

SUPPLEMENTAL DATA

METHODS

Study population

The impact of DGF on 10-year graft survival was evaluated in 6635 deceased-donor kidney transplantations performed between 2000 and 2018 (Netherlands Organ Transplant Registry (NOTR)). Combined organ procedures, procedures in recipients younger than 12 years and uncontrolled circulatory death donor procedures were excluded.

The impact of DBD and DCD donor type on DGF phenotype and functional recovery dynamics was assessed for 287 DBD and 312 DCD kidney transplantations performed at the Leiden University Transplant Center between 2007 and 2018. In addition to previously described exclusion criteria, we excluded grafts with primary non-function. DGF was defined as the need for dialysis in the first postoperative week(s), followed by functional recovery and with exception of a single dialysis performed for elevated potassium levels or fluid overload. The MDRD (Modification of Diet in Renal Disease) Study equation was used to estimate glomerular filtration rate (eGFR). For recipients with DGF, the first autonomous eGFR ('week 1') was defined as the first week following last dialysis. Factors included in the DGF phenotype were duration of dialysis, number of dialysis and the first autonomous eGFR.

Histology and gene expression.

Routine pre-reperfusion graft biopsies are used for graft quality control. All biopsies were collected after static cold storage, and prior to reperfusion. Biopsy samples from 80 donor kidneys were randomly selected based on donor type and the presence or absence of DGF (n=20 per group, Supplemental Table 1). One biopsy contained non-renal tissue and was excluded. Tissue sections (4µm) were cut and immunohistochemical staining was performed for BCL2, IGF-1R, p53, PCNA, phospho-EGFR, phospho-MAPK14, phospho-mTOR, PPARγ. Details of the antibodies and procedures are summarized in Supplemental Table 2. Anti-rabbit/mouse EnVision+ (Dako, Amstelveen, The Netherlands) and 3,3'-diaminobenzidine (DAB) substrate chromogen system (Dako, Amstelveen, The Netherlands) were used for antibody visualization. Tissue sections were counterstained with hematoxylin (Klinipath, Duiven, The Netherlands). All tissue sections were semi-quantitatively and independently reviewed by two observers (M.K. and J.T.N) and scored as: 0 (none), 1 (minimal), 2 (slight), 3 (moderate) and 4 (abundant). Scoring disagreements were identified and resolved by joint review to achieve consensus.

Gene expression profiling of pre-reperfusion renal biopsies and Ingenuity®Pathway Analysis (IPA®, QIAGEN, USA) was used to identify differentially regulated pathways in 23 DBD and 16 DCD grafts (Supplemental Table 3). All biopsies were collected after static cold storage, and prior to reperfusion. Biopsies were either immediately snap frozen in liquid nitrogen or stabilized in RNAlater.^{5,6} Samples were all stored at -80°C. Total RNA was isolated from renal tissues using RNeasy (Qiagen, Venlo, The Netherlands)⁵ or using TRIzol Reagent according

to the manufacturer's instructions (Invitrogen, UK),⁶ cleaned and DNase treated (RNA Clean & Concentration, #R1015, Zymo Research, USA) then stored at -80°C for further analysis. RNA integrity was determined (RNA Nano kit and 2100 BioAnalyzer, #5067–1511, Agilent Technologies, Inc. USA) and samples with $\text{RIN} > 6.0$ were used for further

analysis. Briefly, total RNA was used to create libraries using ribosomal depletion (TruSeq Stranded Total RNA Ribo-

Zero H/M/R Gold, Illumina). Libraries were further assessed by Qubit® (Life Technologies, Inc. USA) and Bioanalyser (High Sensitivity DNA kit [#5067–4626, Agilent Technologies, Inc., USA]). Libraries were sequenced on a

NextSeq500 (Illumina) using a paired-end 2×75 bp run. Detailed methods and analysis approaches were described

previously.⁶ Raw count data were transformed to log₂ scale to normalize expression counts. Multiple testing correction was performed using the Benjamin–Hochberg approach to control false discovery rate (FDR) at 10% ($\text{FDR} \leq 0.1$ was considered significant). Differentially expressed gene targets were analysed using Ingenuity®Pathway Analysis (IPA®, QIAGEN, USA).

