Donor-specific Cell-Free DNA as a Biomarker in Solid Organ Transplantation. A Systematic Review.

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Authorship

SK conceived the study, authored the study protocol, developed and performed literature searches, screened references, analysed the data and wrote the manuscript.

AT participated in study design, screened references, analysed the data and participated in the writing of the manuscript.

MLF participated in study design and participated in the writing of the manuscript.

Disclosures

The authors declare no conflicts of interest relating to the content of this article.

Funding

This study received no external sources of funding.

Abbreviations

ABMR	Antibody mediated rejection
AR	Acute rejection
ATN	Acute Tubular Necrosis
AUC	Area under the curve
BOS	Bronchiolitis obliterans syndrome
BPAR	Biopsy Proven Acute Rejection
cfDNA	Cell-free Deoxyribonucleic acid
CLAD	Chronic lung allograft dysfunction
dd-cfDNA	Donor Derived Cell Free Deoxyribonucleic acid
ddPCR	Digital droplet polymerase chain reaction
DIP	Deletion insertion polymorphism
DTA	Diagnostic test accuracy
EVLP	Ex-vivo lung perfusion
GE	Genomic equivalents
HLA	Human leucocyte antigen
IF/TA	Interstitial fibrosis/tubular atrophy
MPSS	Massive parallel sequencing
NFAT	Nuclear factor of activated T-cells
PCR	Polymerase chain reaction
PCT	Procalcitonin
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
qPCR	Quantitative polymerase chain reaction
QUADAS	Quality assessment tool for diagnostic accuracy studies
RH	Rhesus
ROC	Received operating characteristic
SDC	Supplementary digital content
SNP	Single nucleotide polymorphism
SOT	Solid organ transplant
SPK	Simultaneous kidney-pancreas
SRY	Sex-determining region Y
ssDNA	Single stranded deoxyribonucleic acid
TCMR	T-cell mediated acute rejection
TSP-Y	Testes specific protein Y-linked

Abstract

Background

There is increasing interest in the use of non-invasive biomarkers to reduce the risks posed by invasive biopsy for monitoring of solid organ transplants (SOT). One such promising marker is the presence of donor-derived cell-free DNA (dd-cfDNA) in the urine or blood of transplant recipients.

Methods

We systematically reviewed the published literature investigating the use of cfDNA in monitoring of graft health following SOT. Electronic databases were searched for studies relating cfDNA fraction or levels to clinical outcomes, and data including measures of diagnostic test accuracy (DTA) were extracted. Narrative analysis was performed.

Results

95 manuscripts from 47 studies met the inclusion criteria (18 kidney, 7 liver, 11 heart, 1 kidney-pancreas, 5 lung, and 5 multi-organ). The majority were retrospective and prospective cohort studies, with 19 reporting DTA data. Multiple techniques for measuring dd-cfDNA were reported, including many not requiring a donor sample. dd-cfDNA falls rapidly within 2 weeks, with baseline levels varying by organ type. Levels are elevated in the presence of allograft injury, including acute rejection (AR) and infection, and return to baseline following successful treatment. Elevation of cfDNA levels are seen in advance of clinically apparent organ injury. Discriminatory power was greatest for higher grades of T-cell mediated and antibody-mediated AR, with high negative predictive values.

Conclusions

cfDNA is a promising biomarker for monitoring the health of solid-organ transplants. Future studies will need to define how it can be used in routine clinical practice and determine clinical benefit with routine prospective monitoring.

Introduction

The gold standard for monitoring the health of solid organ transplants has traditionally been through biopsy of the donor organ. Biopsies can be performed in response to suspicion of graft pathology (clinically indicated) or as part of a routine monitoring protocol (protocol or surveillance biopsies). Protocol biopsies are a form of screening test, and therefore must fulfil the criteria outlined for successful screening. They must be safe and acceptable to the patient, detect a clinical condition at a stage where intervention can change the course, and detect abnormalities sufficiently frequently to justify the cost and risks associated.

