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POINT OF VIEW

Biomarkers as diagnostic tests for delayed graft function in kidney transplantation

Christina Lai[1,2], Seow Yeing Yee[3], Tracey Ying[1,2] & Steve Chadban[1,2]

1 Renal Medicine, Royal Prince Alfred Hospital, Sydney, NSW, Australia
2 Kidney Node, Charles Perkins Centre, The University of Sydney, Sydney, NSW, Australia
3 Nephrology Department, Hospital Kuala Lumpur, Kuala Lumpur, Malaysia

Correspondence
Professor Steve Chadban BMed (hons), FRACP, PhD, FAHMS, Department of Renal Medicine, Kidney Centre, Professor Marie Bashir Centre, Royal Prince Alfred Hospital, Missenden Rd, Camperdown, NSW 2050 Australia.
Tel.: +612 9515 1713;
fax: +612 9515 1744;
e-mail:
Steve.Chadban@health.nsw.gov.au

SUMMARY

Delayed graft function (DGF) after kidney transplantation is associated with inferior outcomes and higher healthcare costs. DGF is currently defined as the requirement for dialysis within seven days post-transplant; however, this definition is subjective and nonspecific. Novel biomarkers have potential to improve objectivity and enable earlier diagnosis of DGF. We reviewed the literature to describe the range of novel biomarkers previously studied to predict DGF. We identified marked heterogeneity and low reporting quality of published studies. Among the novel biomarkers, serum NGAL had the greatest potential as a biomarker to predict DGF, but requires further assessment and validation through larger scale studies of diagnostic test performance. Given inadequacies in the dialysis-based definition, coupled with the high incidence and impact of DGF, such studies should be pursued.

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Introduction

Delayed graft function (DGF) is a common postoperative complication of kidney transplantation. DGF is characterized by suboptimal allograft functioning as a result of ischaemia-reperfusion injury incurred during retrieval, storage, and transplantation surgery. DGF is very uncommon in living donor transplantation, but frequent among recipients of kidneys from deceased donors, with incidence reported at 20-50% worldwide [1]. With increasing use of kidneys from expanded-criteria donors and donors after cardiac death, the incidence of DGF is growing and its diagnosis and management is a major unmet need in contemporary kidney transplantation [2].

DGF has a significant impact on transplant outcomes and overall economic costs, being associated with increased length of hospital stay, increased risks of rejection, and inferior long-term graft survival [1–4]. Efforts to reduce the incidence and severity of DGF are a current focus of clinical trial activities [5–8]. Published literature includes studies of both donor- and recipient-directed strategies. Donor pharmacological treatments, such as dopamine infusion [9], and recipient interventions, including complement inhibition [10] and erythropoietin [11], have produced little or no difference in DGF rates or improvement in the graft outcome. On the other hand, T cell depletion therapy in recipients [12], induction of hypothermia in deceased donors, and the use of hypothermic machine perfusion have yielded significant reductions in DGF rates and improved graft survival and outcomes at 1 and 3 years [13–16]. As DGF is an intense area of ongoing research, having an objective, simple, and reproducible diagnostic test for DGF has never been more important.

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Multiple definitions and methods to assess early graft dysfunction have been published in the literature. Between 1984 and 2007, Yarlagadda *et al.* identified 18 different definitions used in the literature [17]. These definitions were divided into dialysis-based, creatinine-based, or a combination of both. The ongoing debate on definition reflects the complexity of the underlying pathophysiology of DGF. The current "gold" or "reference" standard and most frequently used definition of DGF is the requirement for at least one dialysis treatment in the first week after kidney transplantation, as defined by the United Network for Organ Sharing (UNOS) and U.S. Food and Drug Administration (FDA) [18,19]. The diagnosis of DGF can be delayed for a week and the decision for patients to undergo dialysis is based on the subjective opinion of the treating physician, who may include hyperkalaemia, acidosis, and volume status as indications for dialysis, none of which are specific to DGF. This lack of objectivity in the reference standard makes it debatable as to whether it can correctly identify patients with DGF or accurately predict clinical outcomes.

