Clinical Kidney Journal, 2023, vol. 16, no. 3, 447–455

https://doi.org/10.1093/ckj/sfac227
Advance Access Publication Date: 15 October 2022
CKJ Review

CKJ REVIEW

Donor liquid biopsy and outcomes in kidney transplantation

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ABSTRACT

Kidney transplantation is the treatment of choice for patients with kidney failure. Priority on the waiting list and optimal donor–recipient matching are guided by mathematical scores, clinical variables and macroscopic observation of the donated organ. Despite the increasing rates of successful kidney transplantation, maximizing the number of available organs while ensuring the optimum long-term performance of the transplanted kidney remains both key and challenging, and no unequivocal markers are available for clinical decision making. Moreover, the majority of studies performed thus far has focused on the risk of primary non-function and delayed graft function and subsequent survival and have mainly analysed recipients' samples. Given the increasing use of donors with expanded criteria and/or cardiac death, predicting whether grafts will provide sufficient kidney function is increasingly more challenging. Here we compile the available tools for pre-transplant kidney evaluation and summarize the latest molecular data from donors that may predict short-term (immediate or delayed graft function), medium-term (6 months) and long-term (≥12 months) kidney function. The use of liquid biopsy (urine, serum, plasma) to overcome the limitations of the pre-transplant histological evaluation is proposed. Novel molecules and approaches such as the use of urinary extracellular vesicles are also reviewed and discussed, along with directions for future research.

Keywords: acute kidney injury, biomarkers, chronic kidney disease, delayed graft function, donor, extracellular vesicles

INTRODUCTION

Kidney transplantation is the recommended treatment in patients with kidney failure. During 2019, >42 000 patients received a kidney transplant in Europe and the USA [1, 2]. Kidney transplants are available from both living (LD) and deceased (DD) donors, with the latter including donors after brain death (DBD) and donors after circulatory death (DCD) [3]. While kidney transplantation clearly improves outcomes compared with remaining on dialysis, there is a worldwide shortage of kidney donors and current methods to select or reject kidneys for transplantation and for donor–recipient matching remain suboptimal. We herein review the available tools for pre-transplant kidney evaluation and optimal donor–recipient matching,

Received: 28.6.2022; Editorial decision: 13.10.2022

including donor molecular data (e.g. urinary extracellular vesicles) and other pathological or clinical variables that may predict short- and, especially, long-term graft function. Finally, we propose directions for future research. The search strategy is summarized in the supplemental material.

Current risk stratification tools in kidney transplantation donor–recipient matching

Kidney donor–recipient matching is guided by histocompatibility, clinical variables and computational scores, but also by the recipient's vintage on the waiting list and the distance between the donor's and the recipient's hospitals. The Kidney Donor Risk Index (KDRI) estimates the relative risk of graft failure for a kidney from a particular DD compared with the median risk for all DDs in the prior calendar year. The KDRI calculation considers the donor's age, weight, height, ethnicity, (high) blood pressure, diabetes, exposure to hepatitis C, donation after circulatory death status, cause of death and kidney function estimated from the serum creatinine concentration (SCr). The Kidney Donor Profile Index (KDPI) remaps the KDRI from a relative risk scale to a cumulative percentage scale in which a lower percentage is associated with a higher donor quality. The KDPI assesses expected kidney graft survival, i.e. how long a kidney may function once transplanted. Similarly, the estimated post-transplant survival (EPTS) score assesses the expected patient survival for each potential recipient, which marks the priority on the waiting list. The KDPI and the EPTS are used in combination to ensure the best possible match between recipient and donor when the donor's KDPI is <20%. Candidates with longer expected survival (those with an EPTS score ≤20%) will be preferentially offered higher-quality kidneys (KDPI ≤20) [4]. Those risk stratification scores have been developed based on data from USA implanted kidneys and the tools' applicability to other countries with different allocation systems, outcomes and demographic characteristics is not well established. Therefore, they may not achieve the quality standard for outcome predictions.

