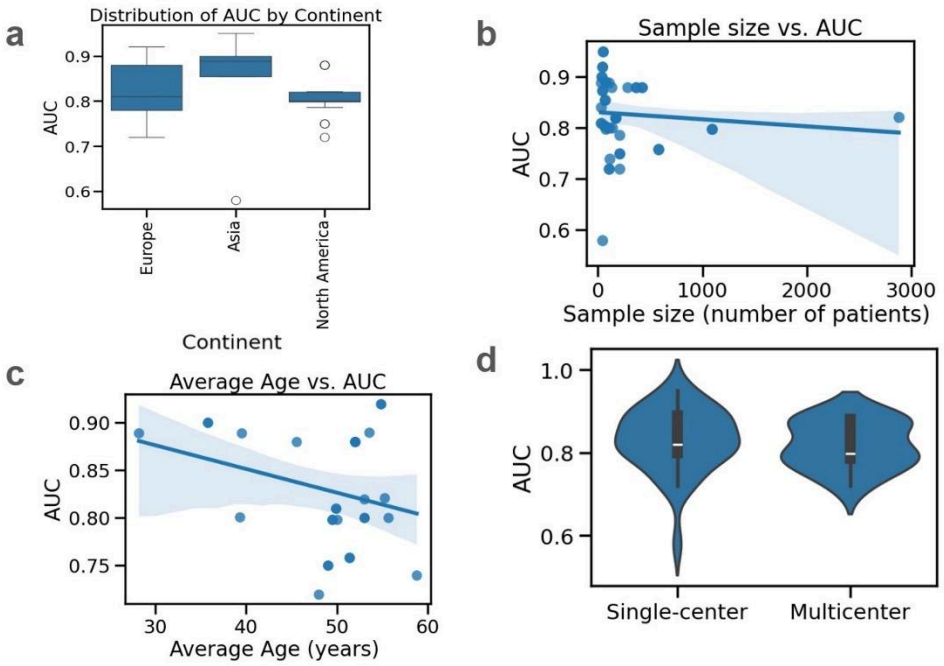

Figure 8: Analysis of dd-cfDNA Diagnostic Accuracy Across Different Study Contexts.

Note: (a) A box plot showing the distribution of reported AUC values, stratified by continent. (b) A regression plot illustrating the relationship between study sample size and reported AUC. (c) A regression plot showing the relationship between the average age of study participants and reported AUC. (d) A violin plot comparing the distribution of reported AUC values between single-center and multicenter studies.

Beyond the overall quality of the studies, it is also important to understand how the performance of dd-cfDNA varies across different study contexts, as detailed in Fig. 8. An analysis of performance by geographic region reveals a consistently high

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level of diagnostic accuracy globally, with the median AUC for studies from Europe, Asia, and North America all above 0.80 (Fig. 8a). Furthermore, an analysis of study scale shows no significant correlation between a study's sample size and its reported AUC (Fig. 8b); the nearly flat regression line indicates that high performance is not simply an artifact of small studies, a conclusion reinforced by the finding that the largest study with nearly 3,000 patients still reported a strong AUC.

dd-cfDNA's performance is further evaluated by an analysis of demographic and methodological factors. The regression plot of average participant age versus AUC shows a slight downwards trend (Fig. 8c). This indicates that in the age group between ages 30 to 60, the AUC decreased as age increased, thereby signifying the need for more studies to further validate this relationship. Finally, while both single-center and multicenter studies reported similarly high median AUCs, the violin plot reveals that the results from multicenter studies were more consistent, with a tighter distribution and less variability (Fig. 8d). One aspect to note about Fig. 8d is that a violin plot was utilized over a standard box plot because it visualizes the full probability density of the data.

4.11. Relationship Between Study Quality and Diagnostic Accuracy

The relationship between the study scores was also analyzed, as determined by my 5-point relevance criteria, and their reported diagnostic accuracy (AUC). The box plot of overall quality scores reveals that studies with higher scores (3, 4, and 5) consistently reported high median AUCs above 0.80 (Fig. 9a). This indicates that the score of a study is not directly related to the reported AUC, as high diagnostic performance was observed across all quality tiers. A more granular analysis shows that studies incorporating more rigorous methodologies, such as longitudinal monitoring, not only reported a high median AUC but also showed more consistent results, evidenced by a tighter distribution of values compared to studies that did not meet this criterion (Fig. 9b).