In seeking to compare potential new biomarker-based tests with the current reference standard test for DGF, such limitations of the reference standard must be borne in mind. A caveat of this is that in conducting trials comparing biomarkers with the reference standard, the aim is actually to determine the ability of the biomarker to predict requirement for dialysis within 7 days of transplantation, rather than to truly diagnose ischaemia-reperfusion injury.

Ideally, we need a biomarker that can diagnose ischaemia-reperfusion injury with greater objectivity, timeliness, and gradation of severity than the dialysis-based diagnosis. This biomarker should have utility in both clinical and research settings. As a first step toward this goal, demonstration that a biomarker can predict DGF, as defined by requirement for dialysis, must be demonstrated and prospectively validated.

Biomarkers other than creatinine have been proposed for the diagnosis of DGF in clinical settings and clinical trials. These include neutrophil gelatinase-associated lipocalin (NGAL), cystatin C, liver-type fatty acid binding proteins, and interleukin 18. These biomarkers have also been evaluated in other clinical settings associated with acute kidney injury, such as postcardiothoracic or intra-abdominal surgery [20–22]. Among studies of novel biomarkers for DGF, the most commonly evaluated were NGAL and cystatin C. We performed a review of published literature, initially seeking studies comparing novel biomarkers with the "gold standard" dialysis-based definition of DGF (Table 1). Secondarily, we reviewed studies comparing biomarker-based definitions of DGF with nongold standard definitions (Table 2) or no comparator (Table 3).

Neutrophil gelatinase-associated lipocalin (NGAL)

Measurements of NGAL in blood or urine show the greatest potential as an alternative to the reference standard. NGAL is expressed in renal tubular epithelial cells during inflammation and after exposure to nephrotoxic or ischaemic insults [23]. After kidney injury, NGAL is released to serum and urine and has been extensively assessed as a biomarker for acute kidney injury. After transplantation, NGAL is detectable at 6 h in urine and 12 h in plasma [24,25]. However, NGAL has not been adopted into widespread clinical practice.

Studies that evaluated serum NGAL have reported persistent elevation of NGAL in DGF, whereas significant reductions were evident by 24 h post-transplant in recipients with immediate graft function. Serum NGAL performed well as a biomarker at day 1, with an area under the curve (AUC) of 0.94 and 0.97, reported by Cantaluppi *et al.* and Bataille *et al.*, respectively [25,26]. However, we need to take into consideration the effect of immunosuppression on serum NGAL concentrations. In particular, Cantaluppi *et al.* [26] measured NGAL before and after tacrolimus was introduced and found a significant increase in NGAL levels, suggesting the rise in NGAL may be caused by drug toxicity rather than DGF.

Among the tested urine biomarkers, urine NGAL was superior to others with the highest reported sensitivity and specificity. The reported sensitivity of urine NGAL varied from 77% to 100% and specificity from 62% to 95% [24,27–30]. In particular, Fonseca *et al.* [27] and Nikolov *et al.* [31] demonstrated a nearly flawless AUC (0.99 and 0.91, respectively) on day 4 post-transplant. Nevertheless, urinary NGAL may be unreliable in the post-transplant period because of the presence of haematuria, as assay interference by haemoglobin is well recognized [32]. The usefulness of a urine test for DGF is also limited as the most severely affected patients are typically anuric post-transplant.

Cystatin C

Serum cystatin C has also been investigated widely in the literature as an alternative marker to creatinine as a measure of kidney function in the setting of acute and

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Table 1. Studies on novel biomarkers.

