

Figure 1. Box-Whisker-Plots of the four biomarkers in the tubulointerstitium. Boxplots show the median and the 1.5 interquartile range of the log₂ (relative expression) measured in the qRT-PCR experiment. A value of zero equates same expression level like in the reference RNA.



Figure 2. ROC curves: Discrimination for DGF after transplantation using donor age (solid blue line), expression features (dashed red line), or the combination of both (dashed green line).

Table 1

Demographic data of transplant donors and recipients stratified by treatment assignment. Continuous data are provided as median (1st quartile, 3rd quartile), categorical data are given as counts.

	PF group	DGF group	p-value
Number of donors		24	na
Number of donor organs	17	17	na
Donor age (years)	44.0 (39 , 47)	50.0 (45 , 60)	0.005
Donor sex (f/m)	9/8	4/13	0.078
Last creatinine of donor (mg/dl)	0.82 (0.65 , 1.01)	1.34 (0.92 , 1.57)	0.011
Vasopressors used (n/y)	2/15	5/12	0.203
Multiorgan donors (n/y)	14/3	13/4	1.000*
Cause of death (trauma / intracranial hemorrhage) ^a	5/12	2/15	0.398*
Number of recipients	17	17	na
Recipient age (years)	47.7 (28.82 , 56.71)	51.6 (42.87 , 59.82)	0.302
Recipient sex (f/m)	5/12	7/10	0.473
Transplant number (1/2/3)	15/2/0	13/31	0.656*
Cold ischemic time (hours)	18.6 (15.26 , 23.10)	19.3 (16.24 , 22.35)	0.757
PRA latest (%)	0.0 (0.0 , 0.0)	0.0 (0.0 , 5.0)	0.521
Sum of HLA mismatches (0/1/2/3/4/5/6)	1/0/4/9/3/0	0/1/2/8/4/2	0.560*
Number of dialysis treatment (0/1/2/3/4/5)	17/0/0/0/0/0	0/0/10/4/1/2	<0.001*
Immunosuppression (CNI/else)	17/0	17/0	na
Induction therapy (none/antiCD25/ATG)	10/6/1	12/5/0	0.721

na ... not applicable, * Fisher's exact test

Table 2

Multivariable logistic regression model. The discriminative power of this model is indicated by a c-statistics of 0.83 (AUC). Given is the odds ratio (OR), the confidence interval, and the p-values.

Predictors	OR	95% CI		p-value
Donor age (per year)	1.108	1.005	1.221	0.040
LCN2 (AU)	1.269	0.851	1.890	0.242
HAVCR1 (AU)	1.443	0.943	2.208	0.091

AU: arbitrary units (log2-ratio of the amount of mRNA as found in the sample with respect to the concentration of the respective mRNA given by the universal reference mRNA used in the qRT-PCR experiments).

Table 3

Discrimination of the models and optimism (3A) derived from the re-sampling procedure (34-fold cross validation). Calibration of the DGF prediction model by the Hosmer-Lemeshow goodness of fit test (3B). The expected to observed number of cases in each of the deciles of patients were not statistically different suggesting good calibration (p=0.76, chi-square test).

Model	AUC (c-statistic)	AUC (X- validation)	Optimism
Donor age	0.78	0.74	0.04
LCN2, HAVCR1	0.80	0.71	0.09
LCN2, HAVCR1 and donorage	0.83	0.75	0.08

Group	Total	Total DGF number of patients Observed Expected		PF		
Group	patients			Observed	Expected	
1	3	0	0.20	3	2.80	
2	3	0	0.46	3	2.54	
3	3	1	0.79	2	2.21	
4	3	2	1.06	1	1.94	
5	3	1	1.30	2	1.70	
6	3	2	1.64	1	1.36	
7	3	2	1.91	1	1.09	
8	3	2	2.28	1	0.72	
9	3	3	2.66	0	0.34	
10	2	2	2.77	1	0.23	



Figure S1. Bias Test of pre-amplification technique: Dilution series from a microdissected nephrectomy sample (T: Tubulointerstium, G: glomeruli part) were measured with qRT-PCR for ACTB (ß-actin), LCN2 (lipocalin 2) and PPIA (Cyclophilin A) after a 10 cycle pre-amplification. All regression lines show excellent correlation between a wide dynamic range (125ng/µl to 1ng/µl cDNA concentration before pre-amplification). The regression lines of PPIA and LCN2 show similar slopes, therefore we decided to use PPIA as endogenous control gene.



Figure S2. Bias test for reverse transcription, pre-amplification and realtime PCR of the reference RNA: Correlation of fourteen different genes in the standard RNA (Stratagene Human reference RNA) is almost one, suggesting reference RNA is an excellent calibrator.

Table S1

Multivariable logistic regression model: LCN2 and HAVCR1. The discriminative power of this model is indicated by a c-statistics of 0.80 (AUC). Given is the odds ratio (OR), the confidence interval, and the p-values.

Predictors	OR	95% CI		p-value
LCN2 (AU)	1.328	0.918	1.920	0.133
HAVCR1 (AU)	1.356	0.948	1.941	0.096

AU: arbitrary units (log2-ratio of the amount of mRNA as found in the sample with respect to the concentration of the respective mRNA given by the universal reference mRNA used in the qRT-PCR experiments).

Table S2

Multivariable logistic regression model: Donor age, cold ischemic time (CIT), panel reactive antibodies (PRA), donor last creatinine, LCN2 and HAVCR1. The discriminative power of this model is indicated by a c-statistics of 0.86 (AUC). Given is the odds ratio (OR), the confidence interval, and the p-values.

Predictors	OR	95% CI		p-value
Donor age (year)	1.078	0.956	1.216	0.222
CIT (hours)	1.009	0.809	1.258	0.939
PRA (%)	1.031	0.968	1.100	0.342
Donor last creatinine (mg/dl)	4.592	0.226	93.367	0.321
LCN2 (AU)	1.146	0.727	1.808	0.558
HAVCR1 (AU)	1.445	0.889	2.348	0.137

AU: arbitrary units (log2-ratio of the amount of mRNA as found in the sample with respect to the concentration of the respective mRNA given by the universal reference mRNA used in the qRT-PCR experiments).

Table S3

Multivariable logistic regression model: Donor age, cold ischemic time (CIT), panel reactive antibodies (PRA) and donor last creatinine. The discriminative power of this model is indicated by a c-statistics of 0.85 (AUC). Given is the odds ratio (OR), the confidence interval, and the p-values.

Predictors	OR	95% CI		p-value
Donor age (year)	1.078	0.967	1.201	0.175
CIT (hours)	0.961	0.783	1.180	0.704
PRA (%)	1.036	0.975	1.101	0.255
Donor last creatinine (mg/dl)	10.797	0.922	126.451	0.058