META-ANALYSIS

A systematic review to identify whether perfusate biomarkers produced during hypothermic machine perfusion can predict graft outcomes in kidney transplantation

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SUMMARY

There is good evidence to support the use of hypothermic machine perfusion (HMP) over static cold storage as the favoured preservation method for deceased donor kidneys. However, the utility of HMP as a tool to assess the viability of kidneys for transplant is unclear. There is a need to determine whether perfusate biomarkers produced during HMP can predict post-transplant outcomes and assess the suitability of organs for transplantation. Three different databases (MEDLINE, Embase, Transplant Library) were screened to 31 May 2019. Articles were included if a relationship was reported between one or more perfusate biomarkers and post-transplant outcomes. Studies were assessed and graded for methodological quality and strength of evidence. Glutathione S-transferase was the most promising biomarker for predicting delayed graft function, but its predictive ability was at best moderate. Analysis of primary nonfunction rates was challenging due to low occurrence rates and small sample sizes. Existing studies are limited in quality and have not yielded biomarkers for kidneys undergoing HMP that are able to predict post-transplant outcomes with sufficient accuracy to support routine clinical use. Further studies with larger samples and more robust methodology are needed. (PROSPERO registration: CRD42019121161).

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Introduction

Kidney transplantation is the treatment of choice for the majority of patients with end stage kidney disease, improving both quality of life and survival. However, the shortage of deceased donor organs leads to long waiting times and there is a need to identify strategies to increase the donor pool. Increasing evidence suggests that hypothermic machine perfusion (HMP) is the best preservation method compared with simple static cold storage (SCS) for extended criteria donor and older kidneys [1,2]. HMP reduces the rate of delayed graft function (DGF) and increases 1-year graft survival in these groups when a higher risk donor organ was used and may also improve outcomes in all donor types [3]. HMP has become standard practice in some centres and also in

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some countries, that is in the Netherlands, where all deceased donor kidneys are placed on an HMP device at the retrieval centre. While perfusion will allow better assessment of viability during the period when bridging from donor to recipient, the role of perfusate biomarkers produced during HMP prior to implant remains unclear yet. There is a clear need for better and more objective markers of viability to reduce discard rates on the one hand but also avoid transplantation of poor-quality kidneys [4]. Better assessment will help to obtain improved outcomes and survival after transplantation.

A systematic review published in 2012 assessed the role of biomarkers in HMP perfusate and in the donor urine [5]. This review was limited by the small overall number and even smaller number of good quality studies included, although it did report a link between rates of DGF and perfusate levels of lactate dehydrogenase (LDH), glutathione S-transferase (GST) and aspartate transaminase (AST). The authors also reported a significant association between primary nonfunction (PNF) and GST (in two out of three studies) as well as LDH (1/3), with no association found between any biomarkers and graft survival. In the last seven years, there have been further publications on the impact of biomarkers related to organ viability during HMP. In this study, we have undertaken a systematic review of the current literature to update findings and to consider the role of new biomarkers reflecting kidney injury. The objectives of this review are to assess whether perfusate biomarkers can predict post-transplant outcomes (namely DGF, PNF and graft survival) and to determine the strength of the biomarkers' association with outcome. In addition, we aim to establish whether there are any differences between biomarkers in the donation after brain death (DBD) and donation after circulatory death (DCD) settings.

Materials and methods

Reporting of this review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. The analysis methods, search strategies and inclusion criteria were specified in advance and documented in a shared protocol. The review protocol was prospectively registered with PROSPERO and can be found online (registration number: CRD42019121161).

Literature search

MEDLINE (1946 – present), Embase (1974 – present) and the Transplant Library (2004 – present) were searched through the Ovid platform for studies comparing HMP perfusate biomarker levels and outcomes after transplantation. Outcomes of interest included DGF (usually defined as the need for one or more haemodialysis sessions within the first week post-transplantation, as opposed to immediate graft function), PNF (defined by permanent need for haemodialysis after transplantation or the failure of resolution of DGF) and long-term outcomes including graft function [serum creatinine or estimated glomerular filtration rate (eGFR)] and graft survival. All articles published before the 31st May 2019 were considered. The search terms are detailed in the search strategy document (Table S1). Reference lists were scanned for relevant articles overlooked by the literature search.

Inclusion/exclusion criteria

Eligibility screening by title and abstract was independently performed by two authors (FG and JH) and any discrepancies were discussed and resolved. Studies were included if they utilized HMP in human kidneys intended for transplantation, and related perfusate biomarkers to one or more of the following clinical outcomes: graft survival; PNF; DGF; and graft function. Biomarkers were always measured from perfusate samples, drawn at various time point after perfusion start. Abstracts, reviews and commentaries were excluded. If there were no biomarker analyses or transplant outcomes, or no association between them was reported, the articles were excluded. Duplicates still present were identified by juxtaposing author names, articles titles and affiliations. The remaining articles were independently reviewed by two investigators (FG, JH). Articles from the same research groups considering overlapping populations were included and grouped to easily detect the most recent results for each outcome of interest. Data regarding the study characteristics were collected by one author (FG) with the support of an extraction table previously refined by all authors. A second author (JH) subsequently checked all the extracted data and disagreements were resolved by discussion between all the authors.

