The Value of Machine Perfusion Perfusate Biomarkers for Predicting Kidney Transplant Outcome

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Background. Retrospective evidence suggests that lactate dehydrogenase, aspartate aminotransferase, total glutathione-*S*-transferase (GST), alanine-aminopeptidase, *N*-acetyl-β-D-glucosaminidase (NAG), and heart-type fatty acid binding protein (H-FABP) measured during kidney machine perfusion (MP) could have predictive value for posttransplant outcome. However, these data may be biased due to organ discard based on biomarker measurements, and previous analyses were not adjusted for likely confounding factors. No reliable prospective evidence has been available so far. Nevertheless, some centers already use these biomarkers to aid decisions on accepting or discarding a donor kidney.

Methods. From 306 deceased-donor kidneys donated after brain death or controlled cardiac death and included in an international randomized controlled trial, these six biomarkers were measured in the MP perfusate. In this unselected prospective data set, we tested whether concentrations were associated with delayed graft function, primary nonfunction, and graft survival. Multivariate regression models investigated whether the biomarkers remained independent predictors when adjusted for relevant confounding factors.

Results. GST, NAG, and H-FABP were independent predictors of delayed graft function but not of primary nonfunction and graft survival. Lactate dehydrogenase, aspartate aminotransferase, and alanine-aminopeptidase had no independent prognostic potential for any of the endpoints. Perfusate biomarker concentrations had no relevant correlation with cold ischemic time or renal vascular resistance on the pump.

Conclusions. Increased GST, NAG, or H-FABP concentrations during MP are an indication to adjust posttransplant recipient management. However, this study shows for the first time that perfusate biomarker measurements should not lead to kidney discard.

Keywords: Machine perfusion, Kidney transplantation, Perfusate biomarkers, Delayed graft function, Graft survival.

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Recently, we conducted an international randomized controlled trial (RCT) investigating the effect of hypothermic machine perfusion (MP) versus static cold storage in deceased-donor kidney transplantation. We found that MP reduced the risk of delayed graft function (DGF) with an odds ratio of 0.57 for all common donor types equally, and the duration of DGF was 3 days shorter in MP kidney recipients. In addition, graft survival (GS) after MP was significantly better already at 1 year posttransplant, and MP reduced the risk of graft failure with a hazard ratio of 0.52 (*1*). Together with evidence coming from earlier studies (*2*, *3*), these findings may lead to an increased usage of MP.

In addition to the beneficial effect that MP preservation has on postoperative outcome, many centers have advocated the method as a diagnostic tool to evaluate graft quality before

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transplantation. Several groups have suggested that perfusion characteristics, such as intrarenal vascular resistance, could have a predictive value for posttransplant outcome (*4*, *5*). In addition, evidence points out that perfusate biomarkers during MP may have a prognostic potential (*6–8*), and as a result, some centers use such measurements to aid decisions on transplanting or discarding a kidney. Nevertheless, the published data are scarce, using only retrospective data, and suffer from selection bias. Moreover, statistical analyses have been univariate, so far. Hence, likely confounding factors may have had an effect on the reported association between perfusate biomarkers and posttransplant results. No previous studies have addressed the question whether such measurements have a truly independent prognostic relevance.

In this study, we have analyzed data from the MP arm of our RCT to investigate whether six important perfusate biomarkers that have been advocated and are already in use by various centers have any true independent predictive value for renal transplant outcome. We deliberately chose not to search for novel biomarkers but to test established biomarkers for the first time with multivariate analyses in a unique and unselected prospective data set.

