Analysis of proteins secreted into donor kidney perfusion fluid for the prediction of delayed graft function

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Introduction

Kidney transplantation is the preferred option for end-stage kidney disease patients but shortage of donor kidneys is an increasing problem. Zero-hour biopsies analysis can assess kidney quality and have predictive value for short- and long-term transplantation outcome but require an invasive procedure and may not reflect the entire organ. Alternative, non-invasive and more representative methods to assess graft quality are required. We hypothesized that kidney preservation fluid contains secreted stress factors associated with graft quality, allowing prediction of kidney function and risk for complications after transplantation.

Protein profiling

Preservation fluid from donation circulatory death (DCD) kidney donors was collected by infusion of 40-60 mL saline solution into the renal artery. The flow-through, coming out from the renal vein, was collected in a sterile manner. Remaining blood cells were counted and the fluid was centrifuged at 2000 x g. The remaining cell-free supernatant was stored at -80°C until analysis (figure 1).

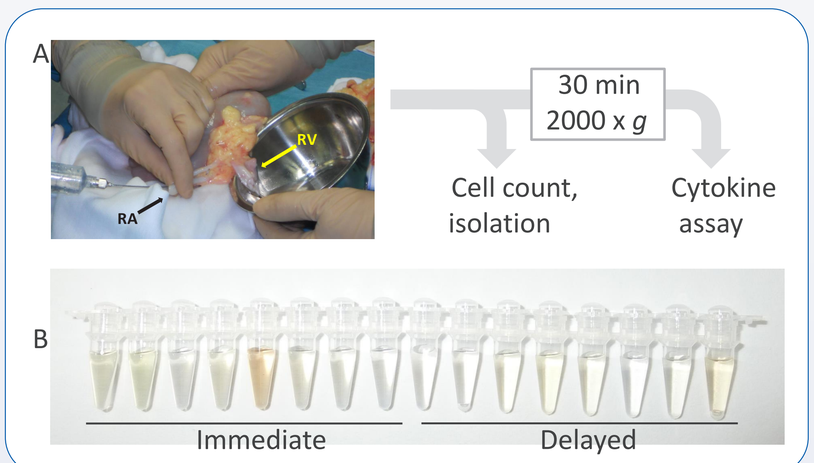

Figure 1: Isolation procedure. (A) Isolation procedure and (B) collected samples from the Immediate function (IF) and Delayed Graft Function (DGF) kidneys.

Levels of 158 secreted proteins were determined in samples from kidneys showing delayed graft function (DGF) and immediate function (IF; Table I). Nine proteins showed an association with DGF and were selected for further verification (figure 2).

Parameter	IF (n=8)	DGF (n=8)	P-value
Donor			
Age (years)	53,13 (14,54)	54,25 (17,40)	0.890
BMI	25,89 (2,21)	26,26 (4.19)	0.827
Hypertension (%)	3 (37,5%)	1 (12.5%)	0,278
Diabetes (%)	1 (12.5%)	1 (12.5%)	1.000
Creatinine (uMol)	80,13 (30,23)	54,75 (17,89)	0,060
KDRI (kidney donor risk index)	1,34 (0,42)	1,32 (0,27)	0,928
Recipient			
BMI	24,74 (2,71)	24,81 (3,50)	0,962
Male (%)	6 (75%)	5 (62.5%)	0,619
Peak PRA (%)	0 (0)	8,375 (13,60)	0,104
Dialysis duration (months)	26,0 (17,34)	51,63 (36,65)	0,116
Diabetes (%)	1 (12.5%)	2 (25%)	0,554
Organ			
HLA mismatches	3,50 (1.41)	2,63 (1.30)	0,219
CIT (hrs)	14.43 (3.79)	14.72 (5.34)	0.902
WIT (min)	30,86 (4,10)	27,50 (6,35)	0.253

Table I: Donor/recipient characteristics discovery panel. In both the immediate function (IF) and delayed graft function (DGF) groups 8 organs with matched donor, recipient and donor kidney characteristics were included.