Statistical analysis

STATA/SE version 12.0 (StataCorp, Texas, USA) and IBM SPSS Statistics 23.0 (Amsterdam, The Netherlands) were used for statistical analysis. Comparisons between groups were performed using the Mann-Whitney rank test and Kruskal-Wallis test for non-parametric data, independent t-test for normal-distributed data, and the Chi-Square test for categorical data. Cox proportional hazards models, censored for early graft loss (defined as functional graft loss within 90 days after transplantation) and recipient death, were used to evaluate differences in impact of DGF on 10-year graft survival. The model adjusted for donor/recipient age and sex, and cold ischemic period. An interaction (Wald) test was used to test the differences between the two models in DCD and DBD grafts. Factors associated with DGF were identified by multivariate regression analysis. The model included all variables with a p-value < 0.10 in the univariate analysis. P-values < 0.05 were considered statistically significant.

Supplemental Table 1. Patient and transplant characteristics of the biopsies used for immunohistochemical evaluation.

		DBD DGF -	DBD DGF +	DCD DGF –	DCD DGF +	p-value
		n = 20	n = 20	n = 20	n = 19	
Donor	Age (years)	55 [49-63]	58 [44-64]	61 [52-64]	63 [52-65]	0.53
	Sex (% male)	11 (55%)	9 (45%)	12 (60%)	11 (58%)	0.79
Recipient	Age (years)	58 [46-69]	54 [44-67]	65 [54-69]	55 [46-73]	0.42
	Sex (% male)	10 (50%)	10 (50%)	16 (80%)	15 (79%)	0.06
Transplant	First warm ischemic period (min.)	NA	NA	14 [12-17]	16 [13-18]	0.28
	Cold ischemic period (hours)	10.7 [8.6 - 14.0]	14.9 [8.9 - 18.5]	11.7 [10.3 - 13.9]	12.1 [10.2 - 15.8]	0.31
	Graft anastomosis time (min.)	29 [22-33]	33 [28-38]	32 [27-37]	31 [23-38]	0.36
	Number of dialysis after transplantation	NA	4 [2-5]	NA	5 [3-7]	0.09

Data are presented as number (%) or as median [25 and 75 IQR]. NA: not applicable.

Supplemental Table 2. Details of antibodies used in this study.

Primary Antibody	Clone	Source	Retrieval	Dilution	Manufacturer	Catalog no.
BCL2	124	Monoclonal mouse	Tris EDTA	1:300	Dako	M0887
IGF-1R	3G5C1	Monoclonal mouse	Citrate	1:1000	ThermoFisher Scientific	MA5-15354
P53	DO-7	Monoclonal mouse	Tris EDTA	1:50	ThermoFisher Scientific	MA5-12557
PCNA	PC10	Monoclonal mouse	Citrate	1:300	ThermoFisher Scientific	13-3900
Phospho-EGFR	S.684.2	Monoclonal mouse	Tris EDTA	1:900	ThermoFisher Scientific	MA-15199
Phospho-MAPK14	-	Polyclonal rabbit	Citrate	1:300	Merck	SAB4300201
Phospho-mTOR	Ser 2481	Monoclonal mouse	Citrate	1:100	SantaCruz Biotechnology	Sc-293089
PPAR γ	-	Polyclonal rabbit	Citrate	1:300	Bio-Rad	AHP1461

Supplemental Table 3. Patient and transplant characteristics of the biopsies used for gene expression profiles and Ingenuity Pathway Analysis.

		DBD	DCD	p-value
		n = 23	n = 16	
Donor	Age (years)	54.2 (15.7)	47.8 (15.8)	0.22
	Sex (% male)	11 (47.8%)	7 (43.8%)	0.70
Recipient	Age (years)	53.7 (12.9)	58.3 (8.3)	0.23
	Sex (% male)	12 (52.2%)	11 (68.8%)	0.38
Transplant	Cold ischemic period (hours)	12.5 [9.4 - 16.8]	11.6 [8.8 - 15.3]	0.51
	Graft anastomosis time (min.)	31.5 [24.8 - 33.5]	28.5 [24.0 - 30.8]	0.40
	Delayed graft function (% yes)	7 (30.4%)	12 (75.0%)	0.02

Data are presented as mean \pm standard deviation (SD) or as number (%) or as median [25 and 75 IQR].

Supplemental Table 4. Baseline patient and transplant characteristics.