Year	References	Study Design	Subjects	Prevalence of DGF (%)	Sample	Biomarkers
2018	Kaminska *et al*. [50]	Retrospective	33	18	Biopsy	MMP9, TGFβ-1
	Nikolov *et al*. [31]	Prospective	50	NR	Urine	NGAL
2017	Capelli *et al*. [51]	Prospective	43	42	Urine	NGAL
	Oda *et al*. [52]	Retrospective	46	76	Biopsy	HIF-1α
	Trailin *et al*. [53]	Retrospective	45	20	Blood	sCD30
	Williams *et al*. [54]	Retrospective	52	40	Urine	C4BPA, GUC2A, IGSF8, SAMP
	Yang *et al*. [30]	Prospective	72	31	Urine	NGAL, TIMP-2, IGFBP-7
2015	Borst *et al*. [49]	Prospective	113	5	Blood	IL-8, S100A8
	Cantaluppi *et al*. [26]	Prospective	50	28	Blood	NGAL
	Fonseca *et al*. [55]	Prospective	40	45	Blood	Creatinine, cystatin C, MDA, Leptin
	Fonseca *et al*. [56]	Prospective	40	45	Blood	Leptin
	Pianta *et al*. [57]	Prospective	56	39	Urine	TIMP-2, VEGF-A, IGFBP-7
	Van Den Akker *et al*. [58]	Prospective	20	85	Blood	NGAL
2014	Buemi *et al*. [59]	Prospective	97	25	Blood	NGAL
	Fonseca *et al*. [60]	Prospective	40	45	Blood	MDA
	Kawai *et al*. [61]	Retrospective	67	40	Blood	L-FABP
	Wohlfahrtova *et al*. [62]	Prospective	38	32	Biopsy	NTN1
	Zaza *et al*. [63]	Retrospective	24	50	Blood	Karyopherin
2013	Fonseca *et al*. [27]	Prospective	40	45	Urine	NGAL
	Kanter *et al*. [28]	Prospective	38	40	Urine	NGAL
	Welberry, *et al*. [38]	Prospective	138	30	Blood	Cystatin C, ACY-1
2012	Andrade-Oliviera *et al*. [64]	Retrospective	91	51	Urine	MyD88, TLR-4
	Lee *et al*. [65]	Retrospective	59	24	Blood	NGAL, IL-18, Creatinine
	Mahdavi-Mazdeh *et al*. [66]	Prospective	33	18	Blood	NGAL
2011	Bataille *et al*. [25]	Prospective	41	37	Blood	NGAL
	Hall *et al*. [37]	Prospective	78	33	Blood	Cystatin C
	Hall *et al*. [67]	Prospective	91	36	Urine	Cystatin C, Creatinine
2010	Dolegowska *et al*. [68]	Retrospective	69	35	Blood	Xanthine Oxidoreductase
	Hall *et al*. [24]	Prospective	91	37	Urine	IL-18, NGAL
	Sadeghi *et al*. [69]	Retrospective	112	35	Blood	sIL-1RA, sIL-6R, IL-10, Neopterin
	Kotsch *et al*. [39]	Retrospective	89	21	Biopsy	Transcriptome
	Kusaka *et al*. [70]	Prospective	34	29	Blood	TIMP-1
2009	Dolegowska *et al*. [71]	Retrospective	69	35	Blood	TxB2
	Lebkowska *et al*. [33]	Prospective	41	10	Blood	NGAL, Cystatin C
2008	Kusaka *et al*. [72]	Prospective	16	31	Blood	NGAL
	Mueller *et al*. [73]	Prospective	87	13	Biopsy	Transcriptome
2006	Parikh *et al*. [29]	Prospective	53	19	Urine	IL-18, NGAL

NGAL, neutrophil gelatinase-associated lipocalin; TIMP-2, tissue inhibitor of metalloproteinases-2; IGFBP-7, insulin-like growth factor-binding protein-7; HIF-1α, hypoxia-inducible factor 1α; MDA, malondialdehyde; L-FABP, liver fatty acid-binding protein; NTN1, netrin-1; ACY-1, aminoacylase-1; TLR-4, toll-like receptor-4; TxB2, thromboxane A2; MMP9, matrix metallopeptidase; TGFβ-1, transforming growth factor beta 1; sCD30, soluble CD30; C4BPA, C4b-binding protein alpha; GUC2A, Guanylin; IGSF8, Immunoglobulin superfamily member 8; SAMP, Serum amyloid P-component; VEGF-A, vascular endothelial growth factor A; IL-18, interleukin-18; sIL-1RA, soluble interleukin-1 receptor antagonist; sIL-6R, soluble interleukin-6 receptor; IL-10, interleukin-10, and IL-8, interleukin-8.