These criteria can be questioned for the monitoring of organ transplants. Biopsies represent an invasive procedure and are uncomfortable and inconvenient for patients. Large series of renal transplant protocol biopsies demonstrate a major complication rate of 1%, with a 3.5% risk of gross haematuria (1). Around 25% biopsies yield an inadequate specimen; this risk may be larger with a smaller needle size. With modern immunosuppression, detection of subclinical rejection may be too infrequent to justify these risks, and many units no longer perform routine biopsies (2). In non-renal transplants, there is a lack of a reliable biochemical marker, equivalent to serum creatinine, to detect changes in graft function to guide clinically indicated biopsies. Similar biopsy complication rates are seen with endomyocardial biopsy following cardiac transplantation and transjugular liver biopsy (3,4), although protocol biopsies are more commonly used in these groups due to a higher potential for the detection of clinically relevant events and lack of alternative monitoring tools.

The disadvantages of histology for the routine monitoring of transplant organs has led to a great deal of interest in non-invasive strategies to detect graft injury and/or rejection. The ideal non-invasive test would be measureable in urine or plasma, relatively cheap for routine use, have a rapid turn-around, and be sensitive and specific for graft injury. Current commonly used methods include measurement of organ function where available (e.g. serum creatinine), or therapeutic drug monitoring of immunosuppressive drugs as a surrogate for adequate immunosuppression and therefore rejection risk. More sophisticated strategies involve monitoring the level of immune system activity, such as nuclear factor of activated T-cells (NFAT)-regulated gene expression, immune cell function assay or urinary/plasma chemokine levels (5–7). Alternatives include the measurement of damage-related gene expression in injured/rejecting organs (e.g. Allomap or kSORT) (8,9).

One proposed method for detecting allograft injury is in the measurement of cell-free DNA (cfDNA) in the plasma or urine of transplant recipients. Presence of cell-free DNA was first identified by Mandel and Metais in 1948, and is released during apoptosis or necrosis in response to injury (10). cfDNA assays have found use in prenatal diagnosis and in the detection and monitoring of malignancy (11,12). A number of groups have now investigated the use of cfDNA as a marker of transplant graft injury. In particular, the ability to differentiate recipient cfDNA and donor-derived cfDNA (dd-cfDNA) makes for a promising tool for the early and sensitive detection of allograft injury. Proof of concept has now been published for all solid organ types, and this study aims to systematically review the techniques and evidence for the relationship between dd-cfDNA levels and clinical outcomes in solid organ transplant recipients.

Materials and Methods

Protocol and registration

This systematic review was conducted in accordance with the *Preferred Reporting Items for Systematic Reviews and Meta-analyses* (PRISMA) statement (13). The protocol was prospectively registered with the *International Prospective Register of Systematic* Reviews (PROSPERO; registration number CRD42017082273).

Inclusion/exclusion criteria

All studies that reported dd-cfDNA levels in the urine or plasma of adult or paediatric solid organ transplant recipients and related these levels to one or more transplant-related outcomes were eligible for inclusion. Recipients of cell or bone-marrow transplants were excluded, as were studies reporting laboratory techniques with no relationship to clinical outcomes of interest. All study designs were eligible for inclusion. No restrictions were placed on publication language or date.

Literature searches

A systematic literature search was performed in OVID MEDLINE and EMBASE, the Transplant Library and the Cochrane Library for studies that met the inclusion criteria. Search terms included keywords and free text terms for solid organ transplantation and cell-free DNA (see SDC 1 for a sample search strategy). The final date for searches was June 12th 2018. Reference lists of included studies and relevant reviews were screened for potentially relevant references not identified by the initial search.

In order to identify unpublished and ongoing studies, the clinicaltrials.gov, EudraCT and ISRCTN trial registries were searched using similar terms to the primary literature.