Study evaluation

Data regarding the strength of relationship between biomarkers and perfusion parameters and clinical outcomes including receiver operator curve (ROC) area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV)

and correlation coefficients were extracted, and each article was graded according to the strength of evidence. Strength was determined by the breadth of the confidence intervals and the method of statistical analysis. Biomarker thresholds were considered when present and articles were assessed for sensitivity and specificity and evidence of biomarker validation. Due to the limited amount of available data, a narrative synthesis was employed to discuss the results.

Each study was evaluated for risk of bias using a modified version of the checklist by Moga *et al.* [6]. This is a quality appraisal tool for case series developed in 2012 by the Institute of Health Economy of Alberta (Canada). Since no specific interventions were evaluated and no adverse events were expected, a maximum of 15 points were assigned to each paper consequently graded as 'good' (14–15), 'fair' (11–13.5) or 'poor' (<11).

Results

Included studies

Literature searches identified a total of 552 citations. After eliminating duplicates, 450 remained. Of these, 390 were discarded after evaluating title and abstract for eligibility. Full-text versions of the remaining 60 articles were reviewed to ensure they met the inclusion criteria. 31 studies were excluded following review as detailed in the search flow diagram (Fig. 1), and 29 articles published from 1973 to 2017 were identified for inclusion. Articles from the same research groups and/or considering overlapping populations were included, and consequently flagged, so important findings were not overlooked.

Study characteristics

The most relevant characteristics of each study are summarized in Table 1. The included studies were heterogeneous in terms of sample size with the largest presenting data from 670 and the smallest from 11 transplants. Of the 29 studies, only two analysed data collected from a randomized controlled trial (RCT) from Moers *et al.* (MP-Trial) [1]. Both were secondary analyses from the machine perfused arm of the trial, whose primary objective was to establish the ability of HMP to reduce DGF occurrence. Both were included in our review: Moers *et al.* [7] considered all the kidneys from the trial and Nagelschmidt *et al.* [8] limited the analysis to only DBD donors older than 55 years. Two additional studies, Hall *et al.* [9] and Parikh *et al.* [10], collected data from a large prospective multi-centre cohort (n = 671). Data from an additional two studies were prospectively collected, not randomized nor blinded and limited by a small sample size (11 to 50 transplants). The remaining were retrospective studies, of these, the work by Hoogland *et al.* [11] (n = 335), de Vries *et al.* [12] (n = 231) and van Smaalen *et al.* [13] (n = 390) had large sample sizes and scored 'good/fair' overall for quality.

The majority of the included studies were conducted in Europe (22/29) and almost a third were published in the last 5 years. Sixteen studies considered grafts from either DCD (10/16) or DBD (6/16) donors; the

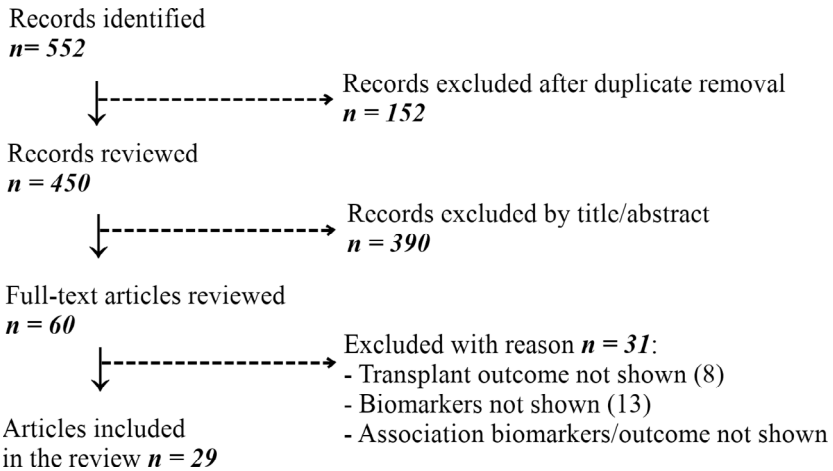

Figure 1 Search flow chart. 552 studies were identified by searching MEDLINE, Embase and the Transplant Library through the Ovid platform, updated to the 31st May 2019. After duplicate removal and screening by title and abstract, 60 studies remained eligible for inclusion. After full-text revision, 31 studies were excluded and 29 were included in the analysis.

Table 1. Study characteristics.