Methods and Results

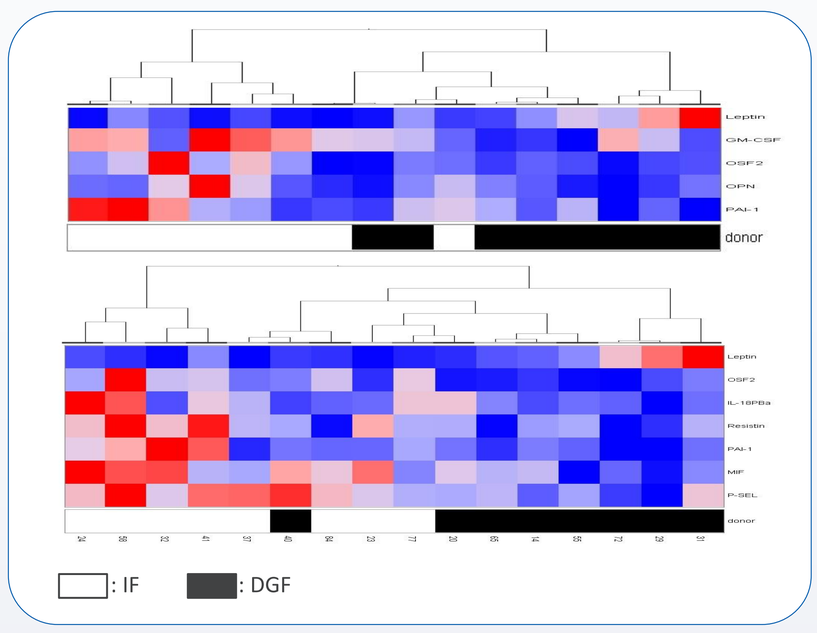

Figure 2: Identification of proteins associated with post-transplantation kidney function. Without correction for total protein content, 5 proteins showed an association with DGF (upper panel). After correction, 7 proteins, of which 3 overlapping protein that show an association with DGF (lower panel)

Verification of potential biomarkers

In total 9 protein were selected as potential biomarkers. We collected a verification panel of 40 additional perfusion fluids (Table 2) to verify identified associations of these proteins with post-transplantation kidney function and to develop a DGF prediction model (figure 3).

Parameter	IF (n=14)	DGF (n=26)	P-value
Donor			
Age (years)	52.29 (14.08)	56.81 (10.08)	0.247
BMI	24.72 (4.62)	27.29 (4.24)	0.084
Hypertension (%)	2 (14,29)	9 (34,62)	0,117
Diabetes (%)	0 (0)	2 (7,69)	0,299
Creatinine (uMol)	62,29 (22.81)	63,58 (21,04)	0,858
KDRI (kidney donor risk index)	1,22 (0,36)	1,33 (0,30)	0,315
Recipient			
BMI	25,38 (4,26)	28,50 (4,36)	**0,036**
Male (%)	9 (64.29%)	17 (65,38%)	0,946
Peak PRA (%)	7,07 (14,46)	6,145 (10,42)	0,816
Dialysis duration (months)	16,80 (19,34)	38,77 (19,86)	**0,002**
Diabetes (%)	1 (7.14%)	6 (23.08%)	0,143
Organ			
HLA mismatches	3,57 (1.09)	3.23 (1.36)	0,426
CIT (hrs)	14,66 (6.53)	14.80 (5.15)	0.939
WIT (min)	24.64 (6.02)	29.27 (10.71)	0.145

Table 2: Donor/recipient characteristics of the verification panel. Fluids from kidneys showing immediate function (IF; n=14) and delayed graft function (DGF; n=26) were collected for verification of 9 candidate biomarkers. Please note that recipient BMI and dialysis duration were significantly different between groups.

