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Donor-Derived Cell-Free DNA in Plasma as a Non-Invasive Marker of Graft Rejection in Liver Transplant Recipients: A Review

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Abstract

Rejection after liver transplantation is confirmed by biopsy, an invasive procedure with its own sampling error. Donor DNA circulating in recipient plasma offers a measurement of graft injury that can be repeated as often as a blood test. The accuracy is good; the threshold at which it becomes a decision is not settled.

Objectives: *To review the published evidence on donor-derived cell-free DNA as a marker of graft rejection after liver transplantation: how it is measured, what accuracy it achieves against biopsy, how it compares with liver function tests, and why thresholds differ so widely between studies.*

Material and Methods: *Narrative review of prospective cohort studies, assay validation reports, cross-organ comparisons and methodological papers indexed in PubMed and the major transplantation and clinical chemistry journals. Studies were eligible if they reported the performance of donor-derived cell-free DNA against a defined rejection endpoint in transplant recipients, or the analytical basis of its measurement. No new patient data were generated; every estimate quoted is one published by the original investigators.*

Results: *In a prospective multicentre cohort of 115 adult recipients across three centres, graft-derived cell-free DNA measured by droplet digital polymerase chain reaction discriminated rejection with sensitivity of 90.3 per cent (95% CI 74.2-98.0) and specificity of 92.9 per cent (95% CI 89.3-95.6) at a threshold of 10 per cent, outperforming conventional liver function tests on the same samples. In paediatric recipients a threshold of 28.7 per cent gave an area under the curve of 0.878 (95% CI 0.753-0.954), with positive and negative predictive values of 80 and 92.3 per cent. Serial measurement detected rising concentrations before graft dysfunction was apparent on liver enzymes, and the marker performs comparably in kidney, heart and lung transplantation.*

Conclusion: *Donor-derived cell-free DNA detects graft injury earlier and more specifically than liver function tests, and does so without biopsy. Its principal weakness is that it reports injury rather than its cause, and no threshold has been agreed across studies, with published cut-offs differing almost threefold.*

Abbreviations: *dd-cfDNA – Donor-Derived Cell-Free DNA, GcfDNA – Graft-Derived Cell-Free DNA, cfDNA – Cell-Free DNA, LTx – Liver Transplantation, ddPCR – Droplet Digital Polymerase Chain Reaction, SNP – Single Nucleotide Polymorphism, LFT – Liver Function Test, TCMR – T Cell-Mediated Rejection, AMR – Antibody-Mediated Rejection, AUC – Area Under the Receiver Operating Characteristic Curve, PPV – Positive Predictive Value, NPV – Negative Predictive Value.*

Keywords: *Liver Transplantation; Cell-Free DNA; Graft Rejection; Non-Invasive Biomarker; Droplet Digital PCR*

Introduction

The presence of donor DNA in the plasma of transplant recipients was demonstrated more than twenty-five years ago, in kidney and liver recipients [1]. The principle is simple: cells of the graft release fragmented DNA into the circulation as they die, and because that DNA carries the donor genotype it can be distinguished from the recipient's own [2,3].

The clinical problem it addresses is specific to transplantation. Rejection is confirmed by biopsy, which carries procedural risk, is prone to sampling error, depends on subjective interpretation and cannot practically be repeated often enough to track a graft over time [4,5]. Liver function tests are performed instead, but they are not diagnostic for either cellular or antibody-mediated rejection and rise only once injury is established [4].

Measurement became practical with droplet digital polymerase chain reaction and with sequencing approaches that quantify the donor fraction using a limited panel of informative single nucleotide polymorphisms [6,7]. Methods now exist that do not require the donor genotype at all [8,9]. The marker has been validated as a clinical-grade assay across solid organs [10] and is established in kidney [11], heart [3,12] and lung transplantation [13].

This review sets out what it achieves specifically after liver transplantation, how it compares with the tests it would replace, and why the reported thresholds vary so much.