In addition to the aforementioned clinical scores based on donor characteristics, attention is paid to the kidney itself to facilitate a better decision. For example, one of the most widely used methods for graft assessment is the pre-transplant histological evaluation, although it provides information about the kidney structure and not about kidney function. Isolated histological characteristics such as a high percentage of glomerulosclerosis, the presence and grade of vascular injury and tubulointerstitial damage and some comprehensive semiquantitative score systems have been related to different post-transplant outcomes in a non-consistent way [5]. For instance, studies analysing the relationship between the comprehensive histological score developed by Remuzzi *et al.* and some transplant outcomes such as delayed graft function (DGF), graft survival and renal function have reported conflicting results [6–8]. Also, this histological approach has several limitations: the kidney biopsy may not be representative of the entire kidney, there is an inherent subjectivity associated with the evaluation and, in a worse-case scenario, biopsies might compromise short- and long-term viability [5, 9]. The high rate of kidney grafts discarded due to procurement biopsy findings (up to 32% in 2020 in the USA) [10] and the doubts about the ability of these findings to predict graft outcomes have led to rethinking the usefulness of pre-transplant histological studies [5]. In machine-perfused grafts, some centres have used perfusion parameters such as renal resistance and arterial blood flow as tools to assess the kidney graft microcirculation before transplantation and as a discarding criterion. Although these perfusion parameters relate to DGF and graft survival in some studies [11, 12], their predictive values were low and should not be used alone to assess the viability of a potential kidney graft [12, 13]. Beyond histocompatibility, other clinical variables considered are the recipient's age, cardiovascular profile, proteinuria, estimated glomerular filtration rate (eGFR) and SCr. However, SCr is a late marker of kidney function influenced by age, gender, muscle mass and nutritional status.

While maximizing the number of available organs for transplantation is desirable, selecting the best kidney for each recipient is key. That being said, current evaluation methods of DD kidneys before transplantation are still suboptimal and a non-negligible number of organs considered not suitable for transplantation could have had good outcomes. Indeed, half of all the organs with a KDPI score >80% are not accepted for transplantation, yet they may benefit some older patients with CKD, and a high KDPI score does not automatically justify rejecting a DD kidney [14]. During 2019, >20% of the kidneys from DDs in the USA were rejected for transplantation, and even though the number of living and DD transplants increased during that year, the waiting list remained static [1]. Because of the challenging risk–benefit balance, eligibility criteria have evolved over time to include medium-quality organs [15, 16] with limitations. An expanded-criteria donor (ECD) is defined as any donor ≥60 years of age or those between 50 and 59 years of age having at least two of the following conditions: death due to cerebrovascular accident, kidney insufficiency defined as SCr >1.5 mg/dl and a history of arterial hypertension. Standard-criteria donors (SCDs) are brain death donors that do not fulfil expanded donor criteria [3]. While receiving an organ from a standard donor is always the preferred option, recipients of ECD kidney grafts from donors with high KDRI, donors >75 years of age or from standard-criteria DCDs showed better survival than patients remaining in the waiting list [14, 17–20]. On the other hand, the survival benefit of kidney grafts from DCDs with expanded criteria is more controversial. A recent analysis of Scientific Registry of Transplant Recipients (SRTR) data has observed that recipients of kidney grafts from DCDs ≥60 years of age have a 48% lower risk of death than patients who remained on the waiting list [21]. In contrast, the Dutch Organ Transplantation Registry reported that the mortality rate of elderly recipients receiving a kidney graft from a DCD ≥65 years of age was higher than for similarly old patients on the waiting list [22]. For an optimal evaluation of kidney grafts from DCDs and/or ECDs or elderly donors, new tools are needed that more precisely estimate the extent of acute and chronic damage present in the graft and predict its future viability and function.

Expanding possibilities by using biological indicators

Kidney transplantation has both short-term [e.g. DGF, acute kidney injury (AKI) or acute rejection] and long-term (defined as ≥12 months) complications. Meeting only the computational numerical thresholds does not ensure a successful transplantation. This highlights the need for novel parameters that aid in evaluating organs available for transplantation, complementing existing algorithms or even creating new scoring systems based on unique features that guide clinical decision making. Along this line, the 2020 Acute Dialysis Quality Initiative (ADQI) consensus developed recommendations on the use of new biomarkers to prevent and manage AKI in clinical practice, including stress markers (e.g. tissue inhibitor of metalloproteinases 2 (TIMP-2) and insulin-like growth factor–binding protein 7 (IGFBP-7) combination), functional markers (e.g. cystatin C) and damage biomarkers (e.g. neutrophil gelatinase-

associated lipocalin (NGAL) or liver-type fatty acid binding protein (L-FABP)) [23]. Assessment in biological fluids (liquid biopsy) of these and other molecular markers that reflect physiological or pathological changes specifically in the kidneys and, more generally, in the whole organism will expand the monitoring and translation capacity of biomarker-based tools.

