

**SUPPLEMENTARY MATERIALS FOR**  
**Hypothermic Oxygenated New Machine Perfusion System in Liver and Kidney**  
**Transplantation of Extended Criteria Donors: First Italian Clinical Trial**

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**SUPPLEMENTARY METHODS**

**Liver graft histopathological analysis.**

Liver biopsies were sent to our Transplant Pathology Unit without any fixation media for the frozen-section analysis for graft suitability. After the frozen sections, tissue was fixed in formalin, embedded in paraffin and routinely processed. From paraffin blocks, 2- $\mu$ m-thick sections were cut for permanent Haematoxylin-Eosin and Reticulin stains.

In liver grafts, according to the guidelines adopted in our Institution<sup>1</sup>, 9 variables were separately evaluated:

- Percentage of macrovesicular steatosis;
- Percentage of microvesicular steatosis;
- Portal fibrosis according to Metavir<sup>2</sup>;
- Amount of portal inflammatory infiltrate and fibrosis according to Ishak<sup>3</sup>;
- Amount of lobular necrosis/inflammatory infiltrate according to Ishak;
- Arteriolar myointimal thickening, scored as absent, mild, moderate and severe;

- Biliocyte regressive changes, scored as absent, focal or diffuse;
- Presence of ductular reaction/neoductulogenesis;
- Presence of cholestasis.

### **Kidney graft histopathological analysis.**

Kidney biopsies were sent to our Transplant Pathology Unit in Serra solution for the histological analysis for graft suitability. Tissue was rapidly processed in microwave oven. From paraffin blocks, 2- $\mu$ m-thick sections were cut for permanent Haematoxylin-Eosin, Masson's Trichrome and Periodic Acid Schiff stains. Thirteen variables were separately evaluated:

- Glomerulosclerosis, according to Karpinski<sup>4</sup>;
- Tubular atrophy, according to Karpinski;
- Interstitial fibrosis, according to Karpinski;
- Vascular damage, according to Karpinski;
- Total Karpinski's score<sup>5</sup>;
- Glomerular ischemic changes, scored as absent, focal or diffuse;
- Mesangial matrix thickening, scored as absent, mild or moderate;
- Presence of thrombotic microangiopathy<sup>6</sup>;
- Acute tubular necrosis, scored as absent, focal or diffuse<sup>23</sup>;
- Isometric vacuolization of tubulocytes, scored as absent, focal or diffuse;
- Presence of intraluminal calcifications' tubuli;
- Prevalence of sclerotic or hyaline changes in arterial/arteriolar walls;
- Presence of interstitial inflammatory infiltrate.

### **Hypothermic oxygenated perfusion**

Organ perfusion has been started during the graft preparation at the surgical back-table in flushing at controlled pressure and flow (20 ml/min) and with new perfusion solution. Concluded the surgical preparation after 30-40 minutes, HOPE has been continued normally at controlled pressure only.

## REFERENCES

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**Supplementary Table S1 | Frequencies and percentages of the 9 histopathological variables evaluated in HOPE-L and SCS-L. No differences were found between the two groups.**

	<b>Sperimental Group</b>	<b>Control group</b>	<b>P value</b>
	<b>HOPE</b>	<b>SCS</b>	
<i>Liver Transplantation</i>	<i>N = 10</i>	<i>N = 30</i>	
Fibrosis (>2 Ishak)	2 (20%)	6 (20%)	0.524
Moderate portal inflammation	1 (11%)	5 (17%)	0.408
Lobular necrosis	2 (20%)	4 (13%)	0.352
Moderate/severe myointimal thickening	5 (50%)	14 (47%)	0.682
Biliocyte regressive changes	6 (60%)	19 (63%)	0.458
Ductular reaction	1 (10%)	6 (20%)	0.483
Cholestasis	2 (20%)	5 (17%)	0.527
Mean macrovesicular steatosis	3.1 ± 2.4	3.9±5.2	0.677
Mean microvesicular steatosis	8.11 ± 8.4	8.9±8.1	0.921

**Supplementary Table S2 | Frequencies and percentages of the 13 histopathological variables evaluated in HOPE-K and SCS-K. No differences were found between the two groups.**

