O1

FUNCTIONAL MRI TO MONITOR RENAL PERFUSION ALTERATIONS IN A MODEL OF RENAL IRI INDUCED HYPERTENSION AND TREATMENT EFFECTS

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Background: Many patients undergoing kidney transplantation (ktx) need antihypertensive therapy. It is still a controversy when and how much blood pressure control is needed after ktx. We investigated the effect of early antihypertensive therapy in a model of renal IRI induced hypertension.
Methods: Transient renal pedicle clamping for 35 min in CD1 mice resulted in IRI with systemic hypertension. Mice were treated with the antihypertensive soluble epoxy hydroxylase (sEH) inhibitor, an ACE-inhibitor or received vehicle. By functional MRI renal perfusion was quantified longitudinally. Histology and immunohistochemistry were done to quantify inflammation and fibrosis. By flow cytometry inflammatory cells were classified in the affected renal tissue. Blood pressure was measured by tail cuff.
Results: IRI caused blood pressure elevation of +20 mmHg in the vehicle group. Antihypertensive therapy normalized blood pressure and reduced mesangial matrix expansion which correlates with glomerulosclerosis. However, functional MRI revealed that antihypertensive treatment aggravated renal perfusion impairment at d1 after IRI. Antihypertensive treatment did not effect interstitial fibrosis and correlated with enhanced leukocyte infiltration into the IRI kidney.
Discussion: The sEH inhibitor and the ACE-inhibitor effectively normalized blood pressure elevation and prevented glomerulosclerosis but did not affect overall interstitial fibrosis. Importantly, early renal perfusion was negatively affected by lowering systemic blood pressure and was linked to more severe Inflammation.
Conclusion: Careful, decision making is necessary in order to find the optimal time point for antihypertensive treatment after IRI or ktx. Functional MRI offers a non-invasive method to monitor renal perfusion alterations and allows longitudinal follow up in drug discovery studies.

O2

TREATMENT OF RATS WITH THE OMEGA FATTY ACID 3 FORMULATION EPA:DHA 6:1 DECREASES THE LEUKOCYTE MICROPARTICLES-INDUCED ENDOTHELIAL PRO-INFLAMMATORY RESPONSES AND SENESCENCE

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Introduction: Microparticles (MP) are plasma membrane vesicles shed by cells, and vascular effectors. We showed that MPs shed by leukocytes in response to LPS or PMA/ionophore act as procoagulant, pro-inflammatory and pro-senescent endothelial effectors involving the local angiotensin system. Endothelial cells (ECs) are main targets of ischemia reperfusion injury impairing organ perfusion. The possibility that a 7-days treatment of rats with an omega 3 formulation affects MP shedding from isolated leukocytes and their property.
Methods: Male 48 weeks-old Wistar rats received 500 mg/kg/day of either EPA:DHA 6:1, EPA:DHA 1:1, or vehicle for 7 days. Porcine coronary artery young ECs at passage 1 were incubated for 48 h with leucocyte-derived MPs (10 nM of $MP_{6:1}$, $MP_{1:1}$, MP_{CTL}) isolated from the rat spleen and cultured for 24 h. Senescence-associated β-galactosidase activity (SA-b-gal) was assessed by C12FDG probe, proteins expression by Western blot, and procoagulant MPs by prothrombinase assay.
Results: Omega 6:1 and 1:1 ingestion decreased leukocyte MP shedding by 24% and 14% ($p = 0.01$, 0.05 vs CTL). In ECs, MP_{CTL} increased SA-b-gal by 28%, the expression of senescence markers p16, p53, p21 and down-regulated endothelial NO synthase whereas no such effects were observed with $MP_{6:1}$. MP_{CTL} but not $MP_{6:1}$ significantly up-regulated pro-inflammatory COX-2 but not COX-1, and adhesion proteins ICAM-1 and VCAM-1 by a 2, 1.2, and 1.4-fold respectively, and angiotensin-converting enzyme and angiotensin type 1 receptor by 1.4 and 1.7-fold, respectively.
Conclusion: Our findings indicate that the EPA:DHA 6:1 treatment prevents leukocyte MP-driven inflammatory responses leading to endothelial dysfunction and senescence. They suggest that omega free fatty acids might be useful in graft pre-conditioning to avoid ischemia reperfusion injury. Moreover, spleen appears to be a convenient source of leukocyte MPs as surrogate markers of the impact of therapies on immune cells.