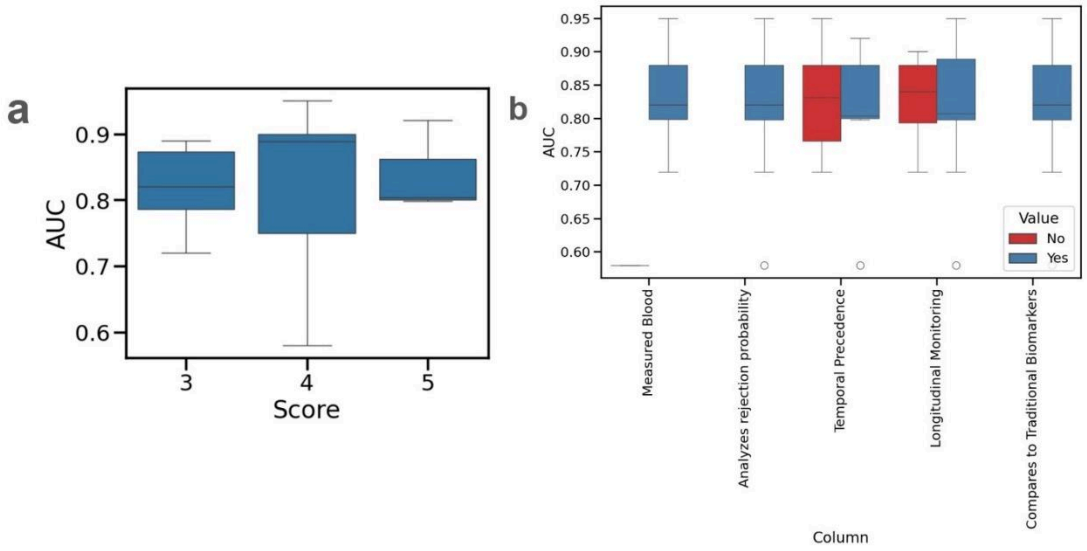

Figure 9: Relationship Between Study Quality and Diagnostic Accuracy (AUC).

Note: (a) A box plot showing the distribution of reported AUC values, stratified by the overall 5-point quality score of the studies. (b) A box plot comparing the distribution of reported AUC values for studies that did ("Yes") or did not ("No") meet each of the five specific methodological quality criteria.

4.12. Performance in Specific Rejection Subtypes

Lastly, to understand the evidence base for different rejection pathways, the included studies were analyzed based on their inclusion of either antibody-mediated rejection (ABMR) or T-cell mediated rejection (TCMR). The analysis shows that the literature provides robust coverage of both primary rejection subtypes. A substantial majority of the included studies, approximately 48, investigated patient cohorts that included cases of ABMR (Fig. 10a), while a similarly large number of studies, approximately 45, included cases of TCMR (Fig. 10b), though many included both.

Furthermore, an analysis of the reported diagnostic accuracy (AUC) shows that the high performance of dd-cfDNA is consistent regardless of a study's specific focus on rejection subtypes. The median AUC for studies that included ABMR was approximately 0.82, a value nearly identical to the median AUC for studies that did not specifically focus on ABMR (Fig. 10c). A similar pattern was observed for TCMR, where the median AUC was also consistent at approximately 0.82 in studies that investigated this rejection type (Fig. 10d).