Ref.	Study design	Years	Donors	Ischaemia time*	Perfusion characteristics machine – solution – temp. – press	Start-to-sample	Transplants (*n*)	Biomarkers	Outcomes	Outcomes frequency	Statistical analysis or test
13	R	Jan '97–Mar '13	DCD	C: 25 h W: 25 m	Gambro PF-3B - Belzer UW - n/a - 55 mmHg; Lifeport - KPS-1 - 4° - 30/40 mmHg	4 h	390	Extracellular histone H3	DGF; PNF; 1-year graft survival	DGF 228/390 PNF 76/390	LogR (M) CoxR (M) AUROC
40	R	2002–2014	DCD	C: 20 h W[1]: 20 m W[2]: 40 m	LifePort - n/a - n/a - 30 mmHg	3 h	155	t-GST	Graft survival and eGFR	n/a	CoxR (U)
17	Unclear	Jul '12–Jun '13	DBD (62%)	C: 11–17 h W: n/a	LifePort - KPS-1 - 3/5° - 30 mmHg	End (10–15 h)	24	NGAL, LDH, MMP-9 and MMP-2	DGF	DGF 5/24	Student's *t*-test
10	P	May '10–Dec '13	DBD (74%)	C: 18 h W: n/a	LifePort - KPS-1 - 4° - 30 mmHg	Baseline, End (10 h)	671	NGAL, KIM-1, IL-18, L-FABP	DGF; PNF; 6-month eGFR	DGF 230/671 PNF 15/671	Wilcoxon sum rank LinR (M)
41	P	n/a	DCD (91%)	C: 18 h W[1]: 13 m	LifePort - KPS-1 - 4° - n/a	1 h	11	miR-21	6 to 12-month eGFR	n/a	Pearson's correlation
18	Unclear	Jul '12–Aug '13	DBD (88%)	C: 23 h W: n/a	LifePort - KPS-1 - 4° - 30 mmHg	45 m, 4 h	26	H-NMR spectroscopy	DGF	DGF 7/26	Mann–Whitney *U*-test AUROC
9	P	May '10–Apr '12	DBD (67%)	C: 18 h W: n/a	LifePort - KPS-1 - 4° - 30 mmHg	Baseline, End (12 h)	428	a-GST, p-GST	DGF	DGF 141/428	Wilcoxon sum rank LBR (M) AUROC
11	R	Jan '97–Jan '08	DCD	C: 27 h W: 26 m	Gambro PF-3B - Belzer UW - n/a - 55 mmHg	1 h, 2 h, 4 h	335	t-GST, LDH, H-FABP, redox-active iron, IL-18, NGAL	DGF; PNF; eGFR; 1-year graft survival	DGF 205/335 PNF 67/335	LogR CoxR (M) KM, log-rank AUROC
8	P	Nov '05–Aug '07	DBD	C: 14 h W: n/a	LifePort - Belzer UW - 1/8°C - 30 mmHg	1 h, End (n/a)	111	t-GST, a-GST, p-GST, LDH, Lac, LPOP	DGF; PNF; 1-year graft survival	DGF 28/111 PNF 2/111	Mann–Whitney *U*-test LogR (M)
7	P	Nov '05 - Aug '07	DBD (75%)	C: 15 h W: n/a	LifePort - Belzer UW - 1/8°C - 30 mmHg	1 h, End (15 h)	306	t-GST, LDH, H-FABP, AST, Ala-AP, NAG	DGF; PNF; 1-year graft survival	DGF 76/306 PNF 7/306	LogR (M) CoxR (M) KM, log-rank AUROC
12	R	May '98–Nov '02	DCD (88%)	C: 27 h W: 25 m	Gambro PF-3B - Belzer UW - n/a - n/a	Baseline, 1 h, 4 h, 6 h	231	t-GST, LDH, redox-active iron	DGF; PNF	DGF 134/231 PNF 43/231	LogR (M) AUROC
42	R	1998–2003	DCD	C: 24 h W[1]: 20 m W[2]: 36 m	Newcastle Hypothermic Preservation System - Newcastle UW - 4/8°C - n/a	4 h	74	t-GST, H-FABP, Ala-Ap	Best postoperative eGFR	n/a	Spearman, s correlation
15	P	n/a	DBD	C: 36 h W: n/a	Waters Mox 100 - Belzer UW - 6*C - 50 mmHg	End (n/a)	50	MDA (LPOP)	DGF; 1 to 4-year serum creatinine	DGF 18/50	Mann–Whitney *U*-test LogR (M)
43 a	R	1994–1999	DBD	C: 33 h W:33 m	Waters Mox 100 - Belzer UW - 6*C - 50 mmHg	4 h	234	a-GST, LDH, Lac	DGF	DGF 84/234	Mann–Whitney *U*-test LogR (M)
30 a	R	1994–1999	DBD	C: 34 h W: n/a	Waters Mox 100 - Belzer UW - 6*C - 50 mmHg	4 h	234	Lac	DGF	DGF 84/234	unclear

Table 1. Continued.