We have studied six biomarkers that are commonly associated with renal cellular injury in general and tubular damage in particular. The extent of such injury is believed to be a major cause of DGF and graft failure (*9–11*). Lactate dehydrogenase (LDH) is a nonspecific cellular injury marker, but because perfusate samples were collected from an isolated kidney perfused on the pump, LDH release could reflect general renal injury. Aspartate aminotransferase (ASAT) is an enzyme that facilitates the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate. Although clinically most often associated with the liver, ASAT is also found in renal parenchymal cells. ASAT is associated with acute damage to parenchymal cells (*12*). Glutathione-*S*-transferase (GST) is an enzyme localized in the renal tubules. It is involved in deconjugation of waste products and excreted into the urine (*13*). Although α-GST is most directly associated with proximal tubular injury, total GST (the sum of α-GST and π-GST) is easier to measure. Total GST has been shown to also reliably reflect renal tubular injury and has become the most often used biochemical marker for kidney injury assessment during MP. In this article, the abbreviation GST refers to total GST. Alanine-aminopeptidase (Ala-AP) is an exopeptidase with a role in cell regulation and is also excreted into the urine (*14*). Ala-AP release is associated with renal tubular injury. *N*-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme present in various tissues in the body, including the kidney, and its release is also associated with ischemic tubular damage (*15*). Heart-type fatty acid binding protein (H-FABP) is a cytosolic protein, located in the distal renal tubules and involved in free fatty acid transport from the cytosol to mitochondria, and is mainly found in the urine (*16*). Increased H-FABP release has been associated with ischemic kidney tissue injury (*17*).

RESULTS

In 306 of 376 kidney transplants in the MP arm of the RCT, suitable perfusate samples were available for biomarker analysis. Table 1 presents baseline characteristics and outcome of these transplants. The baseline values did not differ significantly from the characteristics of the whole MP arm of 376 cases.

We found that the concentration of most biomarkers, except for Ala-AP, did not change considerably after 4 to 6 hr of MP (Fig. 1). This finding is further supported by the observation that there was no relevant correlation between cold ischemic time (CIT) and the concentration of any of the six perfusate biomarkers measured at the end of MP.

Univariate Tests

Table 2 presents that kidneys that developed DGF after transplantation were those that had significantly higher GST and H-FABP concentrations already after 1 hr of MP. Because these two biomarkers seemed to show such an early discriminative potential, we also tested whether their concentrations in donor plasma just before organ retrieval were higher for kidneys that developed DGF after transplantation versus kidneys with immediate function. No significant difference was detected (also see **Supplemental Digital Content 1,** http://links.lww.com/TP/A243). At the end of MP, all biomarkers except Ala-AP had a significantly higher median perfusate concentration for kidneys that developed DGF versus grafts with immediate function. In contrast, at both time points, there was no difference in biomarker release between kidneys that did and did not develop primary nonfunction (PNF). The Kaplan-Meier analyses (see **Figures, Supplemental Digital Content 1,** http://links.lww.com/TP/A243) showed that death-censored GS up to 1 year after transplantation was not significantly different for grafts with any biomarker concentration (end of MP) above the median versus those with concentrations below the median. Receiver-operator curves (ROCs) investigating each biomarker's predictive accuracy for DGF yielded areas under the curve of 0.60 for LDH, 0.61 for ASAT, 0.67 for GST, 0.57 for Ala-AP, 0.64 for NAG, and 0.64 for H-FABP (Fig. 2). In the **Supplemental Digital Content** (http://links.lww.com/TP/A243), we show Pearson's correlation coefficients between the six biomarkers measured and for each biomarker's correlation with renal vascular resistance at the end of MP and with CIT. None of the biomarkers had a relevant correlation with renal resistance or with CIT. The strongest correlation that we found was the one between LDH and ASAT (0.56). Supplemental figures (see **Supplemental Digital Content 1**, http://links.lww.com/TP/A243) show that (except for Ala-AP) the curve of each biomarker's

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TABLE 1. Donor, recipient, and transplant demographics and overall posttransplant outcome for the study group