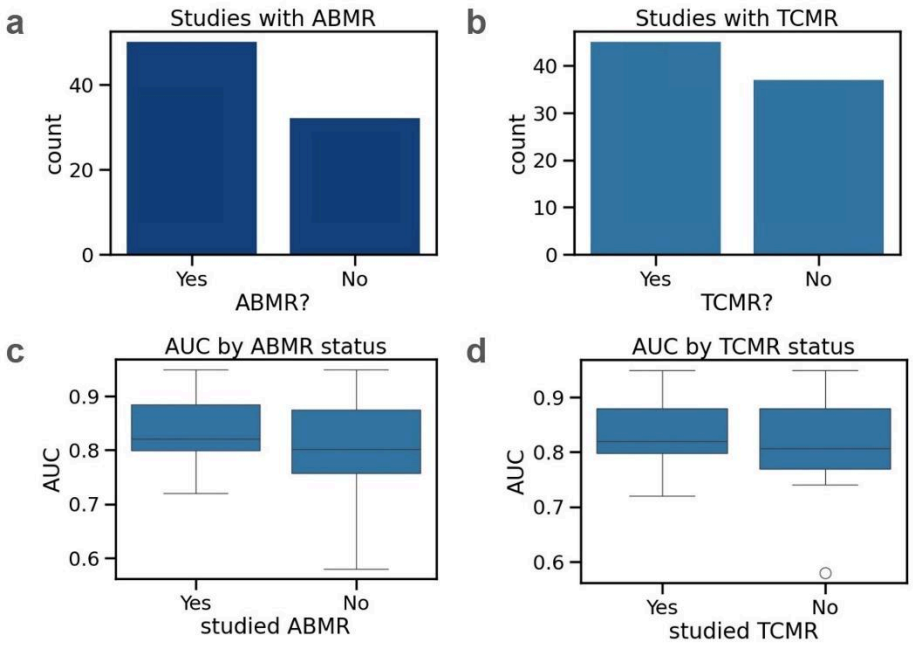

Figure 10: Research Focus on Rejection Subtypes and Corresponding Diagnostic Accuracy.

Note: (a) A bar chart showing the number of studies that did ("Yes") or did not ("No") investigate antibody-mediated rejection (ABMR). (b) A bar chart showing the number of studies that did ("Yes") or did not ("No") investigate T-cell mediated rejection (TCMR). (c) A box plot comparing the distribution of reported AUC values between studies that did or did not investigate ABMR. (d) A box plot comparing the distribution of reported AUC values between studies that did or did not investigate TCMR.

4.13. Methodological Gaps in Evaluating Rejection Timing

Despite this, there appears to be a large methodological gap in the literature regarding timing of rejection. While a reasonably sized number of studies (n=45) utilized some form of longitudinal monitoring for timing, a much smaller, and specific subset

(n≈29) stated the temporal precedence of dd-cfDNA elevation before a rejection diagnosis was confirmed. This indicates a clear opportunity for future research to focus more rigorously on study designs that can quantify this early detection window.

5. Conclusions

The central finding of this systematic review—that donor-derived cell-free DNA (dd-cfDNA) serves as a robust biomarker for kidney allograft rejection with high diagnostic accuracy—is largely consistent with the conclusions of previous literature reviews and meta-analyses. Multiple reviews have affirmed the biomarker's high potential as a "valuable tool" for non-invasive monitoring (Gisch et al., 2025) and as a reliable liquid biopsy for detecting allograft injury (Oellerich et al., 2021). Furthermore, our findings on diagnostic accuracy align with several recent meta-analyses that have reported high pooled AUC values, such as 0.84 for acute rejection (Zhang et al., 2021), 0.86 for overall rejection (Zhang et al., 2025), and 0.88 for antibody-mediated rejection (Yang et al., 2024). However, the present review builds upon this established consensus by providing a more granular, quantitative synthesis of the evolving evidence base. While prior work has established the biomarker's general utility, our analysis is the first to systematically document the rapid maturation of the field, including the significant increase in study sample sizes and the shift towards more rigorous prospective cohort designs. Most importantly, our review addresses a critical knowledge gap by quantifying the significant geographic and ancestral disparities in the literature. Previous reviews have qualitatively mentioned the need for more diverse cohorts, but our analysis provides concrete evidence that data for Black and Hispanic populations are almost exclusively derived from North American studies, underscoring a limitation in the global generalizability of current findings that was not the primary focus of prior meta-analyses.

This systematic review serves as a thorough synthesis of recent and robust research on donor-derived cell-free DNA that helps solidify its importance in the post-transplant period (Huang et al., 2023). The overall body of evidence supports that dd-cfDNA is consistently more accurate diagnostically than traditional biomarkers, such as serum creatinine, because it can detect rejection in those with stable creatinine levels (Gupta et al., 2022). The overall implication is that the use of dd-cfDNA represents a shift from reactive to proactive, evidence-based monitoring and screening that enables clinicians to detect allograft injury weeks or months before it presents clinically (Benning et al., 2023a). Although this review did systematically confirm that a number of studies reported a consistently high measure of diagnostic accuracy (e.g., > 0.80 as the AUC; Mantios et al., 2023), it also highlighted an important knowledge gap around the biomarker as most of the data on Black and Hispanic populations came exclusively from North American cohorts. One concrete recommendation based on this analysis is that future meta-analyses should synthesize other core metrics, such as Negative Predictive Value (NPV), in order to clearly define how dd-cfDNA should be used across different clinical scenarios (Aubert et al., 2024). In summary, this systematic review concludes that dd-cfDNA is a milestone in transplant monitoring that provides a foundation for more personalized and non-invasive patient care (Loupy et al., 2024a).