O3

PULMONARY ISCHEMIA-REPERFUSION IN A RAT MODEL: IMPACT OF CARDIOPULMONARY BYPASS ON PULMONARY ENDOTHELIAL DYSFUNCTION AND SYSTEMIC INFLAMMATION

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Introduction: Lung transplant (LT) morbidity remains actually high. Ischemia-reperfusion (IR) of the graft is one of the main causes of these early complications. The routine use of Cardiopulmonary Bypass (CPB) during LT is currently considered deleterious. The objective of this study is to evaluate the impact of CPB on pulmonary endothelial dysfunction and systemic inflammation after pulmonary IR in a rat model.
Methods: This study included a Sham group ($n = 11$), a CPB group ($n = 9$), an IR group ($n = 8$) and a CPB-IR group ($n = 11$). Rats were exposed to 45 min of CPB, 30 min of left pulmonary ischemia and 15 min of reperfusion. Functional endothelial dysfunction was evaluated by measurement of the pulmonary artery reactivity. Systemic inflammation was evaluated by the plasma assay of IL-1ß , IL-10 and TNF-alpha. The endothelial glycocalyx was evaluated by plasma assay syndecan-1 and electron microscopy. The statistics were performed using an ANOVA test, $p < 0.05$.
Results: We showed that CPB associated with IR induce an endothelial vasorelaxation dysfunction mainly mediated by nitric oxide (NO). CPB increased significantly the effects of IR on systemic inflammation with increased plasma levels of IL-1ß (CPB-IR 594.8 ± 113.0 vs IR 109.9 ± 29, $p < 0.05$) and IL-10 (CPB-IR 234.8 ± 16.0 vs IR 149.8 ± 15.4 pg/mL, $p < 0.01$). The level of TNF-alpha increased significantly in all groups compared to the Sham group. CPB increased the effects of IR on glycocalyx degradation with syndecan-1 (CPB-IR 22.1 ± 0.7 μg/mL vs IR 18.3 ± 0.7 μg/mL, $p < 0.001$).
Conclusion: CPB and pulmonary IR combination would increase pulmonary endothelial dysfunction and systemic inflammation. The glycocalyx degradation appear to be one important mechanism. The use of CPB routinely during LT may therefore be deleterious, further studies in humans need to be conducted to confirm these data.

O4

EFFECT OF ENDOTHELIAL GLYCOCALYX SHEDDING IN ORTHOTOPIC LIVER TRANSPLANTATION

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Background: The endothelial glycocalyx (GXL) plays a key role in vascular permeability and modulation of inflammation and coagulation through forming a protective layer on the endothelial surface. Strategies to preserve this protective layer may reduce the effects of ischaemia reperfusion injury (IRI). In this study syndecan-1 levels, a marker of GXL shedding were measured in patients undergoing liver transplantation (LT) with or without post-operative goal-directed fluid therapy (GDFT).
Methods: Twelve randomly selected patients were included [6 GDFT, 6 standard care (SC)]. Serum samples at six time points (pre-operative, reperfusion, skin closure, ITU time 0, 6 and 12 h) were analyzed for syndecan-1, TNFa and proBNP using ELISA procedures. Graft function was assessed by indocyanine green (ICG) clearance and liver function tests at 12 h. Data is presented in median and interquartile range. Mann-Whitney *U* test was used for non-parametric comparison (Minitab 17 software).
Results: Syndecan-1 levels raised significantly in all patients post reperfusion (baseline 124.7 ng/mL ±65.2 vs reperfusion 6000 ng/mL ±3216.4, $p < 0.0001$) and returned to pre-operative levels after 12 h (baseline 124.7 ng/mL ±65.2 vs 12 h 252.3 ng/mL ±256, $p = 0.14$). There was no significant difference in syndecan-1 levels between GDFT and SC at any time-point. proBNP levels increased between baseline and 6 h (baseline 0.5 pg/mL vs 6 h 1.97 pg/mL, $p = 0.035$) and there was a significant difference in