	Overall (n=306)	DBD (n=231)	DCD (n=75)	IF (n=230)	DGF (n=76)	PNF (n=7)
Donor demographics						
Donor age[a] (yr)	50 (16–78)	52 (16–78)	43 (17–65)	50 (16–77)	50 (18–78)	44 (37–63)
Female donor (%)	39	42	28	39	40	57
DCD donor (%)	25	0	100	15	53	29
ECD donor[b] (%)	28	32	16	29	25	43
Traumatic cause of death (%)	23	22	27	24	21	0
Donor history of hypertension (%)	22	26	12	24	18	57
Donor history of diabetes mellitus (%)	5	5	5	4	9	0
Recipient demographics						
Recipient age[a] (yr)	53 (11–79)	54 (11–79)	51 (24–73)	53 (11–79)	54 (13–73)	46 (13–67)
Female recipient (%)	42	43	37	44	36	71
Total time spent on the waiting list[a] (yr)	5 (1–8)	5 (1–8)	5 (2–8)	5 (1–8)	5 (1–8)	7 (3–8)
Previous transplants (% ≥1)	31	24	56	25	53	43
PRA level >5% (%)	13	13	13	13	20	43
Immunosuppressive drugs (%)						
Prednisolone	98	97	99	97	99	100
Cyclosporine	47	47	48	46	53	43
Tacrolimus	51	51	49	51	49	57
Azathioprine	1	<1	1	<1	1	0
Mycophenolate mofetil	88	85	93	87	88	100
Antithymocyte globulin	14	13	15	14	15	29
Transplant demographics						
HLA mismatches (% of 0 mismatches)	13	17	3	13	13	14
Cold ischemic time[a] (hr)	15 (4–30)	15 (4–30)	16 (4–27)	15 (4–30)	16 (8–27)	16 (14–25)
Posttransplant outcome						
Delayed graft function (%)	25	16	53	0	100	100
Duration of delayed graft function (d)[a]	10 (1–93)	9 (3–31)	10 (1–93)	n/a	10 (1–93)	n/a
Primary nonfunction (%)	2.3	2.2	2.7	0	9.2	100
Any acute rejection in first year (%)	23	24	21	19	36	14
1 yr death-censored graft survival (%)	95	95	93	97	87	0

n=306 transplants in the MP arm of the prospective study. Data are presented as those for the whole group (overall), as well as separate characteristics for kidneys derived from donation after brain death (DBD) and donation after cardiac death (DCD), and for patients with immediate function (IF), delayed graft function (DGF), or primary nonfunction (PNF). No statistical tests were performed on the data. Note that the PNF group consists of only seven cases, which makes a comparison with other groups less reliable.

[a] Median (range).

[b] ECD denotes expanded criteria donation, which was defined as donor age more than or equal to 60 yr, or donor age between 50 yr and 60 yr, with at least two of the following additional donor characteristics: (1) history of hypertension, (2) cerebrovascular cause of death, and (3) preretrieval serum creatinine >132 μmol/L.

n/a, not applicable.

evolution over time was significantly higher for kidneys that developed DGF versus those with immediate function and for donation after cardiac death (DCD) versus donation after brain death (DBD) kidneys (see **Figures, Supplemental Digital Content 1,** http://links.lww.com/TP/A243).

Multivariate Models

The logistic regression and Cox proportional hazards models (Table 3) showed that only GST, NAG, and H-FABP levels in the perfusate measured at the end of MP were true independent predictors for the risk of DGF. None of the six biomarkers had any significant independent predictive value for the risk of graft failure in the first year posttransplant.

DISCUSSION

Predicting outcome after kidney transplantation has been the topic of numerous, often retrospective, studies. Well-known pertinent donor and recipient factors as well as cold and warm ischemic time and the organ preservation modality are usually included into such multivariate risk assessments. Recently, Rao et al. (*18*) developed a comprehensive