Most transplantation studies to date have focused on short-term complications, mainly in the first 3 months post-transplantation, and recipient samples are usually assessed to explore the potential of novel molecules to diagnose and monitor graft injury or dysfunction [24, 25]. Few studies have used donor samples, and even fewer in the context of predicting long-term function (beyond 12 months). Very recently, a transcriptomic profile of pre-implantation biopsies was shown to predict 24-month graft function [26]. However, novel biological indicators are needed to more accurately rank available kidneys according to long-term outcome potential [27–29].

PREDICTING SHORT-, MEDIUM- AND LONG-TERM POST-TRANSPLANT KIDNEY FUNCTION

Despite the expanding criteria and the current standards for eligibility, non-desirable early and long-term outcomes still occur. Table 1 compiles the main findings in donor samples and their association with short-, medium- and long-term kidney function. Short-term complications include AKI, primary non-function (PNF), DGF and reduced graft function (RGF); medium-term refers to studies evaluating kidney function at 6 months; and long-term refers to those at ≥12 months.

Donor markers and short-term complications

In data from the Organ Procurement and Transplant Network [30], 7% of adult kidney transplant recipients experienced acute rejection within 1 year and graft survival was lower for kidneys biopsied at the time of transplantation. Inherent kidney transplantation risks may compromise optimal function. As an example, kidney transplantation is associated with ischaemia-reperfusion injury (IRI), which can drive oxidative stress and activate injury and inflammation cascades including apoptosis, necrosis, endothelial dysfunction and vasoconstriction [31]. IRI is inevitable and can lead to allograft failure and loss, poor long-term outcomes and other complications such as AKI, DGF and even acute rejection [25, 32]. DGF is one of the most common early complications requiring dialysis within the first week post-surgery. Clinically considered as a manifestation of AKI, its incidence has increased in recent years owing to the use of ECD and DCD kidneys.

The potential of traditional renal function markers in monitoring early post-transplant complications has been investigated in several studies. In one study analysing urine samples from 182 brainstem-dead multiorgan donors for biomarkers linked to AKI, high urinary levels of kidney injury molecule-1 (KIM-1) correlated with aberrant early function. When corrected by creatinine concentrations, KIM-1 and fractalkine were also higher than in the immediate function group [33]. In a similar study, the urinary concentrations of KIM-1, NGAL, interleukin-18 (IL-18), L-FABP and microalbumin were analysed in 1304 DDs associated with AKI. All five donor markers were higher in recipients with DGF, and NGAL and KIM-1 performed best when adjusted by donor, transport and recipient variables [34]. Urine NGAL (U-NGAL) levels in 95 deceased kidney donors were higher in cases with prolonged DGF compared with those with short DGF, without observing differences for serum NGAL. However, U-NGAL had weak predictive power for the onset of graft function and failed to predict DGF on the basis of receiver operating characteristics curve analysis [35]. In a large prospective cohort study, urinary analysis of 1301 donors and their 2435 recipients revealed higher levels of the repair phase protein YKL-40 (chitinase-3-like protein 1) in donors with AKI than in those without AKI. In adjusted analysis, higher YKL-40 concentration in donor urine were associated with a reduced risk of DGF and graft failure and higher eGFR at 6 months in the event of DGF [36].

Accumulating evidence suggests that the mechanisms of early post-transplant complications, such as AKI and DGF, involve the generation of reactive oxygen species, which initiate immune and complement cascade activation. In a prospective study of 469 DDs and their corresponding 902 kidney recipients, a significant association was observed between donor AKI and urinary C5a levels. Higher C5a values were also found in recipients who developed DGF, although an independent association between donor C5a levels and recipient DGF was observed only in non-AKI donors [37].

Predicting medium- and long-term kidney function by donor liquid biopsy

Predicting organ quality and post-transplant function is as desirable as it is challenging to achieve. Kidney function post-transplant beyond DGF or AKI is of considerable clinical relevance. Optimal kidney function may be compromised by chronic allograft dysfunction associated with progressive interstitial fibrosis–tubular atrophy, chronic calcineurin inhibitor nephrotoxicity or polyomavirus-associated nephropathy, among others. In this regard, gene expression, microRNAs (miRNAs) and proteins (recently reviewed in Quaglia *et al.* [27]) have been studied in recipients' biopsies, urine, plasma and endothelial cells.