levels at 12 h between the two groups (GDFT 1.94 pg/mL vs 4.02 pg/mL, p = 0.03). Syndecan-1 correlated with proBNP levels at 6 h (r = 0.72, p = 0.007), 12 h (r = 0.85, p = 0005) and day two creatinine ratios (r = 0.71, p = 0.009). There was no significant difference between GDFT and SC in TNF-alpha, ICG clearance and liver function at 12 h.
Conclusion: IRI during LT leads to significant GXL shedding. The rise at 12 h is associated with worsening renal function and right heart strain.

O5 *EX VIVO* NORMOTHERMIC RENAL HEMO-PERFUSION: A FUNCTIONAL EXPLORATION OF TRANSPORT AND RESPIRATORY PERFORMANCES OF PIG KIDNEYS AFTER SHORT-TERM COLD-STORAGE

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Background: Graft shortage leads to use extended criteria kidneys. To preserve or recondition such *at risk* organs, *ex vivo* normothermic perfusion (EVNP) gains interest, but mechanisms are unclear; experimentals show wide heterogeneity. We explore hemodynamic, filtration, reabsorption and O_2 consumption of cold-stored kidneys next submitted to EVNP with Krebs (KR) versus whole-blood (WB) at two heparin levels (H, UI/L).
Methods: Kidneys flushed and cold-stored (SCOT15; 2–4 h); blood cold-stored in CPDA-1 bags. Kidneys mounted for 2 h perfusion flow (PEF mL/min), arterial and venous O_2, urine production, (UP, μL/min) monitoring at 37°C, constant 80 mmHg pressure (PP); resistance RR = PP/PEF. Perfusion media equilibrated with 95%O_2/5%CO_2 (KR, 6% BSA). Na fractional reabsorption (FR%) calculated from glomerular filtration rate (GFR; μL/min = creatinine clearance), Na transport (μmol/min) and excretion; av-O_2 consumption (QO_2, μmol/min). Results as mean ± SD(n).
Results (units above, results normalized to 100 g kidney weight): 1. Globally, all values are lower/much lower than physiological ones, PEF being the closest.
2. KR yields the highest perfusion and QO_2, but low GFR and urine flow, and very low Na^+ reabsorption and TNa/QO_2 (respiratory/transport efficiency).
3. When perfused with WB+250H, PEF and QO_2 are reduced but GFR, TNa, FRN and UP increase.
4. With WB-5,000H, PEF remains similar, but GFR, UP, TNa, FRN, and transport efficiency increase 25-, 50-, 10- and 8-fold, vs KR, and 3-, 18-, 4- and 1.3-fold vs WB-250H, respectively.
Conclusion: EVNP optimal conditions remain to be established. Using a Pig preclinical model of static cold storage, we show strong decoupling (with KR) between (high) perfusion and (poor) function: artificial Krebs-like medium appears unsuitable for renal function. Conversely, high-heparin whole-blood yields better function, approaching physiological values, especially in terms of tubular function.