chronic injury. Serum cystatin C has a shorter half-life than creatinine and is freely filtered by glomeruli, then reabsorbed, and catabolized by the tubular cells, making it a potentially more specific marker of glomerular filtration rate [33,34]. Two recent meta-analyses evaluated the predictive value of serum cystatin C for acute kidney injuries and found that it was a more sensitive marker than serum creatinine [35,36]. However, in our review, we found no convincing data, albeit from only two studies, to suggest the superiority of cystatin C over other biomarkers [37,38]. Lebkowska *et al.* [33] found the kinetics of cystatin C to be inferior to serum NGAL, reporting a delayed fall in cystatin C in comparison to serum NGAL in recipients with immediate graft

Table 2. Studies with alternate DGF definitions.

References	Definition of DGF	Timepoint of test	Subjects	Prevalence of DGF (%)	Tests	Sensitivity/ Specificity	PPV	NPV	OR	Area under ROC
Serum										
Lacquaniti *et al.* [74]	The need for dialysis within the first week after transplantation, or when serum Cr increased, remained unchanged, or decreased by less than 10% per day immediately after surgery.	Pretransplant and D1 post-transplant	124	40	NGAL	NR	NR	NR	NR	0.67
Hollmen *et al.* [75]	Plasma Cr concentration >500μmol/l throughout the first week, or oliguria of less than 1000ml/24 h for more than two days, or more than one dialysis session needed during the first week	Pretransplant, D1 and D14 post-transplant	176	38	NGAL day 1	91/83	NR	NR	NR	0.91
Kusaka *et al.* [47]	The need for dialysis within first few weeks after transplantation	Pretransplant, D1, D2, D3 and D5 post-transplant	67	40	NGAL day 1 NGAL day 2 NGAL day 3 NGAL day 5	91/97 86/90 91/93 91/97	NR NR NR NR	NR NR NR NR	NR NR NR NR	0.99 0.94 0.98 0.97
Yang *et al.* [76]	The need for dialysis in the first post-transplant week, as the failure of serum Cr to decrease by >10% on the first three postoperative days or as serum Cr levels >250μmol/L on postoperative day 5 in the presence of scintigraphic evidence of acute tubular necrosis.	Immediately pretransplant	172	31	Anti-LG3 antibodies	NR	NR	NR	NR	NR
Shahbazian *et al.* [77]	One of the following conditions: (1) need for dialysis within the first week after the operation, (2) serum Cr level of 2.5mg/dL or more and the peak activity time of more than 6.5 min on renal isotope scan performed on day 5, and (3) less than 10% decrement in the serum level of Cr within the first 24 h after the transplantation.	Intraoperatively before and after anastomosis	47	17	Hypoxanthine Xanthine	NR NR	NR NR	NR NR	NR NR	NR NR
Alachkar *et al.* [78]	The requirement for dialysis in the first week post-transplantation or serum Cr level of >3mg/dL on day 5 post-transplantation.	48 h post-transplant	61	23	IFN-α2 IL-3 IL-16 MCP-3 SCF SCGF-β	69/58 69/76 62/88 62/74 92/74 58/88	NR NR NR NR NR NR	NR NR NR NR NR NR	NR NR NR NR NR NR	0.69 0.73 0.74 0.71 0.88 0.72

Table 2. Continued.