While assessment tools based on mathematical algorithms, clinical variables and biopsy analyses can provide valuable data, limitations remain, particularly in medium- (6 months) and long-term (≥12 months) predictions [38]. Targeted molecular studies in donor samples have focused on kidney tubular injury–related proteins, particularly KIM-1, NGAL, IL-18 and L-FABP. In a prospective study of 94 DBDs, urinary levels of NGAL and L-FABP at admission and on the morning of surgery were higher in the reduced graft function (defined as delayed or slow graft function) group than in the immediate graft function group. NGAL was also associated with donor AKI, NGAL and L-FABP were negatively correlated with eGFR at 6 and 12 months post-transplantation and KIM-1 and L-FABP were associated with 1-year eGFR. When adjusting for variables such as donor and recipient age and gender, KDPI, cold ischaemia time, diabetes, acute rejection and the use of calcineurin inhibitors, among others, urinary L-FABP levels remained a significant predictor of 1-year graft function [39]. In another study, higher U-NGAL and L-FABP concentrations were associated with slightly lower 6-month eGFRs, but only in the absence of DGF and after adjustment for transport and recipient variables [34]. In contrast, in a cohort of 1298 kidney donors and 2430 transplant recipients evaluated at 4 years post-transplant, no association was found between urinary levels of microalbumin, NGAL, KIM-1, IL-18 and L-FABP in donors and post-transplant graft outcomes [40]. These findings indicate that analyses of traditional markers of kidney injury have suboptimal performance to predict long-term kidney function.

Table 1: Compilation of molecules measured in donor samples and main findings regarding their association with short-, medium- and long-term kidney function.

Measured molecules	Sample type	Sample time point	Clinical outcome	Groups	Cold ischaemia time (h) (*P*-value)	Main findings	Reference
Short-term (AKI, PNF, DGF, RGF)							
KIM-1, NGAL, IFN-γ, TNF-α, cystatin C, fractalkine and VEGF	Donor's urine	Before transplantation	Early graft dysfunction	Brainstem dead multi-organ donor (182). A: IF (133); B: aberrant function (49)	15.8 (A); 16.4 (B). (.399)	↑KIM-1, KIM-1/creatinine and fractalkine/creatinine in B versus A	[33]
C3a, C5a	Donor's urine	Before transplantation	Donor AKI and DGF	DD (469). Recipients (902)	14	↑C5a in AKI donors versus non-AKI donors, and in DGF versus non-DGF in recipients from non-AKI donors	[37]
YKL-40	Donor's urine	Before transplantation	Donor AKI, DGF	DD (1301): AKI (111); non-AKI (1190). Recipients (2435): DGF (756); non-DGF (1679)	14.4 (non-DGF); 17.2 (DGF). (<.001)	↑YKL-40 in AKI-donors versus non-AKI donors. Associates with reduced risk of DGF and graft failure	[36]
KIM-1, NGAL, IL-18, L-FABP, albuminuria	Donor's urine	Before transplantation	Donor AKI, DGF	DD (1304): AKI (112); non-AKI (1192). Recipients (2441): DGF (756); non-DGF (1685)	14.4 (non-DGF); 17.2 (DGF)	↑ KIM-1, NGAL, IL-18, L-FABP and albuminuria in AKI donors versus non-AKI donors and in DGF versus non-DGF. In fully adjusted analyses, only NGAL associated with DGF	[34]
NGAL, KIM-1, L-FABP	Donor's urine	At admission and in the morning before surgery	Donor AKI, RGF	DD (94). Recipients (109): RGF (22); IGF (87)	2.8 (IGF); 3.6 (RGF). (.045)	↑ NGAL and L-FABP in RGF versus IGF. NGAL associates with donor AKI	[39]
Mitochondrial DNA (dmtDNA)	Donor's plasma	Before transplantation	Donor AKI, RGF, DGF, PNF	DCD (75): AKI (35); non-AKI (40). DCD (141): IGF (101); SGF (23); DGF (13); PNF (4)	2 (IGF); 5 (SGF); 8 (DGF); 4.25 (PNF) (.005)	dmtDNA correlates with donor AKI and post-transplant RGF	[44]
Medium-term (6 months) and long-term (≥12 months)							
YKL-40	Donor's urine	Before transplantation	Kidney function at 6-months post-transplantation	DD (1301): AKI (111); non-AKI (1190). Recipients (2435): DGF (756); non-DGF (1679)	14.4 (non-DGF); 17.2 (DGF). (<.001)	If DGF present, YKL-40 associates with higher eGFR at 6 months	[36]
Urinary EVs proteome	Donor's and recipient's urine	Donors (1 day before transplantation). Recipients (immediately after transplantation, 24 hours, 4 weeks, 1 year)	Kidney function 1-year after transplantation	Screening cohort: LD (22); Recipients (22). Validation cohort: LD (22)	2.9	64 EVs-proteins correlate with 12-month eGFR. ↑ PCK2-EVs one day after transplantation positively correlate with eGFR at 6 and 12 months	[54]
Tubular maximum re-absorption capacity of phosphate (TmP-GFR)	Donor's and recipient's urine and plasma	Donors: 6 ± 7 months pre- and 2 months post-donation. Recipients: 12 months post-transplantation	Recipient GFR 1 year after transplantation	LD (165). Recipients	2.55	↑ Donor pre-donation TmP-GFR: ↑ 1-year GFR	[41]
NGAL, KIM-1, L-FABP	Donor's urine	At admission and in the morning before surgery	Kidney function 1 year after transplantation	DD (94). Recipients (109): RGF (22); IGF (87)	2.8 (IGF); 3.6 (RGF). (.045)	NGAL and L-FABP negatively correlate with eGFR at 6 and 12 months. KIM-1 and L-FABP significantly associate with 1-year eGFR	[39]
NGAL, L-FABP	Donor's urine	Before transplantation	Donor AKI and DGF	DD (1304): AKI (112); non-AKI (1192). Recipients (2441): DGF (756); non-DGF (1685)	14.4 (non-DGF); 17.2 (DGF)	↑ NGAL and L-FABP associate with slightly lower 6-months eGFR only in absence of DGF and if adjusted for transport and recipient variables	[34]
Mitochondrial DNA (dmtDNA)	Donor's plasma	Before transplantation	Donor AKI, RGF, DGF, PNF	DCD (75): AKI (35); non-AKI (40). DCD (141): IGF (101); SGF (23); DGF (13); PNF (4)	2 (IGF); 5 (SGF); 8 (DGF); 4.25 (PNF) (.005)	dmtDNA correlates with 6-months allograft function and <1-year graft survival	[44]