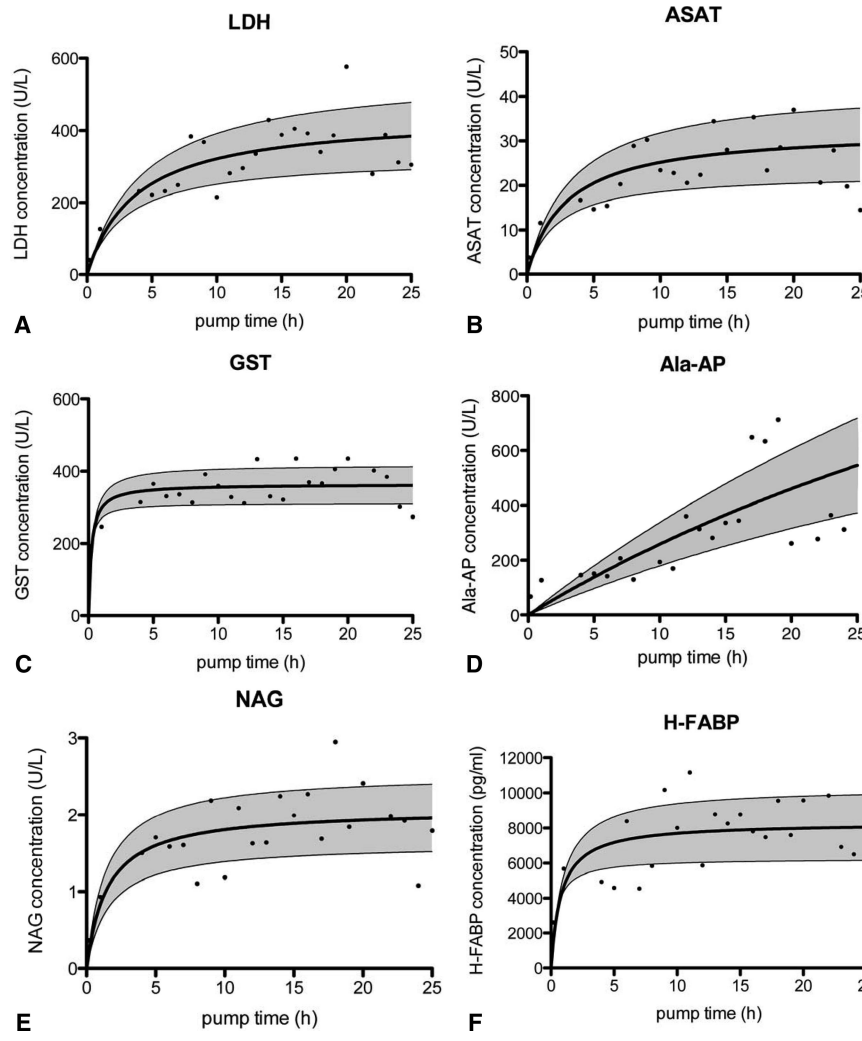

FIGURE 1. Evolution of each biomarker's perfusate concentration in time for A, lactate dehydrogenase, B, aspartate aminotransferase, C, glutathione-S-transferase, D, alanine-aminopeptidase, E, N-acetyl-β-D-glucosaminidase, and F, heart-type fatty acid binding protein. Mean biomarker concentrations per time point after the initiation of machine perfusion (MP) (*bullets*). Least square fit to the plotted data points (*bold lines*), with plus and minus standard error of the mean (*thin upper and lower lines and a gray area*). The baseline function used for each least square fit was a typical equation for molecular saturation in fluids: $y=ax/(x+b)$, where a and b are determined by the least square method. Curves were corrected for outliers using Dixon's Q test.

risk score to predict renal graft failure, and Moore et al. (*19*) conducted a study comparing the predictive value of several existing risk scores for early graft (dys) function. Both studies yielded useful tools to aid decisions on organ acceptance and early recipient management. However, current risk scores are no more than a sophisticated mathematic compilation of routinely collected variables that are already known to the transplant team. In an attempt to add something genuinely novel to the decision-making process, several groups have introduced various biomarkers that may have predictive potential for short- and long-term outcome. For example, evidence suggests that donor serum interleukins could be indicative for posttransplant complications (*20*). In the past, Daemen et al. and Gok et al. have performed analyses of the biomarkers GST, H-FABP, Ala-AP, and LDH in renal MP perfusate. Their studies found that biomarker concentrations were increased in perfusates of those kidneys that were discarded on other grounds and that uncontrolled (Maastricht category II) DCD grafts tended to have higher GST, H-FABP, and Ala-AP concentrations in the perfusate than kidneys recovered from controlled (category III) DCD procedures. In addition, LDH and GST levels correlated with warm ischemic time (*6–8*, *20*, *21*). However, these studies did not investigate whether any of these biomarkers was independently associated with outcome after transplantation. It is plausible that an association between the concentration of a certain substance in the perfusate and posttransplant results is no more than a surrogate marker for another underlying causal factor already known. For example, a longer warm ischemic time could result in an increased biomarker release into the MP perfusate due to more ischemic injury to the kidney. In that case, measuring these markers will not provide any extra information to the clinician, because simply considering warm ischemic time would be sufficient to appreciate the amount of injury to the graft. With this in mind, the univariate results of this study could be biased by confounding factors such as DCD versus DBD. As our supplemental figures show (see **Supplemental Digital Content 1,** http://links.lww.com/TP/A243), DCD kidneys release significantly more injury biomarkers into the perfusate, but such kidneys are already known to have inferior posttransplant outcome in more DGF. Measuring perfusate biomarkers is only worth the extra effort and expense when the biomarker of choice has a truly independent predictive value in the context of traditional prognostic factors. Therefore, multivariate analyses that correct for such likely confounding factors are essential to appreciate any biomarker's true prognostic potential.