Material and Methods

Search strategy: PubMed and the archives of the major transplantation, hepatology and clinical chemistry journals were searched for studies of circulating donor DNA after transplantation. Search terms combined donor-derived cell-free DNA, graft-derived cell-free DNA and donor-specific cell-free DNA with liver transplantation, rejection, droplet digital PCR, biomarker and diagnostic accuracy. Reference lists of retrieved cohorts and methodological reviews were screened by hand.

Eligibility: Studies were included if they reported the performance of donor-derived cell-free DNA against a defined rejection or graft injury endpoint, or the analytical basis of its measurement. Studies in other solid organs are included where they established the method or provide a comparison, and are identified as such.

Data extraction and handling: Design, cohort size, assay platform, how the marker was expressed, the threshold applied and reported accuracy estimates with confidence intervals were extracted as published. Whether the marker was reported as a fraction of total cell-free DNA or as an absolute concentration is stated throughout, because the two behave differently when total cell-free DNA rises for non-graft reasons. No pooling or re-analysis was performed and no patient-level data were obtained. Figures 1 to 3 and Tables 1 to 3 reproduce published values only. This is a narrative synthesis and was not registered as a systematic review.

Results

Performance after liver transplantation: The pivotal study monitored graft-derived cell-free DNA in 115 adult recipients at three German centres in a prospective, observational, multicentre cohort. The marker was expressed as a percentage of total cell-free DNA and measured by droplet digital polymerase chain reaction using four to five informative single nucleotide polymorphism assays selected per patient from a panel of more than forty, giving same-day turnaround [4].

At a threshold of 10 per cent, diagnostic sensitivity was 90.3 per cent (95% CI 74.2-98.0) and specificity 92.9 per cent (95% CI 89.3-95.6) (Figure 1). The area under the curve was higher for the cell-free DNA measurement than for conventional liver function tests on the same samples, and when sensitivity was compared at a fixed 95 per cent specificity the marker retained its advantage [4]. Independent cohorts have reported that concentrations rise before graft dysfunction becomes apparent biochemically [14], and that the marker identifies recipients with biopsy-proven rejection requiring treatment [15].

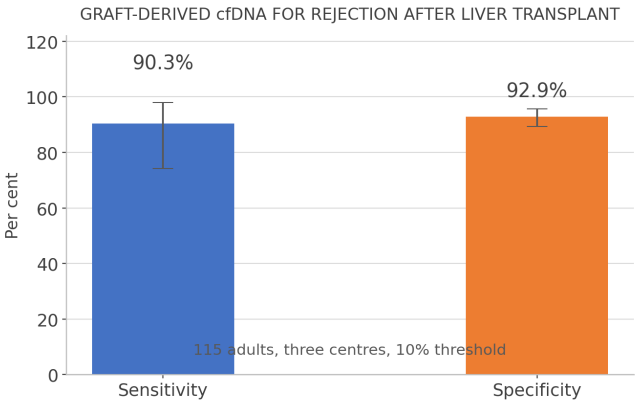

Figure 1: Accuracy of graft-derived cell-free DNA for rejection in 115 adult liver transplant recipients [4]

The same marker in children: Paediatric recipients were assessed separately, which matters because graft-to-recipient size ratio differs and baseline donor fraction with it. A threshold of 28.7 per cent discriminated biopsy-confirmed rejection with an area under the curve of 0.878 (95% CI 0.753-0.954), a positive predictive value of 80 per cent and a negative predictive value of 92.3 per cent (Figure 2) [16].

The asymmetry between those predictive values is the clinically useful part. A negative result makes rejection unlikely and could reasonably defer a biopsy; a positive result identifies graft injury without establishing that rejection is its cause [16,17].