Ref.	Study design	Years	Donors	Ischaemia time*	Perfusion characteristics machine – solution – temp. – press	Start-to-sample	Transplants (*n*)	Biomarkers	Outcomes	Outcomes frequency	Statistical analysis or test
44 a	R	Mar '97–Feb '98	DBD (85%)	C: 32 h W: n/a	Waters Mox 100 - Belzer UW - 6*C - 50 mmHg	1 h, 4 h, 8 h	40	a-GST, p-GST	DGF	DGF 15/40	unclear
16 b	R	Jan '93–Mar '99	DBD	C: 25 h W: n/a	Waters MOX or RM3 - UW - 4°C - <60 mmHg	Every 30 m – 1 h	402	Perfusate ions concentration (Na, Ca, Cl, K)	DGF	DGF 66/402	Student's *t*-test ANOVA
31 b	R	Jan '95–Oct '97	DBD	C: 24 h W: n/a	Waters MOX or RM3 - UW - 4°C - <60 mmHg	Every 30 m – 1 h up to 12 h	150	Perfusate ions concentration (Na, Ca, Cl, K)	DGF	DGF 48/150	Student's *t*-test ANOVA
45 c	R	July '94–Jan '97	DCD	C: n/a W: 50 m	Gambro PF-3B - Belzer UW - n/a - n/a	8 h	71	a-GST, LDH	DGF; PNF	DGF 51/71 PNF 11/71	Mann–Whitney *U*-test
46 c	Unclear	July '93–Nov '95	DCD	C: 30 h W: 41–70 m	Gambro PF-3B - Belzer UW - n/a - 60 mmHg	Baseline, 1 h, 4 h, 8 h	46	a-GST, LDH	DGF; PNF	DGF 26/46 PNF 9/46	Mann–Whitney *U*-test
32 c	R	Mar '93–Jun '96	DCD	C: n/a W: 48–73 m	Gambro PF-3B - Belzer UW - 4°C - 60 mmHg	4 h, 8 h	59	a-GST	PNF	PNF 10/59	Mann–Whitney *U*-test
33 c	R	n/a	DCD	C: n/a W: 19–49 m	Gambro PF-3B - Belzer UW - 4°C - 60 mmHg	6 h	22	a-GST, p-GST	PNF	PNF 3/22	Mann–Whitney *U*-test
34 c	R	Apr '93–Feb '95	DCD	C: n/a W: 30–90 m	n/a	1 h, 2 h, 4 h, 6 h, 8 h	27	LDH	PNF	PNF 6/27	Kruskal–Wallis test (ANOVA)
14	Unclear	n/a	n/a	C: 31–42 h W: 3 m	Waters Mox 100 - CPP, PPF, SAS - 8°C - n/a	End (n/a)	24	GST	ATN (dialysis during the first 3 days post-transplant)	ATN 8/24	Student's *t*-test
47	R	1974–1977	n/a	C: 15 h W: n/a	Gambro preservation unit - SAS - 4/9°C - 60 mmHg	1 h, End (3–19 h)	41	LDH, Lac	DGF (unclearly defined); PNF	DGF 24/41 PNF 9/41	Mann–Whitney *U*-test LinR (U)
48	Unclear	n/a	DBD (90%)	C: n/a W: n/a	Belzer organ preservation system (Model T-1450) - SAS - 4/9°C - 60 mmHg	Every hour	61	Lac	ATN (a period of acute tubular necrosis)	ATN 17/61	unclear
49	Unclear	n/a	DCD	C: 11 h W: 13–35 m	Gambro PF-3A - PPF - 8°C - 50 mmHg	1 h	32	LDH, Lac	DGF (to the postoperative day when the serum creatinine fell by 100 umol/L)	DGF 11/32	Mann–Whitney *U*-test
50	R	Jan '73–Jul '74	DCD (92%)	C: n/a W: n/a	Gambro continuous perfusion - PPF - n/a – 40 –60 mmHg	1 h	37	Lac	DGF (postoperative dialysis)	DGF 21/37	Unclear
51	Unclear	n/a	n/a	C: 7–14 h W: 17–22 m	Watson Marlow pump - PPF - 6°C - flow controlled	Baseline, 1 h, End (4–9 h)	26	LDH, Lac	DGF (dialysis in the post-transplant period)	DGF 16/26	Student's *t*-test

DBD, donation after brain death; DCD, donation after circulatory death; eGFR, estimated glomerular filtration rate; H-NMR, proton nuclear magnetic resonance; LDH, lactate dehydrogenase

AUROC, area under receiver operating curve; CoxR, Cox regression; CPP, cryoprecipitated plasma; LBR, log-binomial regression; LinR, linear regression; LogR, logistic regression; (M), multivariable; P, prospective; PPF, plasma protein fraction; R, retrospective; SAS, serum albumin solution; (U), univariable; n/a, not available. Biomarkers' abbreviations in the text.

Articles (a–c) from the same research group/overlapping populations.

*Mean (or median) cold ischaemia time (C; h - hours) and warm ischaemia time (W; W^1 – first WIT; W^2 – second WIT; m - minutes).

remaining studies evaluated grafts from a mixed, mainly DBD donors ($n = 7$), or mainly DCD donors ($n = 3$). In three studies, the donor characteristics were not detailed.

Quality assessment

According to the quality checklist, the four previously mentioned prospective studies (Moers *et al.;* Nagelschmidt *et al.;* Hall *et al.;* Parikh *et al.*) [7–10] were graded as of 'good' quality, 11 articles graded as 'fair' and 14 as 'poor'. Detailed results from the quality scoring/risk of bias are reported (Table S2).