O6 THE PHYSIOLOGICAL EFFECTS OF ALTERING OXYGENATION DURING *EX-VIVO* NORMOTHERMIC KIDNEY PERFUSION

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Introduction: Kidney Normothermic Machine Perfusion (NMP) has historically supplied a gas mixture of 95% O_2 and 5% CO_2; however, evidence suggests that supra-physiological oxygenation can be deleterious. We hypothesised that excess oxygenation during NMP may abate its conditioning effects.
Methods: In a "minimal injury" model, porcine kidneys underwent 10 min warm ischaemia (WI) and 2 h static cold storage (SCS) before 1 h of NMP with 95% (n = 8), 25% (n = 4), 12% (n = 5) O_2 with 5% CO_2 and N_2 balance. In a "clinical injury" model, kidneys underwent 10 min WI and 17 h SCS before 1 h NMP with 95% (n = 8) 25% (n = 6) or 12% (n = 9) O_2; then were reperfused with whole blood for 3 h. A control group underwent 18 h SCS before reperfusion. We took continuous functional measurements and interval samples of blood, urine and cortical tissue.
Results: There were no significant differences in functional measurements between groups receiving different perfusate oxygenation, in either minimal or clinical injury experiments. During reperfusion following clinical injury, oxygen consumption, fractional sodium excretion and urine output were numerically higher in the 25% O_2 group. Tissue concentrations of High Motility Group Box 1 (HMGB-1) was significantly lower in 12% vs 95% at 3 h reperfusion (25.67 ± 4.06 vs 16.43 ± 2.99 ng/mL, p = 0.012).
Discussion: Normothermic machine perfusion of porcine kidneys with lower oxygen concentrations is not detrimental to renal function or assessment. Reducing P_pO_2 may reduce tissue injury, though further work is required to characterise any underlying change in oxidative stress or differential intrarenal blood flow.

O7 VASCULAR OBSTRUCTION IN DCD KIDNEYS IS REDUCED BY NORMOTHERMIC MACHINE PERFUSION AS TOLD BY ROBUST METHOD OF QUANTIFICATION

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Microvascular and glomerular thrombi have historically been observed in deceased donor kidneys post-transplantation. The mechanisms by which they accumulate are currently unclear and it is unknown how this affects transplant outcomes. It is possible that they disrupt renal blood flow and add to the severity of ischaemia reperfusion injury, increasing the likelihood of delayed graft function. The aim of this study was to determine if normothermic machine perfusion (NMP) could reduce microvascular and glomerular thrombi in donation after circulatory death (DCD) kidneys. Pre-implantation and 30-min post reperfusion wedge biopsies from kidneys receiving static cold storage (CS) n = 14 and NMP n = 11 included in a randomized controlled trial were collected and fixed in 10% formalin. Within the cohort, there were 5 pairs, one kidney undergoing CS and the other NMP. The paraffin sections were stained with Martius Scarlet Blue to isolate areas of fibrin and erythrocyte accumulation. 10 images/sample were then analyzed by an automated script in ImageJ to isolate fibrin rich thrombi. Pre-implantation biopsies from CS (M = 2270 pixels of fibrin/image (pfi), SEM = 2528) and NMP treated kidneys (M = 21,179 pfi, SEM = 2803) presented with similar levels of microthrombi (t_{248} = 0.403, p = 0.6873). Kidneys receiving CS had a significantly larger accumulation of microthrombi post-reperfusion (M = 49,503 pfi, SEM = 4350) compared to pre-implantation (M = 22,701 pfi, SEM = 2528) (t_{278} = 5.327, p < 0.0001). Post reperfusion accumulation was also significantly higher in CS kidneys compared to NMP (M = 20,652 pfi, SEM = 2749) (t_{248} = 5.35, p < 0.0001). Within the pairs, NMP kidneys had less accumulation pre-implantation compared to CS kidneys (p < 0.001). There was also a significant increase in thrombi post-reperfusion in CS kidneys compared to NMP treated kidneys. These findings suggest that NMP can be used to reduce the amount of microthrombi before transplantation.