TABLE 2. Univariate characteristics of biomarkers after 1 hr and at the end of MP

Biomarker concentration after 1 hr of MP			Overall
Lactate dehydrogenase (U/L)			95 (57–151)
Aspartate aminotransferase (U/L)			8 (5–13)
Glutathione-*S*-transferase (U/L)			218 (172–280)
Alanine-aminopeptidase (U/L)			80 (43–143)
N-acetyl-β-D-glucosaminidase (U/L)			0.70 (0.46–1.14)
Heart-type fatty acid binding protein (pg/mL)			4340 (2794–5950)
Biomarker concentration after 1 hr of MP	**No DGF**	**DGF**	***P***
Lactate dehydrogenase (U/L)	91 (55–146)	104 (71–167)	0.089
Aspartate aminotransferase (U/L)	8 (5–12)	8 (6–14)	0.070
Glutathione-*S*-transferase (U/L)	214 (169–278)	235 (202–297)	0.026
Alanine-aminopeptidase (U/L)	81 (38–147)	75 (45–131)	0.61
N-acetyl-β-D-glucosaminidase (U/L)	0.70 (0.45–1.14)	0.70 (0.47–1.26)	0.98
Heart-type fatty acid binding protein (pg/mL)	4018 (2692–5832)	4914 (3422–6244)	0.028
Biomarker concentration after 1 hr of MP	**No PNF**	**PNF**	***P***
Lactate dehydrogenase (U/L)	95 (57–148)	145 (44–175)	0.54
Aspartate aminotransferase (U/L)	8 (5–13)	8 (5–13)	0.98
Glutathione-*S*-transferase (U/L)	218 (172–281)	227 (164–307)	1.00
Alanine-aminopeptidase (U/L)	80 (42–145)	49 (25–97)	0.27
N-acetyl-β-D-glucosaminidase (U/L)	0.70 (0.46–1.14)	0.49 (0.44–1.42)	0.72
Heart-type fatty acid binding protein (pg/ml)	4339 (2804–5966)	4885 (2115–5656)	0.82
Biomarker concentration at end of MP			**Overall**
Lactate dehydrogenase (U/L)			304 (185–456)
Aspartate aminotransferase (U/L)			19 (12–33)
Glutathione-*S*-transferase (U/L)			324 (261–398)
Alanine-aminopeptidase (U/L)			246 (137–423)
N-acetyl-β-D-glucosaminidase (U/L)			1.44 (0.93–2.49)
Heart-type fatty acid binding protein (pg/mL)			5851 (4442–8608)
Biomarker concentration at end of MP	**No DGF**	**DGF**	***P***
Lactate dehydrogenase (U/L)	285 (173–415)	358 (227–529)	0.015
Aspartate aminotransferase (U/L)	18 (12–28)	25 (14–43)	0.006
Glutathione-*S*-transferase (U/L)	302 (256–382)	379 (308–465)	<0.0005
Alanine-aminopeptidase (U/L)	241 (122–420)	253 (184–444)	0.10
N-acetyl-β-D-glucosaminidase (U/L)	1.33 (0.91–2.22)	1.98 (1.21–3.39)	0.001
Heart-type fatty acid binding protein (pg/mL)	5178 (4120–7980)	7325 (5020–12248)	<0.0005
Biomarker concentration at end of MP	**No PNF**	**PNF**	***P***
Lactate dehydrogenase (U/L)	304 (182–456)	276 (213–675)	0.88
Aspartate aminotransferase (U/L)	19 (12–33)	21 (8–26)	0.71
Glutathione-*S*-transferase (U/L)	324 (261–401)	291 (213–368)	0.39
Alanine-aminopeptidase (U/L)	243 (133–422)	313 (182–470)	0.25
N-acetyl-β-D-glucosaminidase (U/L)	1.43 (0.93–2.44)	2.50 (1.23–3.76)	0.11
Heart-type fatty acid binding protein (pg/mL)	5855 (4442–8582)	5,833 (4,315–10,253)	0.80

Values are expressed as median (interquartile range).
MP, machine perfusion; DGF, delayed graft function; PNF, primary nonfunction.