Biomarkers

The main results from each study are outlined in Table 2. Glutathione S-transferase (GST) as total-GST (t-GST) or its isoforms (alpha-GST and pi-GST) was the most commonly evaluated biomarker (14/29), followed by LDH (13/29) and lactate levels (9/29). Fatty acid-binding protein (FABP, in its isoforms heart-FABP and liver-FABP, 4/29), neutrophil gelatinase-associated lipocalin (NGAL, 3/29), interleukin-18 (IL-18, 2/29) lipid peroxidation products (LPOPs, 2/29) and perfusate ionized calcium (iCa, 2/29) were considered in at least two articles. Single-study evaluations were present for: histones H3, kidney injury molecule-1 (KIM-1), aspartate transaminase (AST), alanine-aminopeptidase (Ala-Ap), matrix metalloproteinase 9 and 2 (MMP-9, MMP-2), n-acetylglucosamine (NAG), redox-active iron, mirco-RNA 21 (miR-21) and perfusate proton nuclear magnetic resonance (H-NMR) spectroscopy.

Outcomes

The most commonly evaluated outcome was DGF (23/29). Only twelve articles provided data on PNF. Finally, nine articles studied the relationship between one or more biomarker levels and long-term graft function or patients' survival up to 5-year post-transplantation.

Glutathione S-transferase

GST was the most studied and the most promising biomarker, particularly for DGF prediction, confirming the previous finding by Bhangoo *et al.* [5]. To our knowledge, the first attempt to establish a correlation between GST levels during HMP and transplant outcome in the clinical setting was published in 1981 [14]. In this pioneering work, Cho *et al.* established a statistically significant correlation between GST (previously known as ligandin) levels at the end of perfusion and the development of acute tubular necrosis defined as the need of one haemodialysis session in the first three days post-transplantation. GST levels increased throughout perfusion in all the included studies and they were associated with DGF in 7/10 articles three of which were 'good' quality studies with two (Moers *et al.* [7] and Hall *et al.* [9]) demonstrating a significant association after multivariable analysis. However, the strength of the association of GST levels and DGF was less convincing and the different isoforms and assays used meant that direct comparison of values between studies was not possible. ROC analysis in the Moers paper based on GST levels at the end of perfusion was 0.67, in the Hall paper the authors demonstrate unadjusted ROC of 0.61 and ROC of 0.70 when adjusted for donor, transport and recipient variables. These values were based on the 'log of the optimal cut-off value'. In the Nagelschmidt paper [8] a cut-off of 10 ug/ml of alpha-GST had a PPV of 71.4% and a NPV of 77.9% for DGF. For t-GST, a cut-off of 450 U/l yielded a PPV of only 37.5% and a NPV of 79.1%. When data were available, a statistically significant association between elevated GST levels and PNF was reported in 6/8 papers, but not confirmed in the population studied by Moers *et al.* and Nagelschmidt *et al.* Moreover, four of these articles reporting a positive association were all performed by the University of Maastricht group with data collected between 1993 and 1997. Significant association of GST levels with long-term outcome has never been reported.

Lactate dehydrogenase

Our results show that LDH elevation was associated with DGF in 8/11 articles, but only in Hoogland *et al.* [11] was the association maintained after multivariable analysis. However, the strength of the association was weak (OR, 1.002 (1.001–1.004) $P = 0.007$). The association with PNF was again less common with a significant correlation found in only 2/8 papers, both from the Maastricht group (Hoogland *et al.* [11] and de Vries *et al.* [12]). Again the strength of the association was weak with ROC 0.699, and the transferability of these data to future populations is unclear as the PNF rate in the Hoogland study was unusually high at 20%. The only positive association between elevated LDH levels and long-term outcome was reported, again by Hoogland *et al.*, with 1-year eGFR, but not with graft survival.

Table 2. Study results – biomarkers and outcomes association.