O8 FEASIBILITY OF DELIVERING CELL THERAPY DURING KIDNEY *EX VIVO* NORMOTHERMIC PERFUSION

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Introduction: The advent of warm perfusion technologies has brought about exciting new opportunities for the delivery of novel treatments direct to organs prior to transplant. This includes cell therapies that may facilitate immunomodulation or reconditioning of marginal organs to minimise ischaemia reperfusion injury. Multipotent Adult Progenitor Cells are a well characterised, bone marrow-derived cell population with anti-inflammatory properties. They possess an increased capacity for expansion and are minimally immunogenic making them the ideal "off-the-shelf" therapy candidate. The purpose of this pilot series was to investigate the feasibility of MAPC delivery during kidney EVNP.
Methods: Ten declined human kidneys were included in this study; 8 treated with MAPCs and 4 vehicle-treated controls. EVNP was performed for 7 h. Following 1 h of stable perfusion 50 × 10^6 MAPCs were delivered via the arterial cannula as a bolus. Physiological output from the kidney was recorded at 30 min time-points. Contrast enhanced ultrasound (CEUS) was performed before MAPC treatment, 15 min after infusion and at 4 h. Fluorescent microscopy was used to evaluate engraftment and tracking of the fluorescently labelled MAPCs.
Results: There was no impairment to physiology during warm perfusion. Renal blood flow (mL/min/100 g), vascular resistance, acid/base balance, urine production and serum electrolytes were matched in the controls and MAPC treated kidney. CEUS demonstrated no significant impairment of perfusion following MAPC administration. Fluorescent microscopy demonstrated engraftment of the MAPCs.
Conclusion: We have described the first reported series of cell therapy successfully delivered directly to a kidney in an isolated *ex vivo* perfusion platform. MAPC therapy is feasible during EVNP and does not impair kidney physiology providing us with exciting novel opportunities to recondition marginal organs prior to transplantation.

O9

ANTISENSE OLIGONUCLEOTIDE THERAPY IN RENAL TRANSPLANT ISCHAEMIA REPERFUSION INJURY USING AN *EX-VIVO* NORMOTHERMIC PERFUSION MODEL

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Introduction: Kidney transplantation is the most effective treatment for patients with End Stage Kidney Disease. Demand for transplant organs exceeds supply and long waiting times is a feature of many, if not all, transplant programmes. Patients die waiting for a transplant therefore many initiatives are being developed to expand the donor pool. A consequence of this is the increasing use of sub-optimal (or marginal) donor organs, with an inherent risk of inferior short and longer-term recipient outcomes. Interventions to improve or re-condition donor organs are receiving increasing attention, with normothermic machine perfusion currently in phase III clinical studies. This technique, in which a kidney is perfused with oxygenated blood prior to implantation, could potentially reduce the rate of delayed graft function but also allows the delivery of therapeutics to a metabolically active organ.
Methods: Human kidneys, deemed unsuitable for transplantation, were subjected to 6 h of NMP using a red cell-based perfusate. Fluorescently labelled anti-miR-24-3p (n = 5) or scrambled control ASO (n = 5) were introduced in hypothermic perfusate and during NMP. Serial biopsies were taken at t = 0, t = 60 and t = 360 min. Real time q-PCR was used to assess the expression of the potentially protective miR-24-3p downstream targets Haem oxygenase-1 (HMOX-1) and Sphingosine-1-phosphate receptor-1 (S1PR1).
Results: NMP facilitated uptake of antisense oligonucleotides faster than both previous in-vitro and in-vivo animal models (as early as 6 h, p = 0.0006), with fluorescent signal identified in both endothelium and proximal tubules. In kidneys perfused with anti-miR-24-3p (n = 5) we observed a significant increase in mRNA for HMOX-1 (18 fold, p = 0.0033) and S1PR1 (4 fold, p = 0.0217) at 6 h. These effects were not seen when the ASO was introduced in the perfusate during hypothermic perfusion.