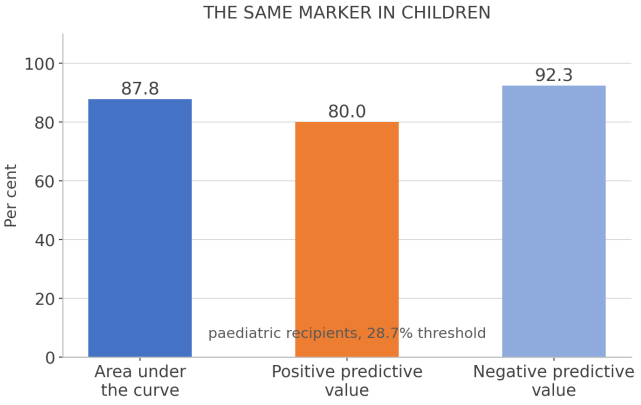

Figure 2: Performance in paediatric liver transplant recipients at a 28.7 per cent threshold [16]

Study	n	Organ	Principal reported finding
Lo et al. 1998 [1]	—	Kidney, liver	Donor DNA present in recipient plasma
Snyder et al. 2011 [2]	—	Heart	Universal non-invasive rejection detection proposed
De Vlaminck et al. 2014 [3]	—	Heart	Circulating cfDNA diagnoses heart rejection
Schütz et al. 2017 [4]	115	Liver	90.3% sensitivity, 92.9% specificity at 10%
Bloom et al. 2017 [11]	—	Kidney	DART study; cfDNA and active rejection
Zhao et al. 2021 [16]	—	Liver	Paediatric threshold 28.7%; AUC 0.878
Levitsky et al. 2022 [14]	—	Liver	Levels rise before graft dysfunction

Table 1: Principal studies reviewed, with the organ studied and the finding as reported by the original investigators

No agreed threshold: The two liver cohorts above applied cut-offs of 10 and 28.7 per cent respectively (Figure 3) [4,16]. That is close to a threefold difference in the value at which a clinician would act, and it arises from real differences in population and assay rather than from error. Graft mass relative to recipient size differs between adults and children; the liver is a large organ and contributes a higher baseline donor fraction than a kidney; and the marker can be expressed either as a fraction of total cell-free DNA or as an absolute concentration in copies per millilitre, which do not behave alike [16,18].

The fractional measure is argued to reflect graft-specific injury better, because it corrects for fluctuations in total cell-free DNA caused by infection, inflammation or surgical stress [18,19]. But that correction cuts both ways: a rise in recipient-derived cell-free DNA from an intercurrent illness lowers the donor fraction without any change in graft injury.

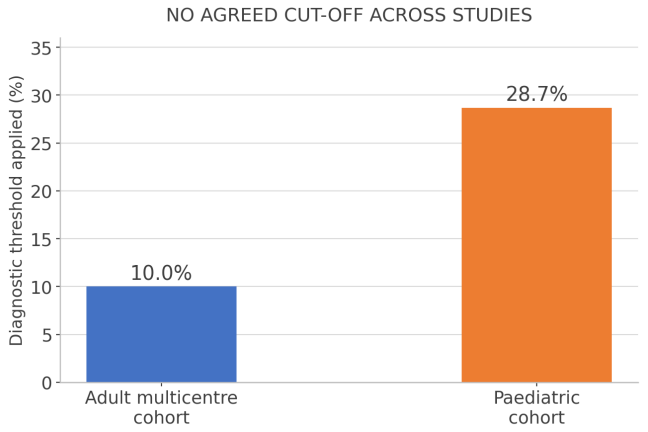

Figure 3: Diagnostic thresholds applied in the two principal liver transplant cohorts [4,16]

What the marker cannot distinguish: Elevated donor cell-free DNA indicates that graft cells are dying, not why. Ischaemia-reperfusion injury raises it early after transplantation independently of any immune process [20], and other causes of graft dysfunction produce elevations that are indistinguishable from rejection on the measurement alone [14,21]. Alternative techniques including short tandem repeat analysis with attention to total cell-free DNA concentration and fragment size have been applied to the same question [17].

Early kinetics carry information beyond the single value. In kidney recipients, the pattern of decline after transplantation predicted graft recovery and longer-term function [22], and absolute quantification early after liver transplantation has been characterised specifically [23]. The practical implication is that a baseline trajectory is needed before an individual value can be interpreted.