In a different approach, the pre-donation tubular reabsorption capacity of phosphate, used as a subclinical tubular function marker, was associated with eGFR at 1 year, although the study was limited to living donors [41]. This study identifies tubular function assessment as an early source of information on subsequent loss of GFR, even when GFR is still preserved. Thus specific changes in tubular function in living donors could predict the risk of lower GFR after transplantation not identified by pre-transplant assessment of donor glomerular function, supporting the study of other makers of tubular function or injury. In agreement with this finding, we recently demonstrated that early subclinical kidney injury resulting in defective tubular reabsorption is reflected in protein and metabolic alterations in urine that identified normoalbuminuric subjects within the high-normal range (albumin:creatinine ratio 10–30 mg/g) that have a higher risk of cardiovascular disease and renal function decline [42, 43]. The tricarboxylic acid cycle was identified as a key biological pathway in urine of high-risk normoalbuminuric subjects and it is also altered in pre-implantation biopsies taken from kidneys that displayed low function 24 months post-transplantation [26]. In a similar manner, the levels of plasma donor mitochondrial DNA (dmtDNA), a surrogate marker of tissue damage, correlated with donor AKI and DGF, as well as with 6-month allograft function and 1-year graft survival [44]. These studies illustrate and support the need for new molecular predictors of post-transplant function, which may define novel pathways and targets of interest. Accordingly, the integration of existing tools with novel techniques and biomarkers may improve prediction performance.

Extracellular vesicles as a new source of pre-transplant donor information

Extracellular vesicles (EVs) are cell-derived membranous structures released into the extracellular space that have multiple physiological roles. Beyond their function in cellular (waste) disposal of toxic materials, EVs participate in immune modulation, cellular regeneration and activation of signalling pathways in target cells by transferring their cargo of messenger RNAs, miRNAs, proteins and metabolites, which serve as messengers in cell-to-cell communication. EVs are released from a wide variety of cellular types and can be detected in biofluids under physiological and pathological conditions. In the kidney, EVs mediate intercellular communication within the nephron involving intraglomerular, glomerular–tubular and tubular–interstitial communication [45, 46], with critical roles as immune modulators in kidney transplantation [47]. In a rat model of unilateral kidney IRI, EVs obtained from mesenchymal stem cells protected against both acute AKI and later chronic disease when administered immediately after injury [48]. EVs may also be therapeutic agents as drug vehicles, with a robust capacity to suppress renal inflammation and fibrosis when loaded with dexamethasone in murine models of nephropathy [49]. Beyond their utility as potential treatments, EVs have demonstrated their value as a source of biomarkers in kidney diseases when isolated from urine, by directly reflecting pathological changes taking place in the kidney in an accessible and non-invasive manner [50, 51]. Indeed, a urine-based device was recently developed to detect kidney transplant rejection by analysing EVs released from immune cells as a surrogate marker for inflammation [52]. Higher levels of $CD3^+$ EVs were found in transplant patients undergoing cellular rejection, and receiver operating characteristics curve analysis revealed a sensitivity of 92.8% and specificity of 87.5%, illustrating its diagnostic potential. A recent study provided proof-of-principle that EVs can be detected in donor kidney preservation fluid [53]. In 11 perfusate fluids from three different donor types (DCDs, DBDs and LDs), the relative abundance of miRNAs in EVs could distinguish DBDs from DCDs and living donors. Analysis of EV miRNAs in a complementary cohort of 16 perfusate fluids from DCDs with known short-term outcome revealed no significant correlation with DGF, however, the EV miRNA profiles were associated with graft function in the first 7 days post-transplantation. Finally, in a cohort of 22 LDs and their 22 recipients, urinary EVs were isolated from donors before transplant and in their corresponding recipients after surgery, at 1 day, 4 weeks and 1 year after transplantation for proteomics analysis. A panel of 64 proteins correlated with eGFR at 12 months and the levels of phosphoenolpyruvate carboxykinase [GTP], mitochondrial (PCK2) in EVs measured 1-day after transplant positively correlated with eGFR at 6 and 12 months, representing a potential predictor of long-term kidney function [54].