Study selection

Duplicates were discarded from the initial search results. The remaining titles and abstracts were reviewed independently by two reviewers (SK and AT) to determine whether they met the inclusion criteria. Full articles of potentially relevant studies were reviewed prior to confirming their inclusion. Inter-reviewer agreement was

assessed by means of percentage agreement and Cohen's kappa. Any discrepancies in inclusion were resolved by review and discussion between the authors.

Data abstraction and analysis

Studies are identified by the first author and year of the first full publication (if available) or published abstract. Study-level data including study design, population, quality assessment, organ type, patient and sample numbers, sample type (plasma/urine), laboratory methodology and outcomes reported were extracted into a spreadsheet. Data relating dd-cfDNA to clinical outcomes were identified as acute rejection, other transplant related outcomes and response to treatment. Studies that compared the diagnostic accuracy of dd-cfDNA to other markers were also identified. For studies that reported diagnostic test accuracy (DTA) data (e.g. sensitivity, specificity, area-under the received operator characteristic curve (ROC AUC), positive and negative predictive values), these data were extracted along with the threshold values used. All laboratory values are presented using the units recorded in the original manuscript.

We had initially planned to perform a diagnostic test accuracy (DTA) meta-analysis on studies that reported DTA data, but it became apparent that heterogeneity was too great and study quality too poor to allow reliable analysis. Therefore, all findings are reported in the form of a narrative review.

Risk of bias in individual studies

In studies reporting DTA data, risk of bias was assessed using the QUADAS-2 tool (14). This validated instrument assesses the risk of bias resulting from patient selection, application of index and reference tests, patient flow and test timing. Two reviewers (SK and AT) independently performed the assessment, and any discrepancies were agreed by discussion.

Results

Literature searches identified 4,347 citations across all databases. 161 citations underwent full text review, with a total of 95 manuscripts/abstracts from 47 studies meeting the inclusion criteria (figure 1). It is possible that there is some overlap between the patient cohorts in some studies as studies used samples from the same biobanks with different analysis methods. Trial registry searches identified 6 ongoing studies, 5 of which were actively recruiting (NCT02178943, NCT03326076, NCT02109575, NCT03255265 and NCT02423070). Two of these ongoing studies had preliminary results identified in the main literature search (NCT02178943 and NCT02424227). Inter-reviewer agreement was substantial – Cohen's kappa was 0.69 with 84.7% agreement.

Identified studies are detailed in table 1. Of the 47 studies, 24 were reported in abstract form only. One study was a report of a single case, the rest were retrospective (n=17) and prospective (n=29) cohort studies. 38 studies were from single centre cohorts, with 9 studies reporting on multicentre cohorts. Transplant types were kidney (n=18), liver (n=7), heart (n=11), kidney-pancreas (n=1) and lung (n=5). 5 studies analysed samples from multiple transplant types. Nineteen studies reported data regarding diagnostic test accuracy; no studies validated cfDNA as a test in a separate cohort.

For studies reporting diagnostic test accuracy, risk of bias was variable across the QUADAS-2 domains (SDC2). No studies clearly predefined a threshold dd-cfDNA level for the diagnosis of clinical condition, risking over-estimation of test accuracy. Patient populations in most studies were representative of the transplant population as a whole, although four studies retrospectively selected patients according to their clinical condition (rather than including consecutive patients), which again artificially increases test accuracy. The majority of studies used biopsy-proven acute rejection as a reference standard.

All non-renal transplant studies measured dd-cfDNA levels in plasma samples. Of the 18 kidney transplant studies, 12 measured dd-cfDNA in plasma, 3 in urine, and 3 in both urine and plasma.

Techniques for detection of donor-derived cfDNA

Whilst the extraction of cfDNA was fairly standard across studies, different methods for determining the donor-derived fraction of cfDNA were employed. The most straightforward method used in the included studies is the

detection of the SRY or TSPY Y-chromosome repeat in female recipients of male organs (15–20). Whilst simple to detect and not requiring donor material for genotyping, this limits dd-cfDNA quantification to a small fraction of transplant recipients, so is not suitable for widespread use. A similar technique was used for known mismatches of the Rhesus (RH) gene (21).