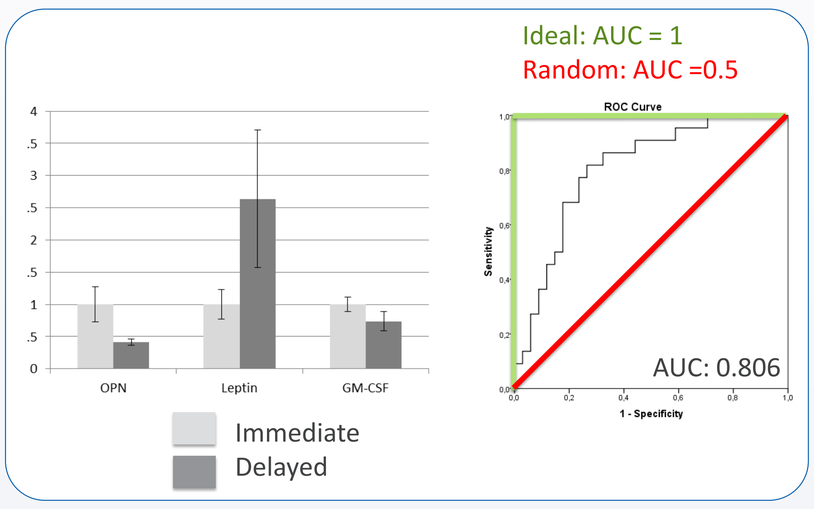

Figure 3: A DGF prediction model based on secreted proteins in perfusion fluid. In the entire cohort, three proteins (osteopontin [OPN], leptin and granulocyte/macrophage colony-stimulating factor [GM-CSF]) showed an association with DGF (left graph). Using logistic regression analysis, a model based on these factor was established. Reciever operated curve analysis (ROC; right graph) shows an area under the curve (AUC) of 0.806.

Clinical parameters

To improve the predictive value of our model, recipient BMI and dialysis duration were tested for their contribution to the model. Including both parameters increased the AUC of the ROC representing the model to 0.889 (figure 4). A model based solely on OPN and dialysis duration showed an AUC of 0.840, indicating the importance of these two factors in the prediction of DGF. Importantly, our models perform better than two algorithms based on clinical parameters alone[1,2], whose AUCs did not significantly differ from 0.500.

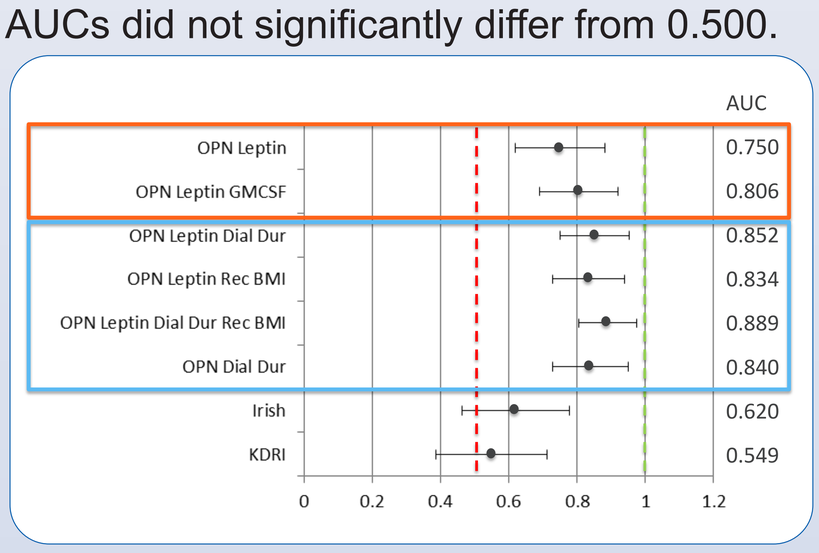

Figure 4: Comparison of different prediction models in our cohort. Prediction models based on secreted proteins alone (orange box) could be improved by the addition of clinical parameters (blue box). All models presented here perform better than models based on clinical parameters alone (Irish, KDRI).

References

1: Irish et al., Am J Transplant 2010 Oct;10(10):2279-86

2: Rao et al., Transplantation 2009 Jul 27;88(2):231-6.

Conclusion

We here describe a non-invasive, easy to implement method for the prediction of short-term kidney function after transplantation, based on levels of osteopontin, GM-CSF and leptin levels in donor kidney perfusion fluid, recipients BMI and dialysis duration. Additionaly, observed associations of osteopontin and GM-CSF levels suggest that recruitment of monocytes and macrophages plays a role in the early engraftment and short-term function of the donor kidney after transplantation.

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