Fig. 1 summarizes the clinical variables, mathematical algorithms and molecular markers identified in donor samples for optimal donor–recipient matching.

Future perspectives: control of variables, omics strategies and multicentric studies

In the decision-making process, molecular features can provide extra information and thus complement clinical data. Some donor markers may represent an adaptive response to prior kidney graft injury, as exemplified by YKL-40 [20]. Other biomarkers may reflect a biological response triggered by and potentially involved in kidney injury. In this sense, urinary C5a levels differed between DDs and healthy volunteers (which may represent living donors) and between DCDs and DBDs, revealing differences in complement activation and baseline injury in donors [37]. Beyond their prognostic biomarker role, these markers may identify active molecular mechanisms of kidney injury that may guide the initiation, adaptation and continuation of specifically targeted therapies that could even be initiated in the donor and continued in the recipient.

The identification, characterization and implementation of these novel molecular features are as challenging as they are necessary, as new approaches are needed. The most common strategy for identifying biomarkers of kidney function is targeted analysis of known indicators of glomerular and tubular damage or of known biological processes involved in kidney transplantation, mainly IRI and oxidative stress. So far, existing markers have little value in predicting allograft function at 6–12 months, or they still need extensive validation. Genomics, transcriptomics, proteomics and metabolomics are non-biased approaches that do not exclude any target molecule in advance and do not focus on any known mechanism of action. These omics techniques can analyse many thousands of molecules simultaneously to identify the major significant biological differences between conditions, which can be further confirmed in larger independent cohorts, by using analytical techniques different from those used in the discovery phase. Very recently, a transcriptomic profile of preimplantation biopsies predicted 24-month graft function [26]. The translation of this and other promising molecular changes to liquid biopsies could provide an accessible source of information associated with improved patient safety and lower costs. However, the clinical use of donor liquid biopsy in kidney transplantation has not yet been implemented. The path from bench to bedside is multidisciplinary and multistage [55]. Pre-analytical and analytical