	DGF								PNF								Long-term outcome							
Ref.	GST	LDH	FABP	LAC	NGAL	IL-18	LPOP	Other	GST	LDH	FABP	LAC	NGAL	IL-18	LPOP	other	GST	LDH	FABP	LAC	NGAL	IL-18	LPOP	other
13								H3 (#)								H3 (–)								H3 (#)
40																	tGST (–)							
17		(–)			(–)			MMP-9 (+); MMP-2 (+)																
10			L-FABP (+)		(+)	(+)		KIM-1 (–)			L-FABP (–)		(–)	(–)		KIM-1 (–)			L-FABP (#)		(#)	(–)		KIM-1 (–)
41																								MiR-21 (+)
18								H-NMR (+)																
9	pGST (#); aGST (+)																							
11	(–)	(#)	H-FABP (–)		(–)	(#)		redox iron (#)	(+)	(#)	H-FABP (+)		(–)	(#)		redox iron (+)	(–)	(+)	H-FABP (–)		(–)	(–)		redox iron (–)
8	tGST (+); aGST (+); pGST (–)	(–)		(–)			(#)		tGST (–); aGST (–); pGST (–)	(–)		(–)			(+)		tGST (–); aGST (–); pGST (–)	(–)		(–)			(–)	
7	(#)	(+)	H-FABP (#)					NAG(#); AST (+); Ala/Ap (–)	(–)	(–)	H-FABP (–)					NAG(–); AST (–); Ala/Ap (–)	(–)	(–)	H-FABP (–)					NAG(–); AST (–); Ala/Ap (–)
12	(+)	(+)						redox iron (+)	(+)	(#)						redox iron (#)								
42																	tGST (–)		HFABP (–)					Ala-Ap (–)
15							MDA (+)																MDA (+)	
43 a	aGST (+)	(+)		(+)																				
30 a				(+)																				
44 a	aGST (+); pGST (–)																							
16 b								iCa (+)																
31 b								iCa (+)																
45 c	aGST (–)	(+)							aGST (+)	(–)														
46 c	aGST (–)	(+)							aGST (+)	(–)														
32 c									aGST (+)															
33 c									aGST (+); pGST (–)															
34 c										(–)														
14	(+)																							
47		(–)		(–)						(–)		(–)												
48				(–)																				
49		(+)		(–)																				
50				(+)																				
51		(+)		(+)																				

LDH, lactate dehydrogenase.

(–) No significant association; (+) elevated biomarker's levels are associated with the corresponding outcome; **(**#) significant association is maintained after multivariable analysis. Biomarkers' abbreviations in the text.

Articles (a–c) from the same research group/overlapping populations.

Lactate

The association between elevated lactate levels and DGF/PNF was repeatedly investigated, mostly in the older articles. In total, 4/8 papers found a significant association between lactate levels and the occurrence of DGF, although there was some overlap between similar populations as highlighted in Table 1. This association was not maintained after multivariable analysis in any of the included studies. Lactate levels were never found to be associated with PNF or long-term outcomes.

FABP, LPOPs and IL-18

FABP (with its isoforms H-FABP and L-FABP), LPOPs and IL-18 were reported to be significantly associated with DGF, respectively, in 2/3, 2/2 and 2/2 articles. H-FABP was identified, together with GST and NAG, by Moers *et al.* [7] as an independent predictor for DGF with a moderate prognostic value (AUC of 0.64 for H-FABP). Lipid peroxidation products were reported to be an independent predictor for DGF in a subgroup of the same population (Nagelschmidt *et al.* [8]), while IL-18 levels were highlighted by Hoogland *et al.* [11] together with redox-active iron. In regard to PNF, FABP and LPOPs did not show any significant correlation in the MP-Trial population. On the contrary in the Maastricht population, IL-18 proved to be independent predictors of PNF (Hoogland *et al.* [11]). None of these biomarkers showed significant correlation with long-term outcome in these studies, with the exception of the work by Parikh *et al.* [10], in which L-FABP levels were modestly associated with 6-month eGFR. In one small retrospective study [15], malondialdehyde (MDA), a particular lipid peroxidation product, was found to be significantly associated with DGF and 1 to 4-year post-transplant serum creatinine.

Neutrophil gelatinase-associated lipocalin

Baseline perfusate NGAL (but not post-HMP NGAL) was reported to be significantly associated with DGF in only 1/3 articles without maintaining significance after multivariable analysis. Parikh *et al.* [10] also described post-HMP NGAL levels to be modestly associated with 6-month eGFR, but not with PNF. The measurement time point after HMP initiation might have a crucial role in determining the efficacy of the studied biomarkers, especially when their synthesis/release could potentially be altered by hypothermia.

Single-studied biomarkers

Promising results appeared in a recent work by *van* Smaalen *et al.* [13]. Extracellular histone H3 concentration was reported to be an independent risk factor for DGF and 1-year graft survival in 390 transplant patients. As already mentioned above, n-acetylglucosamine (NAG) proved to be an independent predictor for DGF in the work by Moers *et al.* [7]. Polyak *et al.* [16], in 2000, demonstrated that iCa concentrations were significantly higher in DGF patients. Interestingly KIM-1, despite promising results as acute kidney injury (AKI) biomarker, was found not to be predictive of either DGF, PNF or long-term graft function in the prospective study by Parikh *et al.* [10]. A positive association with DGF was also described for matrix metalloproteinase 9 and 2 (MMP-9, MMP-2); however, this analysis was performed on only 24 patients, only five of which experienced DGF [17]. Guy *et al.* [18] demonstrated a difference in perfusate metabolomic profiles of kidneys with immediate graft function and DGF. They showed that perfusate glucose, inosine, leucine and gluconate were significantly different in the DGF group with AUC 0.7–0.9; however, the small size ($n = 26$) and study methodology limit the strength of these results.