	<b>Sperimental Group</b>	<b>Control group</b>	<b>P value</b>
	<b>HOPE</b>	<b>SCS</b>	
<i>Kidney Transplantation</i>	<i>N = 11</i>	<i>N = 30</i>	
Glomerulosclerosis (Karpinski's 1-2)	8 (73%)	11 (41%)	0.085
Tubular atrophy (Karpinski's 1-2)	6 (54%)	23 (77%)	0.317
Interstitial fibrosis (Karpinski's 1-2)	6 (64%)	21 (70%)	0.280
Vascular damage (Karpinski's 2-3)	4 (36%)	5 (17%)	0.639
Mean Karpinski's score	3.6±2.0	3.4±1.3	0.639
Ischemic glomeruli	6 (55%)	8 (27%)	0.108
Mesangial matrix thickening	8 (73%)	16 (53%)	0.497
Glomerular microangiopathy	0 (0%)	3 (10%)	0.296
Acute tubular necrosis (diffuse)	4 (36%)	5 (17%)	0.271
Vascular changes:- sclerosis	8 (73%)	21 (70%)	0.591
- hyalinosis	3 (28%)	9 (30%)	
Isometric vacuolization of tubuli	6 (64%)	9 (30%)	0.060
Calcifications of tubuli	1 (9%)	0 (0%)	0.324
Interstitial inflammatory infiltrate	3 (28%)	5 (17%)	0.404

**Supplementary Table S3 | Post-operative complication according to Clavien Dindo grade classification**

<i>Kidney-Clavien Dindo grade</i>	<i>HOPE (N = 10)</i>	<i>SCS (N = 30)</i>
I	0/10 (0%)	2/30 (6.7%)
II	1/10 (10%)	5/30 (16.7%)
IIIa	0/10 (0%)	1/30 (3.3%)
IIIb	1/10 (10%)	1/30 (3.3%)
IVa	2/10 (20%)	12/30 (40%)
IVb	0/10 (0%)	1/30 (3.3%)
V	0/10 (0%)	0/30 (0%)
<b>Total</b>	<b>4/10 (40%)</b>	<b>22/30 (73.3%)</b>
<i>Liver-Clavien Dindo grade</i>	<i>HOPE (N = 10)</i>	<i>SCS (N = 30)</i>
I	1/10 (10%)	4/30 (13.3%)
II	5/10 (50%)	10/30 (33.3%)
IIIa	1/10 (10%)	2/30 (6.7%)
IIIb	0/10 (0%)	1/30 (3.3%)
IVa	0/10 (0%)	3/30 (10%)
IVb	0/10 (0%)	0/30 (0%)
V	0/10 (0%)	1/30 (3.3%)
<b>Total</b>	<b>7/10 (70%)</b>	<b>21/30 (70%)</b>

**Supplementary Table S4 | Description of post-transplant complications**

<i>Kidney complication</i>	<i>HOPE</i>	<i>SCS</i>
Renal Replacement Therapy	2/10	12/30
Anemia	2/10	3/30
Ureteral stenosis	1/10	3/30
Infection	2/10	6/30
Acute rejection	1/10	2/30
Atrial fibrillation	0/10	3/30
Venous thrombosis	0/10	1/30
Bleeding	0/10	1/30
Acute pancreatitis	0/10	1/30
Dysuria	0/10	1/30
<i>Liver complication</i>	<i>HOPE (N=10)</i>	<i>SCS (N=30)</i>
Psychomotor agitation	2/10	1/30
Infection	4/10	4/30
Ascites	1/10	6/30
Biliary stenosis	1/10	0/30
Biliary leak	0/10	1/30
Anemia	1/10	1/30
Pleural effusion	0/10	3/30
Atrial fibrillation	0/10	1/30
Bleeding	0/10	1/30
Acute rejection	0/10	3/30
Renal insufficiency	0/10	1/30
Arterial thrombosis	0/10	1/30
Cholestasis	0/10	1/30



### Supplementary Table S5 | Markers in Liver Perfusate.

Values are expressed as pg/ml (GST P is expressed as MFI)  $\pm$  Standard Deviation.

T0 values were detected in 3 different samples Belzer solutions before starting perfusion

<b>Markers</b>	<b>T0</b>	<b>T1</b>	<b><i>p</i></b>
pGST	2043	42,544 $\pm$ 67,400	0.002
beta2-microglobulin	3	28.6 $\pm$ 22.3	0.002
Albumin	1274	3,202 $\pm$ 4,848	0.002
NGAL	1	9.9 $\pm$ 5.4	0.002
Calbindin	2.84	21.8 $\pm$ 13.0	0.0039
Clusterin	0.16	12.8 $\pm$ 7.5	0.0039
Osteopontin	16	36.8 $\pm$ 10.5	0.002

## Supplementary Figure S1 | Not significant correlations of markers of acute kidney injury and eGFR at 1st, 3rd and 6th month after kidney transplantation.

Markers detected on perfusate do not correlate with moderate-term renal outcomes, in terms of renal function (eGFR). For each markers, patients of HOPE-K groups were divided based on median values.