This is the first prospective study that shows that GST, NAG, and H-FABP, measured in the MP perfusate at the end of MP, are independently associated with the risk of DGF and that LDH, ASAT, and Ala-AP do not seem to have such predictive potential. Therefore, measuring the former three markers will indeed provide an extra piece of information to clinicians who care for a kidney recipient. Nevertheless, because no marker could predict GS, we believe that there is no

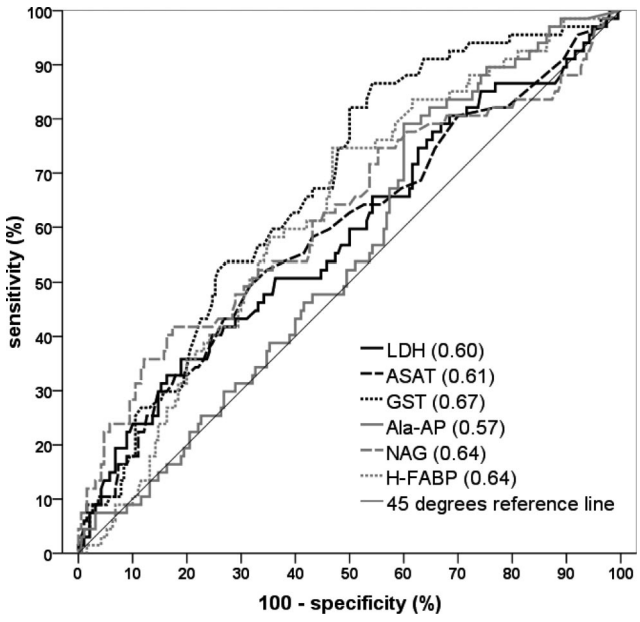

FIGURE 2. Receiver-operator curves for each of the six perfusate biomarkers' concentration at the end of machine perfusion (MP) at a continuous range of cut-off points. The numbers in *brackets* indicate the area under the curve for each line.

rationale to discard a kidney based on such measurements. DGF may be an unwelcome postoperative complication but given the present donor organ shortage a known increased risk of DGF will seldom be the reason to refuse a renal graft. Several centers worldwide already use one of the perfusate biomarkers discussed in this article for pretransplant kidney quality assessment to aid decisions on acceptance or discard of donor kidneys. The results of this analysis are of immediate clinical importance, because our data suggest that this so called "evidence based" decision making is probably not justified. However, prior knowledge of an increased DGF risk could be useful to fine-tune recipient management. Our data set is likely to be more reliable and has no selection bias compared with other studies: Data collection for this study was multicenter, prospective, and no kidneys were discarded based on biomarker measurements or other MP-related characteristics.

With ROC analysis, we sought for relevant cut-off values for each biomarker's concentration (*22*). In this analysis, all areas under the ROC were below 0.8, with the value for GST (0.67) most approaching a reliable predictive test. These results do not allow to determine cut-off values for the three predictive biomarkers because sensitivity or specificity will be poor. A more practical approach could be to consider the biomarker's concentration as a continuous variable in the context of other predictive factors. As usual, it is the clinician's task to make a balanced judgment of DGF risk, taking all such relevant factors into account.

The univariate results of this study suggest that GST and H-FABP have significantly higher levels in the perfusate already after 1 hr of MP in kidneys that develop DGF. However, our multivariate assessment shows that this association does not persist when tested against relevant confounding factors. In addition, GST or H-FABP measured in donor plasma did not predict DGF. Therefore, measuring these biomarkers in the donor or already after 1 hr of MP may be too early to draw reliable conclusions about DGF risk.

Interestingly, no biomarkers correlated with renal intravascular resistance during MP. This finding suggests that perfusate biomarkers reflect a different aspect of renal injury than intravascular resistance does. Perfusate markers in our study are most likely related to tubular injury, whereas vascular resistance may reflect endothelial damage.