DCD and DBD donors

In 10/29 articles, a mixed population of grafts from DCD and DBD donors was analysed (Table 1). When comparing eight DCD (or 'mainly DCD') populations versus seven DBD (or 'mainly DBD') populations, some interesting trends emerged (Table 3). In particular, an association between high GST levels and DGF was reported in 1/4 of DCD studies but 5/5 of DBD; on the contrary, the association between elevated LDH levels and DGF was reported in 5/5 of DCD studies and 2/4 of DBDs. After multivariable analysis, the association GST-DGF was maintained only in two DBD studies, whereas the association LDH-DGF was maintained only in one DCD study. Moreover, the association with PNF largely increased when considering only DCD grafts with GST significantly associated with PNF in 6/6 articles, LDH in 2/5 articles (with maintained association after multivariable analysis) and H-FABP in 1/1 articles. On the other hand, in DBDs populations PNF was never associated with the studied biomarkers (GST 0/2, LDH 0/2, FABP 0/2).

Table 3. DCDs versus DBDs.

DCD	DGF			PNF			DBD	DGF			PNF		
Ref.	GST	LDH	FABP	GST	LDH	FABP	Ref.	GST	LDH	FABP	GST	LDH	FABP
11	(–)	(#)	H-FABP (–)	(+)	(#)	H-FABP (+)	17		(–)				
12	(+)	(+)		(+)	(#)		10			L-FABP (+)			L-FABP (–)
45	aGST (–)	(+)		aGST (+)	(–)		9	pGST (#); aGST (#)					
46	aGST (–)	(+)		aGST (+)	(–)		8	tGST (+); aGST (+); pGST (–)	(–)		tGST (–); aGST (–); pGST (–)	(–)	
32				aGST (+)			7	(#)	(+)	H-FABP (#)	(–)	(–)	H-FABP (–)
33				aGST (+); pGST (–)			43	aGST (+)	(+)				
34					(–)		44	aGST (+); pGST (–)					
49		(+)											

DBD, donation after brain death; DCD, donation after circulatory death; H-NMR, proton nuclear magnetic resonance; LDH, lactate dehydrogenase.
(–) No significant association; (+) elevated biomarker's levels are associated with the corresponding outcome; **(#)** significant association is maintained after multivariable analysis. Biomarkers' abbreviations in the text.

Discussion

The present study has identified GST and LDH as the most studied and promising biomarkers to predict short-term graft function. The GSTs are a well-known enzyme family with hepatocellular activity for glutathione conjugation and the ability to bind proteins and toxins. GST elevation in the urine has been associated with renal injury and AKI in diverse clinical settings. Its isoforms alpha-GST and pi-GST are released during ischaemia, respectively, from the proximal and distal tubule [19,20]. GST accumulation in the perfusate during kidney machine preservation could imply epithelial cell disruption and tubular damage. However, with ROC values of between 0.61 and 0.7 the predictive strength were still only moderate.

LDH is a nonspecific marker of cell injury and extrarenal conditions such as circuit-dependant haemolysis can determine its elevation. Numerous studies have investigated the distribution of LDH in normal renal tissue and its elevation in the serum and urine in patients with renal diseases [21,22]. A recent experimental model combining ischaemia-reperfusion injury with magnetic resonance cortical imaging, demonstrated increased LDH activity and release in the interstitial space from cells undergoing necrosis and apoptosis [23].

NGAL is released by renal tubular cells in response to ischaemic and toxic injury and has therefore gained much attention as biomarker for AKI. Both urine and plasma NGAL have demonstrated to be powerful independent predictors of AKI in different clinical settings but its role in the transplant and preservation settings is still debated [24–26]. Despite this background, its use in the pretransplant assessment has never been extensively studied and preliminary results are not encouraging.

Overall, the included studies span a long time-period, and no articles were found to be included between 1981 and 1995. For studies published prior to 1981, perfusion machines were prototypes and often required external intervention (*e.g.* for pH adjustment), standard HMP perfusion solutions were not yet available with albumin-based plasma protein fraction solutions used as perfusate. Most importantly, the definitions of outcomes were not standardized in these studies and different interpretations of the terms DGF and acute tubular necrosis can be found. Lastly, it is worth considering that post-transplant care, immunosuppression and graft survival in those days was very different.

This current study appears to be the most extensive systematic review on this topic. We have summarized

all the available data relating to biomarkers in kidney HMP and their association with graft outcome from 1973 to today. We selected DGF and PNF as outcomes because they were frequently compared with biomarkers in the studies and are clinically relevant. Additional factors that may also be relevant to outcomes include donor age, cold ischaemia time (CIT) and, in the context of DCD organs, warm ischaemic time. The literature is clear that increasing durations of CIT and donor age are linked to DGF and long-term graft survival [27]. The range of median CIT in this study was 14–42 h (median 24) which is higher than current values in the UK and Europe. In addition, DGF is known to increase the risk of acute rejection and potentially decrease long-term allograft survival. Its occurrence and duration can have a negative impact on the graft outcome [28]. However, recent work suggests that DGF is a different entity in DCD and DBD kidneys. The incidence of DGF in DCD kidneys does not have an impact on graft survival in the same way it does in DBD kidneys. The warm ischaemic insult in DCD kidneys more frequently leads to DGF, and although there are higher rates of PNF, those that recover from DGF do not have significantly different outcomes. However, the brainstem death cascade that leads to DGF in DBD kidneys does not appear to have the same recoverability in the long term [29].