A number of studies employed methods that allow differentiation of host and donor cfDNA in most, if not all, recipients. Two studies used amplification of human leucocyte antigen (HLA) DNA with quantitative PCR (qPCR) or digital droplet PCR (ddPCR), allowing identification of HLA alleles present in the donor but not the recipient (22,23). Recipient genotype can be determined from white blood cells with ddPCR. This works for the majority of recipients may be more challenging in closely related live donor/recipient pairs. Many of the recent studies identified made use of informative single nucleotide polymorphisms (SNPs) or deletion/insertion polymorphisms (DIPs) that are present in the donor but not the recipient. Selection of panels of polymorphisms that are known to be highly variable in the population of interest means that for most donor/recipient pairs, informative alleles will be present. These studies used qPCR (24–27), ddPCR (28–34) or massive parallel sequencing (MPSS) (35) to detect informative SNPs. Whilst earlier studies required initial detection of SNPs in both donor and recipient samples to detect informative mismatches, more recent studies have used computational approaches to determine the minor donor type, removing the requirement for donor samples and typing (28,29,31–34).

Stable values and time-course

Studies variably reported dd-cfDNA as the proportion of total cfDNA (n=32), absolute DNA levels by weight (n=4), copy numbers (n=5) or as genomic equivalents/ml (n=3). 21 studies reported the time-course of dd-cfDNA levels in the immediate post-transplant period, all demonstrating a rapid fall to steady-state baseline levels in uncomplicated patients by around day 7-10 post-transplant. Studies comparing different organ types suggested that the decline in levels seen in cardiac transplant recipients is slower than that after liver transplantation (36). Initial post-transplant levels are also higher in deceased donor compared to live donor transplant recipients, in keeping with lower levels of graft injury in live donor organs (34).

Steady-state plasma dd-cfDNA levels appear to vary by organ type. Mean fraction in liver (dd-cfDNA:total cfDNA range 3.3-5%) and lung (range 2-5%) recipients is higher than that seen after cardiac (range 0.06-0.6%) and renal transplantation (range 0.3-1.2%). This was confirmed in studies that directly compared recipient of different organ types using the same technique, with higher levels seen in liver transplant recipients compared to cardiac or renal transplants (33). The higher levels seen in lung and liver recipients most likely relate to a greater transplanted cell mass, a theory supported by the finding that recipients of double lung transplants have higher levels than recipients of single lungs (27). Dd-cfDNA fraction in the urine of renal transplant recipients was higher than that documented in plasma (8.7-55%) (17,29). Cardiac transplant recipients with a left ventricular assist device (LVAD) pre-transplant demonstrate higher dd-cfDNA levels than those without (37).

Interestingly, De Vlaminck and colleagues reported a slow increase in dd-cfDNA levels in lung transplant recipients from 3 months onward, which they relate to the development of chronic damage related to a loss of lung function (27).

Relationship to acute rejection

Forty-one studies (85%) reported the relationship between dd-cfDNA levels and biopsy-proven acute rejection (BPAR). All but one study reporting on kidney transplant recipients demonstrated significant elevations in dd-cfDNA levels at the time of BPAR. Bloom and colleagues demonstrated higher dd-cfDNA levels in antibody-mediated rejection (ABMR) than T-cell mediated rejection (TCMR) (38). In keeping with this, dd-cfDNA levels are also elevated in recipients developing de-novo donor specific antibodies (DSA) (39). Combined diagnosis using DSA and cfDNA levels may improve diagnostic accuracy (40). Levels also appeared to correlate with the severity of TCMR, with no difference seen between mild rejection (Banff 1A) and controls. This finding was also reflected in the study from Sigdel at al, who demonstrated a correlation between dd-cfDNA levels and Banff i and t scores (16). Elevated levels were reported up to 31 weeks prior to the clinical diagnosis of acute rejection in up to 68% of recipients (15,35,41). Lee and colleagues were the only group who did not find a relationship between either urine or plasma dd-cfDNA levels and presence of BPAR (29). The reason for this discrepancy is unclear, although the sample size in this retrospective study was very small, with high inter- and intra-patient

variability in dd-cfDNA levels seen. A single study in recipients of simultaneous kidney-pancreas transplant recipients demonstrated similar elevations of dd-cfDNA during episodes of rejection.