O10

GENETIC DELETION OF DUSP3 PHOSPHATASE ATTENUATES KIDNEY DAMAGE AND INFLAMMATION FOLLOWING ISCHEMIA/REPERFUSION IN MOUSE

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Background: Renal ischemia-reperfusion (I/R) injury represents an unavoidable event in kidney transplantation. Dual Specificity Phosphatase 3 (DUSP3, also called *Vaccinia*-H1 Related (VHR)) is highly expressed in endothelial cells, in platelets, monocytes and macrophages. Since DUSP3 is a positive regulator of the innate immune response, its inactivation/deletion may attenuate kidney inflammation and damage caused by I/R.
Methods: Ten-weeks-old C57BL/6 wild-type (WT, n = 10) versus systemic knock-out (KO, n = 10) mice underwent unilateral left renal ischemia for 30 min. Right nephrectomy was simultaneously performed. The left kidney was excised and blood sample was collected at 48 h post reperfusion. Renal function was assessed upon Blood Urea Nitrogen (BUN) levels. Expressions of inflammatory and immune markers were comparatively quantified at both mRNA (RT-qPCR) and protein (immune-blotting and –staining) levels in ischemic versus non-ischemic kidneys in DUSP3 WT versus KO mice.
Results: BUN reached 259 ± 51 vs 78 ± 11 mg/dL in WT and KO, respectively ($p < 0.01$). DUSP3 KO ischemic kidneys showed a reduced number of PCNA- (3-fold, $p < 0.001$), CD11b- (3.5-fold, $p < 0.001$) and F4-80-positive cells (1.7-fold, $p < 0.001$) in comparison to WT. The expression levels of CD11b (2.2-fold, $p < 0.01$), HSP70 (2.7-fold, $p < 0.01$) and PCNA (10-fold, $p < 0.001$) were significantly decreased in DUSP3 KO compared to WT ischemic kidneys. By contrast, a 1.5-fold increase of anti-inflammatory M2 CD206-positive macrophages was observed in DUSP3 KO ischemic kidneys. At mRNA levels, DUSP3 WT vs KO ischemic kidneys (normalized to WT sham-operated right kidneys) showed an upregulation of 6.5-fold ($p < 0.05$) vs 10.5-fold ($p < 0.01$) of M2-type macrophage (*Arginase*), 4.6-fold ($p < 0.001$) vs 2.2-fold ($p < 0.05$) of CD11b, 4.5-fold ($p < 0.001$) vs 0.7-fold ($p > 0.05$) of TNF and 111-fold ($p < 0.001$) vs 4.5-fold ($p > 0.05$) of KIM-1, respectively.
Conclusions: Genetic deletion of DUSP3 attenuates renal I/R-associated damage and inflammation.

O11

LESSONS LEARNED FROM THE CLINICAL USE OF NORMOTHERMIC REGIONAL PERFUSION (NRP) IN UNCONTROLLED DONATION AFTER CIRCULATORY DEATH (UDCD)

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The national protocol for the uDCD program restricts the donor age to <55 years, the no flow period to <30 min and the total warm ischemia time (WIT) to <150 min. In situ kidney perfusion is realized by a double-balloon catheter (ISP) or by a nRP. Perfusion machine is systematic. Only non-immunized recipients for a 1st transplantation were eligible. 499 kidney transplantations (KTR) (period 2007–2014) were analyzed. In situ organs perfusion by nRP was performed in 50%, mean WIT was 135 min and mean cold ischemia times (CIT) was 14 h.

Analysis risk factors of primary non function (PNF, n = 37) and graft failure (eGFR <30 mL/min or graft loss at 1 year, n = 66) were performed by logistic regression.

PNF risk factor was donor age [OR = 0.95, p = 0.002] and a sensibility analysis shown a center effect.

Graft failure risk factors (excluding PNF) were donor BMI [OR = 1.2, $p < 0.001$] and ISP (compared to nRP) [OR = 1.2, $p < 0.001$]. No effect of donor age, no flow period, WIT or CIT was found in multivariate analysis.

Graft survival (period 2007–2015 endpoint 31 dec 2016) was significantly different according to donor type with at 5 years 76% [71–79%] for uDCD, 68% [67–69%] for brain death extended criteria donors (DBD ECD) and 84% [84–85%] for optimal DBD. After adjustment by Cox model on recipient age, a significant increased risk of failure remains in uDCD recipients compared to optimal DBD [HR = 0.54, $p < 0.001$]. After the exclusion of failures of less than 2 months and adjustment by Cox model on recipient age, we observed a significant difference risk of failure between uDCD recipients compared to optimal DBD [HR = 0.63, p = 0.02] and DBD ECD [HR = 1.28, p = 0.03].

In conclusion, uDCD kidneys are an additional source of valuable transplants with a graft survival between those with optimal DBD and DBD ECD. The use of nRP seems to decrease graft failure, through restoration of oxygenated blood, acting as the first step of pre-reconditioning.