A possible limitation of this study is that the most important time point at which three biomarkers were independent predictors of DGF (i.e., end of MP) was not standardized and could be anywhere between 4 and 25 hr after initiation of MP. This may have introduced more variance in these data. However, none of the six markers had a relevant correlation with CIT, and we found that five of six curves showing the average accumulation of each of the biomarkers' concentration in time followed an almost horizontal course after 4 to 6 hr of MP. This is in line with previous findings (*21*). Therefore, any time point after 4 to 6 hr is likely to be suitable to take a representative perfusate biomarker sample. This will add to clinical applicability, because organ transport logistics will not always allow perfusate sampling at a fixed time point. Because an increased perfusate GST, NAG, or H-FABP concentration will at best lead to adjusted recipient management and not to kidney discard or reallocation, the late availability of test results at the end of MP should not be a major concern for clinicians.

It is important to note that the incidence of PNF was low in our data set. Hence, comparisons between groups are unreliable for this endpoint. Nevertheless, because there was a considerable number of graft failures in the first year posttransplant, 1-year GS does provide a reliable endpoint in our data to assess this single most important outcome measure after transplantation.

Another limitation of our data is that this study did not include uncontrolled (Maastricht categories I and II) DCD kidneys. These are the organs that have sustained most ischemic damage, rendering viability testing during MP even more relevant (*21*, *23*). In addition, it is conceivable that a biomarker will be predictive for graft failure in the context of such extremely marginal kidneys. Similar multivariate analyses with cohorts of recipients of these organs are needed to determine whether perfusate markers could predict more than just DGF. Unfortunately, to date, large series of such transplantations remain rare.

In conclusion, this study demonstrated that GST, NAG, and H-FABP released in the perfusate and measured at the end of MP are independent predictors for DGF but not for GS in the first year after kidney transplantation. Because their prognostic value for DGF is at best moderate, these markers should always be considered in the context of other known variables. LDH, ASAT, and Ala-AP do not possess independent predictive potential for posttransplant outcome. Given the results of our analysis, an increased GST, NAG, or H-FABP concentration in the MP perfusate could be an additional trigger to adjust postoperative recipient management. However, in defiance of the practice in various transplant centers worldwide, this prospective study for the first time showed that the values of any of these markers may not

TABLE 3. Multivariate risk analysis[a] for delayed graft function and graft failure

Biomarker covariate	Odds ratio/hazard ratio (95% CI)[b]	P
Risk of delayed graft function (biomarker measured after 1 hr of MP)		
Lactate dehydrogenase (log[U/L])	1.43 (0.94–2.19)	0.10
Aspartate aminotransferase (log[U/L])	1.34 (0.90–2.00)	0.16
Glutathione-*S*-transferase (log[U/L])	1.90 (0.82–4.42)	0.14
Alanine-aminopeptidase (log[U/L])	0.81 (0.57–1.17)	0.26
N-acetyl-β-D-glucosaminidase (U/L)	1.17 (0.78–1.76)	0.45
Heart-type fatty acid binding protein (log[pg/mL])	1.27 (0.84–1.93)	0.26
Risk of delayed graft function (biomarker measured at end of MP)		
Lactate dehydrogenase (log[U/L])	1.09 (0.68–1.74)	0.73
Aspartate aminotransferase (log[U/L])	0.97 (0.63–1.51)	0.91
Glutathione-*S*-transferase (log[U/L])	3.21 (1.37–7.50)	0.007
Alanine-aminopeptidase (log[U/L])	1.03 (0.70–1.49)	0.90
N-acetyl-β-D-glucosaminidase (U/L)	1.31 (1.04–1.66)	0.02
Heart-type fatty acid binding protein (log[pg/mL])	1.91 (1.18–3.08)	0.008
Risk of graft failure within the first year posttransplant[c] (biomarker measured at end of MP)		
Lactate dehydrogenase (log[U/L])	0.94 (0.43–1.97)	0.83
Aspartate aminotransferase (log[U/L])	0.74 (0.36–1.50)	0.40
Glutathione-*S*-transferase (log[U/L])	0.31 (0.06–1.49)	0.14
Alanine-aminopeptidase (log[U/L])	1.05 (0.55–2.02)	0.89
N-acetyl-β-D-glucosaminidase (U/L)	1.06 (0.86–1.32)	0.57
Heart-type fatty acid binding protein (log[pg/mL])	0.81 (0.41–1.60)	0.54

For each of the six biomarkers, a separate multivariate model was built. Only the adjusted odds/hazard ratios and *P* values for the biomarker of interest are given.