All studies in liver transplant recipients demonstrated elevated dd-cfDNA levels during episodes of BPAR. As with kidney transplant recipients, levels appear to rise prior to the clinical manifestations of acute rejection, with two studies demonstrating elevated levels 4-6 days prior to an aminotransferase rise, and 8-15 days prior to biopsy confirmation of rejection (31,42).

The majority of studies in cardiac transplant recipients found a relationship between acute rejection (identified on endomyocardial biopsy) and dd-cfDNA levels. As with kidney transplant recipients, the relationship appears to be stronger for ABMR and more severe TCMR than for mild TCMR (24,25,43,44). Again, elevated levels of dd-cfDNA precede the diagnosis of acute rejection by up to 5 months (24,25). Of note, one study in paediatric recipients of cardiac transplants failed to identify a difference in dd-cfDNA levels between patients with acute rejection or a stable course, albeit with small numbers of recipients experiencing acute rejection episodes (26).

A similar pattern was seen in lung-transplant recipients, with elevated levels of dd-cfDNA seen at the time of endobronchial biopsy demonstrating acute rejection. As in kidney and cardiac rejection, levels related to the severity of rejection, with greater discriminatory power seen for severe rejection (27,43,45,46).

A number of studies followed dd-cfDNA levels after successful treatment of acute rejection, demonstrating a fall to baseline levels in most cases (15,23,28,30,34,47–49). In keeping with the short half-life of dd-cfDNA (less than 1 hour), the majority of studies demonstrated a rapid fall to baseline following successful treatment, However, the time taken to return to baseline was variable, with Bloom et al. reporting persistently elevated levels at 1 month with a return to baseline after 2-3 months, perhaps indicating residual graft injury. At least in cardiac transplant recipients, rebound of dd-cfDNA levels following treatment is a poor prognostic indicator (50).

Diagnostic test accuracy

Nineteen studies reported measures of diagnostic test accuracy (DTA) (table 2). In general, these supported the overall findings from all studies; dd-cfDNA levels were able to predict acute rejection with moderate to good

performance (ROC AUCs ranging from 0.59-0.97). Predictive ability appears similar across all organ types, with better performance seen for higher-grade and antibody-mediated rejection. Threshold values for the liver appeared higher than the other organ types. Negative predictive value was generally superior to positive predictive value, supporting the idea the dd-cfDNA is most useful for excluding graft injury in a stable patient (38).

Sigdel et al. demonstrated good performance for the detection of acute allograft injury in 2 studies, but were unable to differentiate the causes of injury (BK virus nephropathy, pyelonephritis or rejection) (16,51). In keeping with this, Moriera et al. demonstrated improved discriminatory performance for rejection when procalcitonin levels were used in conjunction with dd-cfDNA to differentiate between infection and rejection (15).

Relationship between dd-cfDNA and other clinical events

As suggested in the DTA data above, elevated dd-cfDNA levels were not always related to acute rejection and elevations were seen in response to other causes of acute graft injury. In the kidney, elevated dd-cfDNA levels were seen with BK virus nephropathy and urinary tract infection/pyelonephritis (16,28,51,52). Much smaller rises were seen in recipients with acute tubular necrosis (ATN) (15,28,51), and levels were unable to discriminate between normal histology and other chronic findings such as CNI toxicity or interstitial fibrosis/tubular atrophy (IF/TA) (16,28). In liver recipients, levels elevate with active hepatitis B and C infection (18,31,53), but not with cholestasis (33,53,54). In lung recipients, elevated levels are seen in recipients with infections associated with chronic lung allograft dysfunction (CLAD) (55)

A small number of studies attempted to relate baseline dd-cfDNA levels with long-term outcomes. In the kidney, Goh and colleagues demonstrated a positive correlation between dd-cfDNA levels at discharge from hospital and 1-year serum creatinine level (56). Similarly, Zhang and colleagues also identified a relationship between early dd-cfDNA levels and graft dysfunction at 12 months (57). In particular, a highly variable “peak-spiked” pattern of dd-cfDNA levels in the early post-transplant period was associated with long-term graft dysfunction, suggesting that repeated episodes of acute graft injury may impair long-term function.