Comparison	What it offers	What it cannot do
Liver biopsy	Distinguishes rejection type and grade	Invasive; sampling error; not repeatable often [4,5]
Liver function tests	Cheap, universal, repeatable	Not diagnostic for rejection; rise late [4]
Donor-derived cfDNA	Detects injury early; repeatable as a blood test [4,14]	Reports injury not cause; no agreed threshold [4,16,20]

Table 2: The three approaches to assessing graft integrity and what each contributes

Evidence from other organs: The marker is further advanced outside the liver, which informs what to expect. In kidney transplantation a multicentre study established the relationship between circulating donor cell-free DNA and active rejection [11]; in heart transplantation the donor fraction relates to rejection in both adult and paediatric recipients [12]; and in lung transplantation it detects infection as well as rejection [13]. A clinical-grade assay has been validated across solid organs [10], and methodological reviews have set out the analytical considerations that apply generally [19,24,25].

Liver transplantation differs from these in one respect worth stating. The graft is proportionally much larger, so the baseline donor fraction is higher and the dynamic range within which a rise must be detected is correspondingly compressed [16,18]. Thresholds derived in kidney or heart recipients do not transfer.

Discussion

The evidence supports a clear conclusion about performance and an unclear one about use. On performance, donor-derived cell-free DNA outperforms liver function tests for detecting rejection, achieves sensitivity and specificity above 90 per cent against biopsy in a prospective multicentre cohort, and rises before biochemical dysfunction appears [4,14]. For a test requiring only a blood sample with same-day turnaround, that is a strong result.

On use, the difficulty is that the marker measures graft cell death without attributing it. Rejection, ischaemia-reperfusion injury, infection and other causes of graft dysfunction all raise it [20,21], so a positive result narrows the differential without resolving it. The negative predictive value is where the clinical value most plausibly lies: a low result makes rejection unlikely and could defer a biopsy that would otherwise be performed [16].

Why the thresholds differ

A cut-off of 10 per cent in adults against 28.7 per cent in children is not a discrepancy to be resolved by more data alone [4,16]. Graft mass relative to recipient body size is the dominant determinant of baseline donor fraction, and it differs systematically between the two populations. The same logic explains why liver thresholds sit far above those used in kidney transplantation.

This argues for individualised rather than population thresholds. Serial measurement establishing each recipient's own baseline, with rejection flagged by a rise above that baseline rather than by crossing a fixed value, is consistent with the observation that patients without complications show a transient post-operative peak followed by a stable plateau [14,22,23].

Study limitations

The limitations belong to the underlying literature. The principal adult liver cohort included 115 patients at three centres in one country [4], and the paediatric evidence rests on smaller numbers still [16]. Assay platforms differ between droplet digital polymerase chain reaction, sequencing and short tandem repeat approaches, and results are not interchangeable [6,7,17]. The marker is variously reported as a fraction and as an absolute concentration, which complicates comparison across studies [18,23]. Biopsy serves as the reference standard despite its own sampling error and interobserver variation, which biases accuracy estimates in an unknown direction [4,5]. And most cohorts are observational, with sampling schedules that differ between centres [4,14].

Two gaps deserve naming. No randomised study has tested whether managing recipients by donor-derived cell-free DNA rather than by protocol biopsy changes graft or patient survival, so the demonstrated accuracy has not been shown to translate into benefit [4,14]. And no study reviewed here establishes how the marker

should be interpreted when it rises in a recipient with intercurrent infection, which is the situation in which its lack of specificity matters most [13,21].

Conclusion

Donor-derived cell-free DNA detects graft injury after liver transplantation with sensitivity of 90.3 per cent and specificity of 92.9 per cent against biopsy, outperforms liver function tests on the same samples, and rises before biochemical dysfunction is apparent. It requires only a blood sample and can be repeated as often as clinically useful. Its limitations are equally clear: it reports that graft cells are dying without saying why, and no threshold has been agreed, with published cut-offs differing almost threefold between adult and paediatric cohorts for reasons of graft size rather than error. The most defensible current use is as a rule-out test against each recipient's own baseline trajectory, deferring biopsy when the value is low rather than diagnosing rejection when it is high.

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Conflict of Interest

None of the authors has an interest that competes with this work.

Ethics Statement

The article rests entirely on studies already published. No participant was enrolled for it and no patient data were newly collected, so ethical approval did not arise.

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