[a] Logistic regression models for delayed graft function, and Cox proportional hazards models for graft failure. Other covariates in each model were renal vascular resistance at the end of MP (mm Hg/mL/min), donor age (yr), donor type (DCD vs. DBD), CIT (hr), the duration of pretransplant dialysis (yr), the number of previous transplants of the recipient, recipient age (yr), and the number of HLA mismatches.

[b] Odds ratios apply to the logistic regression model, and hazard ratios apply to the Cox proportional hazards model.

[c] Censored on death with a functioning graft.

MP, machine perfusion; DCD, donation after cardiac death; DBD, donation after brain death; HLA, human leukocyte antigen; CIT, cold ischemic time.

be used for the decision to transplant or discard a DBD or controlled DCD kidney.

MATERIALS AND METHODS

Donors and Recipients

As published previously (*1*), a total of 376 deceased-donor kidney pairs were included in the extended data set of our RCT between November 1, 2005, and August 17, 2007. Of these inclusions, 294 were DBD, and 82 were controlled DCD (Maastricht category III). One graft of each donor's kidney pair was cold stored, and the contralateral organ was preserved by MP. For this study, we analyzed perfusate biomarkers and follow-up data of the recipients in the MP arm of our trial. For detailed information on study design, randomization, logistics, and data collection, we refer to our previous publication (*1*).

Machine Perfusion

LifePort Kidney Transporter machines (Organ Recovery Systems, Itasca, IL) were used for perfusion, delivering a pulsatile flow of University of Wisconsin MP solution (kidney preservation solution-1) (*24*) at 1°C to 8°C, with a systolic perfusion pressure fixed at 30 mm Hg. Kidneys were machine perfused immediately after organ retrieval and flush out, until transplantation. To prevent bias in clinical decisions about transplanting or discarding an organ, intravascular resistance, flow readings, and biomarker concentrations were never revealed to the transplantation team.

Sample Collection and Biochemical Analysis

Perfusate samples of 10 mL were drawn after 10 min, after 1 hr, and at the end of the preservation period just before transplantation. All samples were stored on ice during transport and thereafter at −80°C until further analysis. Details on the methodology of biochemical analysis are provided as Supplemental Digital Content (see **Supplemental Digital Content 1,** http://links.lww.com/TP/A243).

Study End Points

DGF and PNF were analyzed as outcome measures of short-term graft function. DGF was defined as dialysis requirement in the first week posttransplant. PNF was scored when a kidney graft never showed sufficient function to prevent the need for dialysis after transplantation. Death-censored GS served as endpoint for graft performance up to 1 year posttransplant.

Statistical Analysis

First, univariate comparisons were made for each biomarker. The Mann-Whitney *U* test investigated whether biomarker concentrations were significantly different in recipients with and without DGF and PNF. We used the Kaplan-Meier method with log-rank tests to obtain a univariate comparison of GS up to 1 year between recipients of kidneys with a biomarker value below and above the median.

Second, for each individual biomarker, logistic regression models were constructed to find independent risk factors for DGF, and Cox proportional

hazards models were used to identify independent risk factors for graft failure (*25*). Apart from the biomarker of interest, other covariates in these models were renal vascular resistance at the end of MP (mm Hg/mL/min), donor age (years), donor type (DCD vs. DBD), CIT (hr), the duration of pretransplant dialysis (years), the number of previous transplants of the recipient, recipient age (years), and the number of human leukocyte antigen mismatches. These particular covariates were chosen for the models of this study because they were significant predictors of early posttransplant outcome in our data set (*1*). To prevent overfitting of the models, other covariates that had no significant impact on outcome in the RCT were not considered in the multivariate analyses of this study. For all multivariate analyses, except those for NAG, biomarker concentrations had to be log transformed to better approach a normal distribution. Two-sided *P* values under 0.05 were considered to indicate statistical significance.

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