References	Definition of DGF	Timepoint of test	Subjects	Prevalence of DGF (%)	Tests	Sensitivity/ Specificity	PPV	NPV	OR	Area under ROC
Zmonarski *et al.* [79]	The need for at least 1 haemodialysis since the third day after kidney transplantation	On recruitment and follow up (exact timing varies)	143	29	mRNA TLR4 expression	NR	NR	NR	NR	NR
Vinot *et al.* [80]	DGF grafts exhibited delayed function and marked depression of the GFR, measured as the clearance of iothalamate on postoperative day 3.	Within 3 h after graft reperfusion and day 3 postop	23	48	ANP	NR	NR	NR	NR	NR
Urine										
Lacquaniti *et al.* [74]	The need for dialysis within the first week after transplantation, or when serum Cr increased, remained unchanged, or decreased by less than 10% per day immediately after surgery.	Pretransplant and D1 post-transplant	124	40	NGAL	96/92	NR	NR	NR	0.98
Yadav *et al.* [81]	7th post-transplant day serum Cr >2.5mg/dL, and in need of one dialysis within the first post-transplant week	0,6,12,18,24,48 h post-transplant	56	16	KIM-1 18 h post-transplant	89.9/100	NR	NR	NR	0.99
Alvarez *et al.* [82]	Whenever haemodialysis was required in the postkidney transplant period	24, 48, 72 h post-transplant	15	20	NGAL	NR	NR	NR	NR	NR
Salamzadeh *et al.* [83]	The need for dialysis within the first week after transplantation, or when serum Cr increased, remained unchanged, or decreased by less than 10% per day immediately after surgery.	D1 and D3 post-transplant	68	16	NGAL IL-18	NR NR	NR NR	NR NR	NR NR	NR NR
Hollmen *et al.* [84]	oliguria <1L every 24 h for 42 days, or plasma Cr concentration >500μmol/l throughout the first week, or >1 dialysis session needed during the first week	D1 postop	176	40	NGAL	68/73	NR	NR	5.4	0.75
Waikar *et al.* [85]	A < 10% decrease in serum Cr per day for the first 3 days after transplantation.	Immediately post-transplant, and serial daily collections	12	17	Cr KIM-1	NR NR	NR NR	NR NR	NR NR	NR NR
Alachkar *et al.* [78]	The requirement for dialysis in the first week post-transplantation or serum Cr level of >3mg/dL on day 5 post-transplantation.	48 h post-transplant	61	23	IL-2Rα	86/67	NR	NR	NR	0.23

Table 2. Continued.

References	Definition of DGF	Timepoint of test	Subjects	Prevalence of DGF (%)	Tests	Sensitivity/ Specificity	PPV	NPV	OR	Area under ROC
Biopsy										
Bank *et al.* [86]	The serum Cr level increased, remained unchanged, or decreased by less than 10% per day immediately after surgery during three consecutive days for more than one week.	D10 post-transplant	64	77	KIM-1 staining	NR	NR	NR	NR	NR
					NGAL staining	NR	NR	NR	NR	NR
Guerrieri *et al.* [87]	The need for dialysis	D7 post-transplant	34	68	IFNA1	NR	NR	NR	NR	NR
					IL-1R1	NR	NR	NR	NR	NR
					HMOX-1	NR	NR	NR	NR	NR
					TGF-β	NR	NR	NR	NR	NR
Combination										
Schmitt *et al.* [88]	Low diuresis (<1000ml excretion/day) despite forced medical stimulation and/or need for renal replacement therapy up to 7 days following kidney transplantation	Pretransplant, immediately after the end of the surgical procedure, day 1, 3, 5, 7, and 10.	91	21	Plasmatic total K18 + clinical scoring system	NR	NR	NR	NR	d0: 0.81; d1: 0.86
					Urinary TIMP-2 and IGFBP7 + clinical scoring system	NR	NR	NR	NR	d0: 0.65; d1: 0.74
					Plasmatic total K18 + urinary TIMP-2 and IGFBP7 + clinical scoring system	NR	NR	NR	NR	d0: 0.74; d1: 0.86
Cui *et al.* [89]	The need for dialysis during the first week after kidney transplantation or a decrease in serum Cr within 48 of >=10%	4, 12, 24, 48, 72 h post-transplant; serum Cr taken at 24, 48, 72 h post-transplant	129	16	Urine NGAL + Urine IL-18	70/90.6	70	90.6	NR	0.844
					Urine IL-18 + Serum Cr	90/78.1	56.3	96.2	NR	0.916
					Urine NGAL + Serum Cr	100/90.5	83.3	100	NR	0.978
					Urine NGAL + Urine IL-18 + Serum Cr	100/96.9	90.9	100	NR	0.984