O12

NORMOTHERMIC MACHINE PERFUSION PARAMETERS CORRELATE WITH EARLY ALLOGRAFT FUNCTION IN DBD AND DCD KIDNEY TRANSPLANTS

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Introduction: Normothermic machine perfusion (NMP) is a new technique that utilises
extracorporeal circulation technology to assess and re-condition kidneys prior to transplantation. This study is the first comparison of NMP for kidney transplants from brain death donors (DBD) and circulatory death donors (DCD).
Patients and methods: Immediately prior to transplantation, kidneys underwent 60 min of NMP using an oxygenated red cell-based solution at 36°C. During NMP kidneys were scored from 1 (highest quality) to 5 (lowest quality) according to macroscopic perfusion, renal blood flow and urine output. NMP score was correlated with clinical outcome in DBD and DCD kidneys.
Results: 28 DBD and 38 DCD kidney transplants were performed after NMP. Donor and recipient demographics were similar between groups. NMP scores were as follows: DBD group – score 1 (n = 19), score 2 (n = 4), score 3 (n = 5); DCD Group score 1 (n = 15), score 2 (n = 14), score 3 (n = 9); ($\chi^2 = 5.22$, p = 0.07). The delayed graft function rate was 2/28 (7%) in DBD kidneys and 14/38 (37%) in DCD kidneys (p = 0.008). There was one primary non-function (PNF) in the DCD group due to renal vein thrombosis 7 days post-transplant. NMP score was negatively correlated with improved early allograft function measured by recipient serum creatinine at day 7 (r^2=0.179 p = 0.0009) and one-month post-transplant ($r^2 = 0.121$ p = 0.006).
Conclusion: DCD kidneys had poorer quality scores during NMP and this was reflected in a higher rate of DGF. NMP can be used to predict early renal allograft graft function.

O13

THE SYNTHESIS OF COAGULATION FACTORS DURING NORMOTHERMIC MACHINE PERFUSION OF LIVERS IS IMPAIRED BY ISCHEMIA IN PIGS AND MIGHT PREDICT GRAFT VIABILITY

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Normothermic Machine Perfusion (NMP) of livers is a promising tool to test graft viability during preservation. Coagulation factors are synthetized by the

liver, hence are candidate markers of function. We evaluated the production of coagulation factors during NMP, tested if ischemia alters their synthesis, and correlated their levels with markers of hepatocyte damage (AST) and sinusoidal endothelial injury {Hyaluronic acid (HAc)}. Porcine livers exposed to 60 min Warm Ischemia (WI60, n = 5) or not (WI0, n = 5) were NMP perfused for 6 h.

The concentration of Factors V (FV), VIII (FVIII), and X (FX) in the perfusate was measured at 15, 30 min, 1, 2, 3, and 6 h of NMP and compared within and between groups (between-within ANOVA). Levels of AST and HAc were correlated to FV and FVIII. Mean ± SD is given. FV increased significantly over time in both groups, although with a different kinetics, as a plateau was reached at 2 h of NMP in WI0 only. FV concentration was inferior in WI60 vs WI0 ($p < 0.0001$) (Figure 1A). Similarly, FVIII raised over time significantly in both WI0 and WI60, reaching a plateau at 3 h of NMP only in WI0. The production of FVIII was inferior in WI60 vs WI0 ($p = 0.006$) (Figure 1B). FX raised steadily without a plateau in WI0, while no significant increase was observed in WI60, in which the concentration of FX was inferior ($p = 0.001$) (Figure 1C). The area under the curve of AST was greater in WI60 (3077) vs WI0 (405.3, $p = 0.0006$). FV was inversely correlated to AST release at all time points except 6 h. HAc levels increased over time [11.3 ± 5.3 ng/mL at 15 min to 101.8 ± 75.5 ng/mL at 6 h in WI0 ($p = 0.045$), and from 51.5 ± 6.9 ng/mL at 15 min to 303.2 ± 107.7 ng/mL at 6 h in WI60 ($p = 0.01$)] and were higher in WI60 vs WI0 ($p = 0.004$). FVIII was inversely correlated to HAc levels at 1 h (r: −0.58, $p = 0.02$) and 2 h (r: −0.59, $p = 0.03$) of NMP. The production of coagulation factors during NMP of human livers and their role in the assessment of viability deserve to be explored.