There has also been suggestion that average dd-cfDNA levels over longer time-periods may reflect cumulative graft injury and relate to longer-term adverse outcomes. In lung recipients, higher mean dd-cfDNA levels during the first 6 months after transplantation are associated with inferior survival and higher incidence of bronchiolitis obliterans syndrome (BOS) (58). In cardiac recipients, high median levels associate with a combined endpoint of death, retransplantation, hemodynamic compromise or graft dysfunction at 3 years (59).

Comparison with other markers

A few studies have compared dd-cfDNA with other markers of graft injury. It outperforms standard biochemical measures in both the kidney (serum creatinine) and liver (aminotransferases), with levels elevating earlier and providing better discrimination for acute rejection (28,31,35,60).

Two studies compared performance of dd-cfDNA levels with the Allomap score, a panel of gene assays used in the detection of acute rejection (61,62). Little correlation is seen between the Allomap score and dd-cfDNA levels. Combining the two scores provided greater discriminatory power for the detection of acute rejection in cardiac transplant recipients, suggesting that they may provide complementary information.

Discussion

This systematic review has found evidence for the validity of dd-cfDNA as a biomarker in all solid organ transplant types. Donor-derived cfDNA can be reliably detected using a number of techniques and falls rapidly to a baseline level within 2 weeks of transplantation once the initial ischaemia-reperfusion injury has subsided. Baseline levels vary between organ types, relating to the cellular mass of the organ transplanted, with highest levels seen in liver and lung transplantation. The majority of studies show a strong relationship with acute rejection and other causes of acute allograft injury. Discriminatory power is greatest for more severe grades of acute rejection and antibody-mediated rejection, and levels return to baseline following successful treatment.

Donor-derived cell-free DNA meets many of the criteria required to be a useful screening test for acute rejection. Levels have been shown to rise prior to the clinical manifestations of rejection, with elevated levels seen up to a month or more prior to diagnosis in some prospective studies. Refinement of the techniques for quantifying dd-cfDNA mean that it can now be quantified even in the absence of material for donor genotyping, and turn-around time once informative SNPs or alleles have been identified can be as short as one working day (42). These newer techniques also allow accurate detection of dd-cfDNA even in HLA-matched transplants (63).

A recent study investigating dd-cfDNA as a diagnostic test in renal transplant recipients has demonstrated performance similar to the use of Troponin I in the diagnosis of acute myocardial infarction (64). Whilst intraindividual variability is generally lower than inter-individual variability, there is some within-patient variation that must be considered when interpreting results. An increase of 61% or more from the previous value is likely to be clinically significant; smaller increases probably warrant a confirmatory test. Whilst similar in magnitude, the exact threshold for determining a “positive” test varied between studies even in the same organ type, likely reflecting both variability between populations and in the methods and assay used.

In general, studies included in this review identified a higher negative predictive value than positive, suggesting that the test may be most suited to excluding rejection when values are below the threshold. Levels are sensitive for graft injury, but not specific for the cause of injury, with elevated levels also seen in infections such as BK nephropathy and hepatitis. In reality, this means that elevated levels will need to be interpreted in

conjunction with other clinical parameters and laboratory tests such as viral PCRs and urine culture. Indeed, the simple addition of procalcitonin as a marker of infection improves the specificity of dd-cfDNA in the setting of renal transplant rejection (15). In most settings, it is likely that dd-cfDNA will be used as a tool to identify the need for further investigation and to target protocol biopsies in the presence of subclinical changes, reducing the risks and costs associated with a protocol biopsy program whilst increasing yield. It will also find utility as a means of monitoring the response to treatment.