		DBD n = 3744 (56.4%)	DCD n = 2891 (43.6%)	p-value
Donor	Age (years)	50.0 (15.0)	49.6 (15.0)	0.261
	Sex (% male)	1783 (47.6%)	1682 (58.2%)	< 0.001
	Height (cm)	173.0 (9.9)	175.0 (10.3)	< 0.001
	Weight (kg)	76.0 (15.6)	77.9 (16.7)	< 0.001
	BMI (kg/m ²)	25.3 (4.3)	25.3 (4.6)	0.648
	Last creatinine (μmol/L)	70.7 [56.0 - 91.0]	67.0 [53.0 - 83.0]	< 0.001
	Cause of death			< 0.001
	- Trauma	736 (19.7%)	806 (27.9%)	
	- Stroke	2241 (59.9%)	1123 (38.8%)	
	- Cardiac arrest	124 (3.3%)	418 (14.5%)	
	- Other	643 (17.2%)	544 (18.8%)	
	Hypertension (% yes)	984 (30.1%)	579 (21.1%)	< 0.001
	Diabetes (% yes)	168 (6.2%)	149 (5.7%)	0.407
	Smoking (% yes)	1760 (51.4%)	1378 (50.6%)	0.492
Recipient	Age (years)	52.1 (14.6)	54.1 (13.3)	< 0.001
	Sex (% male)	2170 (58.0%)	1806 (62.5%)	< 0.001
	Height (cm)	170.7 (10.2)	171.8 (10.3)	< 0.001
	Weight (kg)	73.9 (15.4)	76.7 (15.4)	< 0.001
	BMI (kg/m ²)	25.3 (4.5)	26.0 (4.4)	< 0.001
	Pre-emptive (% yes)	62 (1.7%)	57 (2.0%)	0.333
	Panel reactive antibodies			< 0.001
	- PRA <6%	3172 (84.7%)	2640 (91.3%)	
	- PRA ≥6 and <85	496 (13.3%)	235 (8.1%)	
	- PRA ≥85	75 (2.0%)	16 (0.6%)	
	Mismatches			
	HLA-DR 0	1558 (41.7%)	922 (32.1%)	< 0.001
	1	1883 (50.4%)	1718 (59.8%)	
	2	292 (7.8%)	231 (8.0%)	
	HLA-A 0	1451 (38.9%)	878 (30.4%)	< 0.001
	1	1828 (49.0%)	1577 (54.7%)	
	2	455 (12.2%)	429 (14.9%)	
	HLA-B 0	978 (26.2%)	476 (16.5%)	< 0.001
	1	1873 (50.2%)	1706 (59.2%)	
	2	883 (23.6%)	702 (24.3%)	
Transplant	First warm ischemic period (min.)	NA	17.0 [14.0 - 21.0]	NA
	Cold ischemic period (hours)	17.0 [13.1 - 22.0]	16.0 [12.6 - 20.1]	< 0.001
	Graft anastomosis time (min.)	34.0 [26.0 - 41.0]	32.0 [26.0 - 40.0]	0.003

Data are presented as mean (± standard deviation) or as number (%) or as median [25 and 75 IQR]. NA: not applicable.

Supplemental Table 5. Multivariate analysis (Odds Ratio (95% CI)): risk factors associated with DGF.