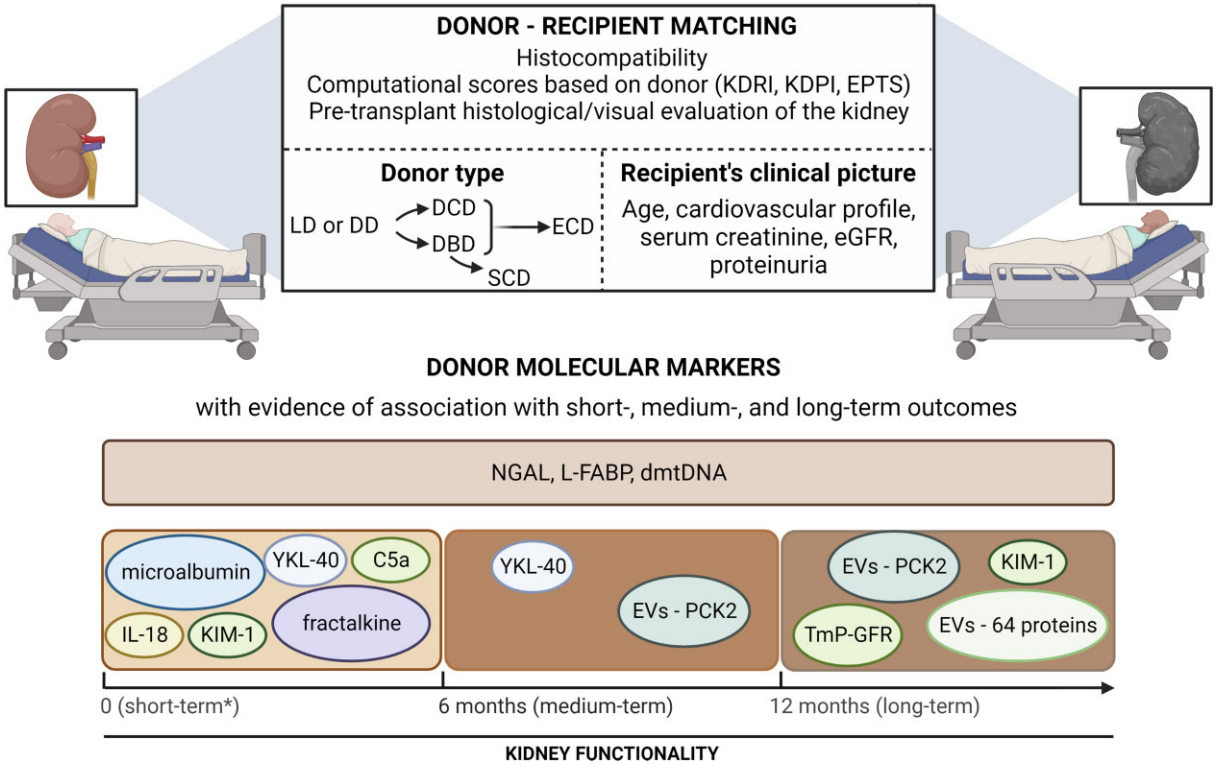

Figure 1: Schematic view of clinical variables and mathematical algorithms used in pre-transplant kidney evaluation for best donor–recipient matching. Molecular markers identified in donor samples are summarized according to their association with short-, medium- or long-term outcomes. *Short-term complications including AKI, PNF, DGF and RGF mainly occur within the first 3 months. Created with BioRender.com.

Table 2: Keynotes for a multicentric study to identify novel markers of long-term kidney function.

Variables control	
External (to be assumed)	To be standardized across centres
Distance donor-receptor	Sample collection and preservation time lapses
Donor type—DCD/DBD	Organ preservation
Multi-organ receptor	Biopsy (yes/no)
Organ procurement and transplantation procedure	Adjustment variables (donor/receptor)
Clinical history 'of damage'—baseline donor/receptor characteristics	
Targeted biological processes and known markers of kidney dysfunction	
Ischaemia-reperfusion injury	
Oxidative stress	
Complement activation/coagulation pathway	
Glomerular injury	
Tubular dysfunction	
Untargeted systems biology	
Non-biased approach without pre-selection of molecular variables to be studied	

factors may limit clinical translation if not properly controlled and the complexity of omics requires specialists technically able to perform the analysis and interpret the results to yield robust and reproducible markers that successfully overcome subsequent validation stages and provide enough sensitivity to ensure flawless adaptation to point of care or clinical analyses laboratories. Even so, biomarkers identified and validated in liquid biopsy are expected to, at the very least, complement current algorithms for optimal matching of donors and receptors.

Multicentric and multinational studies will be needed to confirm the added value of translating the newly discovered markers to daily clinical practice in patients of diverse racial and ethnic backgrounds, gender and age. In these validation studies, it is imperative to adequately control for modifiable variables associated with the identification of diagnostic and prognostic