It is also important to consider the limitations inherent in the designs of the included studies. While this review found a predominance of prospective cohort studies, which is a methodological strength, a notable number of studies were retrospective or single-center designs. Single-center studies may have limited external validity, while retrospective designs can be subject to selection bias and incomplete data. These factors represent a limitation of the overall evidence base and highlight the need for more large, prospective, multicenter trials to confirm the findings synthesized in this review. Furthermore, there was significant variability in diagnostic accuracy reported between different commercial assays used across the studies, representing another key limitation of the current evidence base.

This systematic review confirms that dd-cfDNA is a useful non-invasive biomarker for early allograft injury with long-term prognostic value (Kim et al., 2024; Tian et al., n.d.; Nie et al., 2025; Loupy et al., 2024). While included studies demonstrated its ability to identify rejection sooner than traditional markers (Bromberg et al, 2024; Parajuli et al., 2024), this review identified significant limitations, including a lack of ethnic diversity and variable accuracy across different assays (Nguyen et al., 2025). Furthermore, because dd-cfDNA is a dynamic marker whose levels can become erratic, a single static measurement can be misleading (Nguyen et al., 2025). Therefore, future research must focus on validating performance in diverse populations and establishing standardized protocols for sequential monitoring to best differentiate true injury from transient fluctuations (Stites et al., 2020).

To fully realize the clinical potential of donor-derived cell-free DNA (dd-cfDNA), future research must focus on precision, standardization, and equitable application. This systematic review confirmed the biomarker's high diagnostic accuracy but also highlighted that different commercial assays can have different rejection detection rates for the same rejection subtype. Therefore, robust prospective trials are needed to conduct head-to-head comparisons of existing platforms and establish standardized reporting metrics. Furthermore, to ensure the clinical translatability and applicability of this biomarker, future studies must also assess its cost-effectiveness and the logistical challenges of integrating dd-cfDNA testing into routine clinical workflows. To ensure equitable and effective use, there is an immediate need for future research on underrepresented populations in Europe and Asia, as current evidence for Black and Hispanic populations is almost exclusively from North American studies. The ultimate success of this biomarker will also hinge on its ability to improve long-term patient outcomes, necessitating intervention trials that use dd-cfDNA's early warning signal to guide pre-emptive treatment. This would provide direct evidence on whether a dd-cfDNA-based strategy improves the trajectory of allograft health compared to the current standard of care. This potential for underreporting of negative or inconclusive studies represents a key limitation of the existing evidence base, and future meta-analyses should attempt to quantify the extent of this publication bias.

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Code and Data Availability

Data collected as part of this systematic review is freely available at https://docs.google.com/spreadsheets/d/1KAfEUQF-zXAJviuMSKyAEOpJUpMJbEMjBCOCbhbmgj4/edit?usp=sharing. The Google Colab notebook used for analysis and plot generation is freely available at https://colab.research.google.com/drive/1oq564UU3FZyur_mscmA8rdN5-H1EsuY1?usp=sharing.

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Author Biography

Alan Wu is a student researcher at Westlake High School in California. He is passionate about the intersections of non-invasive diagnostics, clinical nephrology, and equitable patient care, particularly in transplantation medicine. His academic focus is on monitoring diseases and developing new biomarkers for personalized care strategies. His systematic review aimed to quantify the diagnostic strength of a non-invasive biomarker for kidney allograft rejection and assess the generalizability of the current research, noting gaps in demographic and geographic representation. He is also interested in data science, biostatistics, and the ethical integration of new technologies into clinical practice. Alan aspires to become a transplant surgeon who uses molecular tools like dd-cfDNA to transform clinical decision-making, bridging scientific

inquiry with compassionate, technology-enabled patient monitoring to improve long-term graft survival.

Mentor Contribution Statement

Christa Caggiano served as the Research Mentor for this systematic review, guiding the author through the scientific research process and offering her expertise in data science methods and the paper structure. Her support was advisory and technical, focusing on providing insightful and critical feedback on all manuscript drafts and figures to ensure logical flow, content clarity, and factual accuracy. She meticulously reviewed all written sections and figures to confirm coherence and truthful representation of the systematic review data. Furthermore, she provided patient instruction on the fundamentals of Python programming required to transform the raw data into the final figures presented in the Results section. She also offered mentorship during the technical execution of data analysis and constructive suggestions during the revision process.