Our work suggests that elevated perfusate levels of GST, LDH and FABP are often associated with DGF. Also, IL-18 and lipid peroxidation products levels appear to be promising biomarkers in predicting DGF. Despite this, recurring evidence suggests that predictive value of a single elevated biomarker is usually low or, at best, moderate. An attempted sub-analysis comparing the DBD versus DCD populations suggested some differences worth considering. In particular, GST and LDH elevation appeared more often associated with DGF among DBDs and DCDs, respectively. GST elevation in DCDs was frequently associated with PNF. Despite the small number of studies, the frequent overlap between DBD and DCD patients in the same cohorts, and the lack of direct comparisons, it would be helpful to analyse these populations separately in future studies. This may be a reflection on the different pathophysiology of DGF between DCD and DBD kidneys but unfortunately the study numbers are too small to be conclusive.

The ability to predict PNF is perhaps more important as the outcome is devastating for the patient. Unfortunately, less than half of the included articles studied the association between biomarkers and PNF and the majority of them were not powerful enough to establish a significant association after adjusting for confounding factors. Reasons for this were the low rate of PNF, especially when grafts from DBD donors with low warm ischaemia time were considered, and the small sample sizes. GST and LDH were the most common biomarkers associated with PNF, LDH being an independent risk factor in two studies from the Maastricht population. Other independent risk factors, IL-18 and redox-active iron levels, appeared in one single study. As expected, in the light of the numerous variables of a transplant natural history, none of the included studies was able to demonstrate an independent statistically significant association between one single biomarker and long-term outcome with the exception of the recent work by van Smaalen *et al.* [13].

Our review has some limitations. The inclusion of different articles by the same research groups, with possible overlap between populations, was necessary so important results were not overlooked. In addition, five studies [30–34] have to be considered preliminary versions of already included articles. Their results are consequently highlighted and related to the more recent publications by the same research groups in Tables 1 and 2. Limited information was available to assess the optimum time points for assessment of biomarkers during the preservation process and this is reflected in the variation of timings of GST sampling which were between 1 and 15 h of HMP. This makes interpretation difficult. The included articles are graded on behalf of a quality appraisal tool for case series studies modified by Moga *et al.* [6], and although four of the more recent studies were graded 'good', the majority of the others had a high risk of bias due to retrospective or noncomparative study design. Due to the heterogeneity of the included study, a meta-analysis was not performed.

Although this review focusses on HMP, it is important to mention progression in the perfusion field that has not been evaluated. HMP with the addition of oxygen has recently been assessed in two clinical trials as part of the consortium for organ preservation in Europe (COPE) and (unpublished) [35] results suggest that this may be beneficial. It would be interesting to assess biomarkers such as GST, LDH and lactate in this context as the addition of oxygen should theoretically reduce ischaemic/anaerobic injury. Ex vivo normothermic machine perfusion (EVNP or NMP) is a technique that involves pumping the kidney with a body temperature, blood-based, oxygenated solution. Early work by Hosgood and

Nicholson suggested that DCD kidneys undergoing 1 h EVNP immediately prior to transplant had reduced rates of DGF compared with matched controls [36]. This question is currently being assessed in a multi-centre RCT [37]. EVNP has also been used as a means of viability assessment, which has resulted in the successful transplantation of DCD kidneys declined for transplant [38]. There is currently no clinical data on biomarkers of viability although this is a potentially fertile area of research. Preclinical work in discarded human kidneys undergoing 1 h NMP showed urinary NGAL may be a useful measure of kidney quality [39].

Prolonged periods of kidney perfusion may also be useful for viability assessment, repair and regeneration. A phase II clinical trial in deceased donor kidneys using the OrganOx kidney NMP device is due to commence in early 2020 and will assess preservation periods of up to 24 h of NMP (personal communication Profs Friend and Ploeg in Oxford).

Conclusion

This systematic review demonstrates that no single biomarker measured during HMP is able to accurately predict short-term or long-term graft outcome. GST levels represent the most important predictor of DGF, but combinations of multiple biomarkers and new biomarkers should be tested in new studies. To date, our clinical parameters provide a footprint, but to properly assess higher risk donor organs we need a more refined fingerprint, which requires further study.

Authorship

All authors made a significant contribution to the content of this manuscript as per ICMJE recommendations. FG, RJP and JPH: conceived the research idea. FG: participated in the literature systematic research, participated in the eligibility screening and full-text articles revision, participated in articles evaluation and data collection and wrote the article. SRK: provided support to the systematic research and to the evaluation process and critically reviewed the article. RJP: provided support to the systematic research and to the evaluation process and critically reviewed the article. JPH: participated in the literature systematic research, participated in the eligibility screening and full-text articles revision, participated in articles evaluation and data collection and critically reviewed the article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Search strategy.

Table S2. Quality assessment.

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