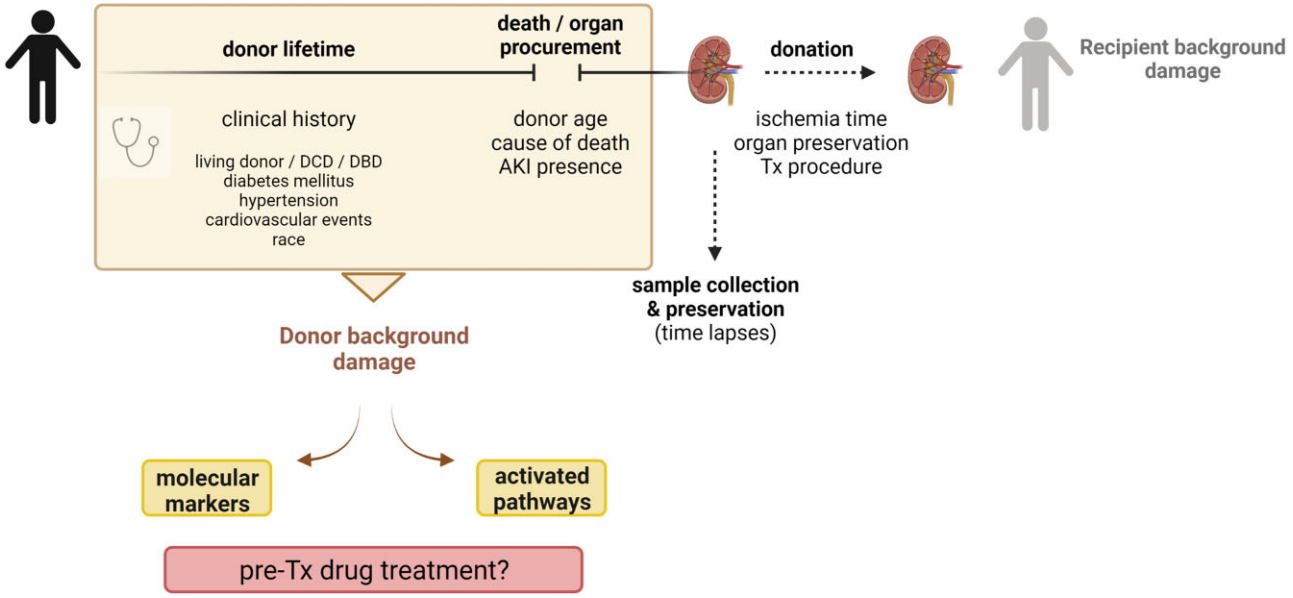

Figure 2: External and modifiable variables conditioning the identification and translation of newly discovered markers to clinical practice. External variables include the donor and recipient background damage. Modifiable variables include sample collection and pretreatment, graft preservation and donation procedure. Created with BioRender.com.

biomarkers (Table 2). However, some external variables cannot be fully controlled. This is the case for the distance between donor and recipient, the post-mortem times, the donor type (cause of death), the possibility of multi-organ donation to one recipient, the procedure itself and the 'background damage' of donor and recipient, only partly evidenced by clinical data (Fig. 2).

Modifiable variables should be controlled and standardized across centres to avoid significant variability that may obscure differences in marker levels (Fig. 2). This is the case for sample collection methods (including time points), pretreatment protocols, subcellular/subproteome fractionation (if applicable) and storage. Kidney graft preservation and reperfusion procedures may also affect the robustness of markers. Whenever possible, candidate biomarkers should be adjusted by relevant cofactors to properly assess their performance during clinical translation. These typically include donor SCr, albuminuria, age, weight, race and ethnicity, history of hypertension and diabetes, stroke as the cause of death, hepatitis C, DCD, KDRI/KDPI and cold ischaemia time, as well as recipient age, race and ethnicity, diabetes as the cause of CKD, body mass index, dialysis, previous kidney transplantation, need for pre-transplant blood transfusion, use of calcineurin inhibitors and number of human leucocyte antigen mismatches.

CONCLUSION

The donor biological samples represent an underestimated source of information on graft kidney outcomes based on the relatively small number of studies available. Their main value lies in their potential predictive capacity, which may contribute to clinical decision making when added to the macroscopic appearance of the kidney, mathematical scales or comorbidities of the donor and the recipient. The analysis of biomarkers in donor liquid biopsies may improve donor–recipient matching by refining the prediction of kidney function in the short-, medium- and long-term.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

FUNDING

This work was supported by Fundacion Mutua Madrileña, Instituto de Salud Carlos III (RICORS2040-RD21/0005/0001) FEDER funds, Fundacion SENEFRO/SEN and Comunidad Autónoma de Madrid (CAM) (2018-T2/BMD-11561, PEJD-2020-AI/BMD-17899).

CONFLICT OF INTEREST STATEMENT

A.O. has received grants from Sanofi and consultancy or speaker fees or travel support from Advicciene, Astellas, AstraZeneca, Amicus, Amgen, Fresenius Medical Care, GlaxoSmithKline, Bayer, Sanofi-Genzyme, Menarini, Mundipharma, Kyowa Kirin, Alexion, Freeline, Idorsia, Chiesi, Otsuka, Novo Nordisk, Sysmex and Vifor Fresenius Medical Care Renal Pharma and is Director of the Catedra Mundipharma-UAM of diabetic kidney disease and the Catedra AstraZeneca-UAM of chronic kidney disease and electrolytes. He is also the former *CKJ* Editor-in-Chief. The rest of the authors have no conflicts of interest to disclose. The results presented in this article have not been published previously in whole or part.

DATA AVAILABILITY STATEMENT

The data are included in the article.

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