Whilst dd-cfDNA has largely been demonstrated as a marker for acute graft injury, there is also some evidence that elevated levels relate to inferior longer-term outcomes. In lung recipients, persistently elevated levels have been associated with CLAD and BOS (58,55). In the kidney, persistently elevated and variable levels during the first 6 months post-transplant are associated with inferior graft function (57). This finding may in part be explained by under-immunosuppression, with low tacrolimus exposure being associated with higher dd-cfDNA levels possibly related to persistent immune activity and chronic graft damage (65).

It must be noted that the majority of studies identified in this review are of limited methodological quality. All studies were retrospective or prospective cohorts, with a number of retrospective cohorts selecting patients based upon clinical manifestation of graft injury. Even in prospective studies, the gold standard test (biopsy) was often only applied in patients with clinical evidence of graft dysfunction, meaning that the utility of dd-cfDNA in detecting subclinical graft injury is uncertain. No studies attempted to validate the determined dd-cfDNA threshold in an external population, and no studies have attempted to determine the impact of prospective monitoring on clinical outcomes. Around half of the identified studies were reported in abstract form only. Whilst this is inevitable in such a rapidly expanding field, the limited space afforded in a conference abstract means that full methodological description is lacking and assessment of study quality is difficult.

It is not yet clear what is the optimum interval for dd-cfDNA measurement for routine transplant monitoring. Prospective studies identified in the present review used minimum intervals of 1 month between tests, with shorter intervals in the early post-transplant period when the risk of acute rejection and infection are at their highest. This would seem reasonable given the finding that levels rise up to a month prior to clinically apparent organ damage.

Searches of trial registries identified a number of ongoing studies that may help to clarify some of these unanswered questions. The majority of ongoing studies are large, prospective multicentre cohort studies that will help to validate the thresholds to prompt further investigation and determine the optimum interval for monitoring. One study (NCT03326076) is aiming to test the clinical utility of routine and for-cause dd-cfDNA monitoring in renal transplant recipients, comparing clinical outcomes in patients undergoing monitoring with a matched retrospective control cohort in whom monitoring was not undertaken. This study will provide the first direct evidence as to whether dd-cfDNA monitoring can actually impact clinical outcomes.

Cell-free DNA may also have a role to play in assessing pre-transplant injury and organ viability in deceased-donor transplantation. Methods have been described to determine the origin of circulating cell-free DNA using organ-specific methylation patterns (66). Use of beta-cell specific cfDNA detection has already been reported in the context of clinical islet cell transplantation (67). Application of these techniques in organ donors may allow the quantification of organ injury prior to procurement, aiding decisions where acute organ dysfunction is present. A recent abstract has provided proof of principle for this concept, with donor plasma mitochondrial DNA levels independently predicting slow-, delayed- and primary non-graft function following renal transplantation (68). Measurement of cfDNA levels during normothermic machine preservation may also help in pre-transplant viability assessment. A recent study exploring perfusate cfDNA levels during ex-vivo lung perfusion (EVLP) demonstrated significantly higher levels after 4 hours of perfusion in patients experiencing post-transplant graft dysfunction (69).

In summary, donor-derived cell-free DNA shows promise as a biomarker for the detection of acute transplant graft injury. It has potential to reduce the need for protocol biopsy surveillance, allowing for a more targeted diagnostic approach. Detection of injury occurs prior to clinical manifestation, meaning that there is a window for earlier detection and treatment of acute rejection and other causes of graft injury with the potential to improve outcomes. It may also facilitate the detection of under-immunosuppression and find use as a tool for monitoring during immunosuppression minimisation. Further studies are required to validate the thresholds for further investigation and intervention, determine the optimum frequency for monitoring, and to identify whether prospective monitoring using dd-cfDNA can indeed improve transplant outcomes compared to current practice.

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