PPV, positive predictive value; NPV, negative predictive value; OR, odds ratio; area under ROC, area under receiver operative characteristics curve; Cr, creatinine; NR, not reported; NGAL, Neutrophil gelatinase-associated lipocalin; IFN-α2, interferon alpha-2; IL-3, interleukin-3; IL-16, interleukin-16; MCP-3, monocyte chemotactic protein-3; SCF, stem cell factor; SCGF-β, stem cell growth factor-beta; mRNA TLR4, mRNA toll-like receptor 4; GFR, glomerular filtration rate; ANP, atrial natriuretic peptide; KIM-1, kidney injury molecule-1; IL-18, interleukin-18; IL-2Rα, interleukin-2 receptor-alpha; IFNA1, interferon alpha-1; IL-1R1, interleukin 1 receptor type 1; HMOX-1, heme oxgenase-1; TGF-β, transforming growth factor-beta; TIMP-2, tissue inhibitor of metalloproteinases-2; and IGFBP7, insulin-like growth factor binding protein-7.

Table 3. Studies with no DGF definition.

References	Tests	Timepoint of test	Subjects	Prevalence of DGF (%)	Sensitivity/ Specificity	PPV	NPV	OR	Area under ROC
Serum									
Caban *et al.* [90]	IL-6	Preop, 4 h and day 4	20	25	NR	NR	NR	NR	NR
	TNF-α				NR	NR	NR	NR	NR
	IL-1β				NR	NR	NR	NR	NR
	CRP				NR	NR	NR	NR	NR
Malyszko *et al.* [91, 92]	Hepcidin	Preop, day 1, 3, 6, and 10	31	NR	NR	67	97	NR	0.78
Urine									
Kwiatkowska *et al.* [92]	IL-8	Day 1 and 14	87	NR	NR	NR	NR	NR	NR
Qurashi *et al.* [93]	NGAL	6 h post-transplant	67	16	NR	NR	NR	NR	NR
Biopsy									
Kusaka *et al.* [94]	Multiple gene expression in graft biopsy	1-h postperfusion	29	NR	NR	NR	NR	NR	NR
Mishra *et al.* [95]	NGAL staining	1-h postperfusion	25	16	NR	NR	NR	NR	NR
Toronyi *et al.* [96]	Necrotic/apoptotic activity in graft biopsy	Immediately pretransplant and 30mins after reperfusion	11	55	NR	NR	NR	NR	NR

PPV, positive predictive value; NPV, negative predictive value; OR, odds ratio; area under ROC, area under receiver operative characteristics curve; IL-6, interleukin-6; NR, not reported; TNF-α, tumour necrosis factor-alpha; IL-1β, interleukin-1beta; CRP, C-reactive protein; IL-8, interleukin-8; and NGAL, Neutrophil gelatinase-associated lipocalin.

function. Future studies of larger cohorts are required to further evaluate the usefulness of cystatin C for the diagnosis of DGF.

Renal biopsy

Renal allograft biopsy is the gold standard for diagnosis of rejection, but the role of a biopsy in diagnosing DGF has not been established. Most studies evaluated the usefulness of renal biopsy taken 30 min to 1 h after revascularisation. Different techniques were used, including immunohistochemistry staining and gene expression analysis. Markers studied included molecules associated with inflammation, hypoxia-related injury, apoptosis, T-lymphocyte activation, T-regulatory cell function, and fibrosis. Data on the diagnostic accuracy of transcriptome measurements or protein expression detected by immunohistochemistry were limited. No specific transcript or single marker has proven useful to differentiate DGF from immediate graft function. Kotsch reported that the combination of clinical variables and a selection of genes was superior to gene analysis alone in predicting the development of DGF, but with a modest AUC of 0.7 (sensitivity of 68% and specificity of 65%) [39]. The invasive nature of renal biopsy further detracts from its clinical utility.