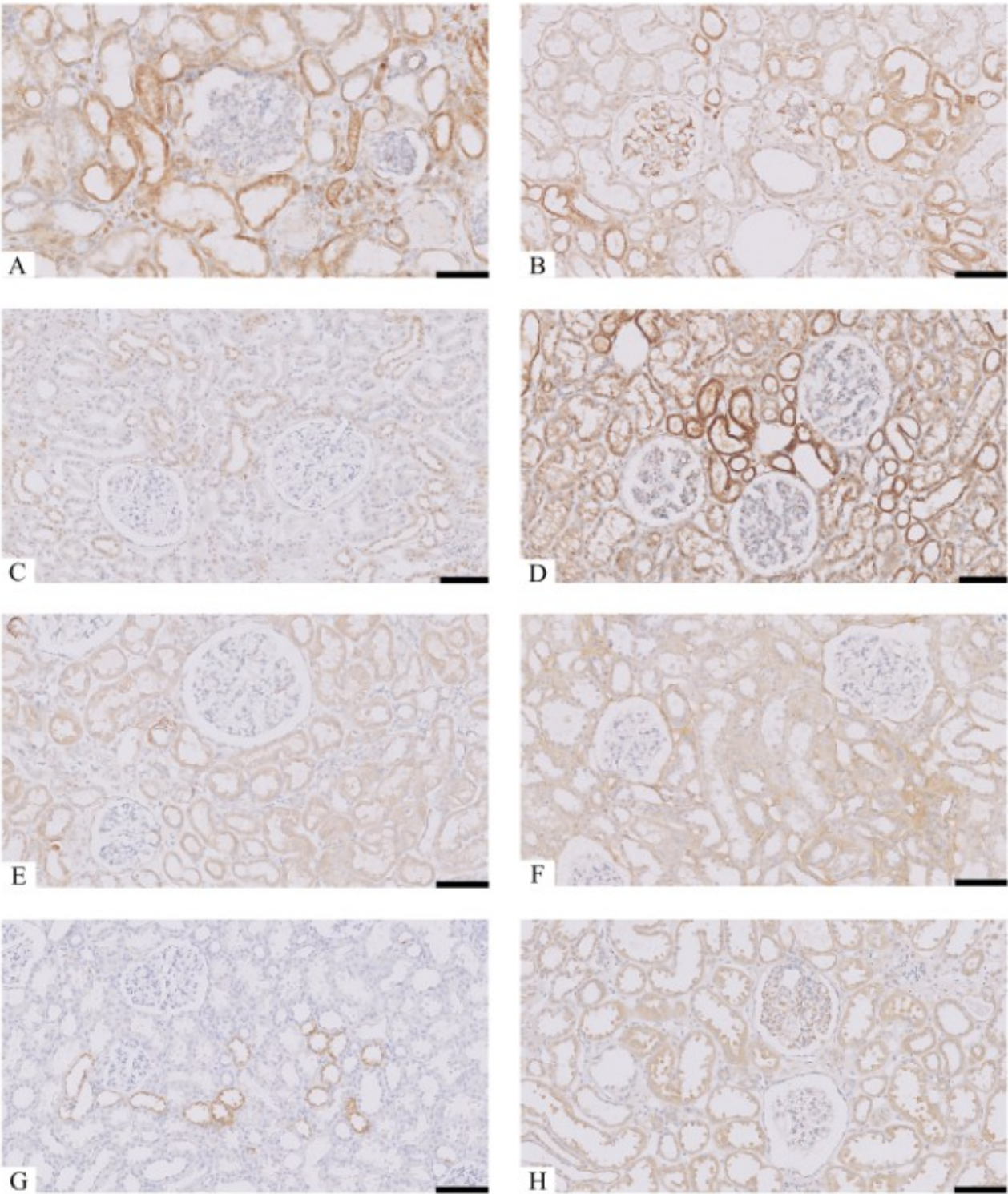
	DBD	DCD
Donor age	1.012 (1.005 - 1.019)**	1.006 (0.999 - 1.012)
Donor last creatinine (μmol/L)	1.008 (1.006 - 1.010)**	1.000 (0.996 - 1.003)
Mismatch HLA-DR	1.215 (1.036 - 1.426)*	1.168 (0.992 - 1.357)
First warm ischemic period (min.)	NA	1.017 (1.004 - 1.031)*
Cold ischemic period (hours)	1.035 (1.021 - 1.049)**	1.019 (1.002 - 1.036)*
Graft anastomosis time (min.)	1.017 (1.010 - 1.024)**	0.996 (0.989 - 1.003)

*p<0.05; ** p<0.005, NA: not applicable.

Supplemental Figure 1. Immunohistochemical scoring of pre-reperfusion kidney biopsies. Bars represent mean \pm standard deviation.

Expression of BCL2 (Kruskal-Wallis test $p = 0.653$), IGF-1R ($p = 0.340$), p53 ($p = 0.268$), PCNA ($p = 0.846$), phospho-MAPK14 ($p = 0.510$), phospho-mTOR (0.554), PPAR γ ($p = 0.350$) did not differ between groups. Expression of phospho-EGFR was lower in DCD grafts without DGF ($p = 0.002$).

Supplemental Figure 2. Immunohistochemical staining of pre-reperfusion kidney biopsies for factors associated with tumor resilience. Bars represent 100μm.



A = BCL-2; B = IGF-1R; C = p53; D = PCNA; E = phospho-EGFR; F = phospho-MAPK14; G = phospho-mTOR; H = PPAR γ .

Supplemental Figure 3. Illustration of Ingenuity Pathway Analysis (IPA). Strong influences were found on pathways collectively labelled by IPA as “cardio-vascular diseases”. The network annotated as “advanced stage peripheral artery disease” constitutes the most differentially upregulated network (p-value <0.001). This network is dominated by members of the heat shock protein family.