Combination of biomarkers

A single biomarker may be inadequate to reflect the complex pathophysiology and heterogeneous clinical manifestations of DGF. A combination of biomarkers may overcome these shortfalls, as Fonseca *et al.* [40] proposed in examining the combination of serum creatinine, malondialdehyde (MDA), and cystatin C, which exhibited superior accuracy to single measures of the same biomarkers and predicted DGF with a high degree of accuracy with a sensitivity of 100%, specificity of 86%, and AUC 0.96. The authors' posed that the combination of a marker of injury (MDA) coupled with markers of dysfunction (creatinine and cystatin C) best addressed the complex pathophysiology of DGF. However, changes in protein binding of MDA in renal failure [41], and the effects of calcineurin-inhibitors [42,43], or MDA measurement may confound its association with DGF. Further studies are required to clarify these issues.

Combining biomarkers inherently increases the cost of testing and the complexity of the test [44,45].

Studies comparing biomarkers to nonstandard definitions of DGF

Twenty-seven studies compared novel biomarkers with nongold standard definitions of DGF, based upon urine output, serum creatinine levels, and dialysis requirement with various time constraints or a combination of the above criteria (Table 2). Seven studies described biomarkers without a comparator (Table 3). Serum and urine NGAL were again the most common tests evaluated. Regardless of the definition of DGF, the reported diagnostic performances of serum NGAL were superior to other biomarkers, with two studies describing near perfect test performance as defined by AUC [46,47]. Again, heterogeneity and poor study quality were common, and all studies were exploratory, with no validation studies reported.

Limitations in the existing literature

We have identified several challenges in studying novel diagnostic tests for DGF. First, significant heterogeneity was evident between studies investigating the same diagnostic tests. Potential explanations include the study population characteristics (e.g., proportion of deceased donor recipients or living donor recipients) and test applications (e.g., diagnostic test methods, test threshold, and timing of tests). Such heterogeneity highlights the need for adequate validation studies. Second, the lack of clinical consensus and objectivity regarding the gold standard definition of DGF made it challenging to correctly identify the target population. In this review, we primarily selected the requirement for dialysis within seven days post-transplant as the reference standard against which biomarkers should be compared. Only three out of 37 studies excluded patients who received dialysis for reasons other than DGF, including hyperkalaemia or fluid overload, in order to increase the specificity [25,48,49]. This may have resulted in overestimation of the incidence of DGF. Furthermore, the threshold used to determine positive results varied among studies. Most studies used receiver operating characteristics curves to define positive test results, in which the cut-off values are driven by the sensitivity and specificity of the tests. This approach potentially led to overly optimistic measures of diagnostic accuracy. A conservative threshold, relative to a liberal threshold, will have different implications for determining the presence or absence of DGF. Finally, most of the studies were single-centre studies involving a relatively small number of patients, and these studies were exploratory in nature with a short follow-up time. Approximately 30% of the included studies were retrospective, raising the potential for bias in reporting diagnostic accuracy. Such weaknesses in the published literature highlight the need for larger prospective cohort studies to validate use of each biomarker, with predefined test thresholds and longer follow-up of patients to determine the relationship between biomarkers and long-term outcomes.

Conclusion

Novel biomarker-based methods for diagnosing DGF remain exploratory. Serum NGAL appears to be the most promising biomarker at present. There is a need for larger-scale diagnostic accuracy studies to define and validate the diagnostic role of biomarkers for DGF, and in particular serum NGAL at day 1. Larger numbers, prospective study design, and use of prespecified test threshold values will be required. Beyond validation of a biomarker(s) that are predictive of DGF, as defined by requirement for dialysis within seven days of transplantation, further work will be required to determine whether such tests can overcome the shortfalls of this dialysis-based definition of DGF. Studies defining the associations between the identified biomarker-based tests and short- and long-term outcomes after transplantation, including acute rejection, graft function, and graft failure, will ultimately be required. Identifying a biomarker-based diagnostic test for DGF to enhance the objectivity and timing as compared with dialysis-based definitions, and to provide a quantitative diagnosis of ischaemia-reperfusion injury, remains an important goal that should be pursued.

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Conflict of interest

The authors of this manuscript have no conflicts of interest to disclose.

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