O14 NORMOTHERMIC REGIONAL CIRCULATION FOR TYPE 3 DONATION AFTER CIRCULATION DEATH (DCD). RESULTS IN LIVER TRANSPLANTATION OF A FRENCH MULTICENTRIC SERIES

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Originality of the French Maastricht 3 DCD (M3) protocol is the use of a normothermic regional circulation (NRC) just after death, in the intensive care unit and before the surgical period. The NRC minimizes consequences of the functional warm ischemia time (FWIT = low flow + no flow/no touch + NRC installation) and makes possible to optimize the organization of the harvesting procedure.

Aim: Evaluate the results of the first consecutive liver transplantations (LT) performed in 6 French pilot centers.

Method: Retrospective study comparing a group of LT from DCD (M3, n = 51) to a control group (CTRL, n = 102) performed during the same period of time (January 2015–June 2017). The CTRL group was paired to the M3 group on the recipient age, the donor age, and the score of priority. This CTRL group was extracted from a pool of 252 LT selected for: recipient age 18–65 years old, MELD ≤25, first LT, not combined, not urgent with a whole graft, from a brain death donor (BDD), 18–65 year old, without temporary cardiac arrest, with initial gGT <50 UI/L and cold ischemia time <9 H.

Results: The FWIT was of 23 ± 1 min. The M3 group was comparable to the CTRL group for the age, the height, the ASAT, the sex of the donor, the cold ischemia time (M3 versus CTRL: 352 ± 11 vs 374 ± 8 min, $p = 0.06$) and for the UNOS status, the tumor on the explanted liver and the MELD score of the recipient (M3 versus CTRL: 12 ± 1 vs 9 ± 1, $p = 0.29$) (Mean ± SEM). However, small differences were observed between the 2 groups for the BMI (M3 versus CTRL: 24 ± 0 vs 25 ± 0 kg/m^2, $p = 0.01$), the ALAT (73 ± 11 vs 59 ± 10 UI/L, $p = 0.04$), the gGT (154 ± 21 vs 42 ± 6 UI/L, $p = <0.001$) and the steatosis of the donor (5 ± 1 vs 13 ± 2 %, $p = 0.02$). The 90 days graft survival probability was 0.98 ± 0.02 for M3 and 0.93 ± 0.03 for the CTRL (graft loss: 1 and 7 ($p = 0.37$) respectively).

Conclusion: The use of NRC in DCD before harvesting the liver, produced 3 months graft survival in LT from a DCD identical to LT from a BDD.

O15 EVALUATION OF OUTCOMES IN RENAL TRANSPLANTATION USING MACHINE PERFUSION FOR THE PRESERVATION OF KIDNEYS FROM EXPANDED CRITERIA DONORS

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Introduction: The shortage of kidney grafts led to retrieve organs from old donors with one or more co-morbidities, considered as "expanded criteria donors" (ECD). In France, since 2012, the Agency of biomedicine (ABM) has recommended the use of machines perfusion (MP) to preserve kidneys from this donor population to improve kidney preservation and the transplantation outcomes, with the creation of a specific lump sum financing the additional costs of this strategy. This study evaluates the impact of MP vs cold storage (CS), for the period 2011–2014 with kidneys from ECD.

Methods: From the ABM database (Cristal), the effect of MP on the delayed graft function (DGF) was analyzed using a multivariate logistic model excluding pre-emptive transplants and primary non functions (PNF). In addition, transplants from the same donor, whose one kidney preserved by MP and the other by CS (population of twins), were analyzed using a mixed model.

Results: Co-morbidities of recipients are more frequent and the age of donors and recipients is significantly higher for kidney preserved by MP (n = 801) versus CS (n = 3515). With 16% of DGF for MP versus 29% for CS, MP has a protective effect on the DGF (OR adjusted = 0.45, CI [0.36, 0.56]). In the population of the twins (84 pairs, 168 grafts), we observed 7% of DGF for MP versus 33% for CS and an adjusted OR 0.19 (CI [0.06; 0.58]). The durations of hospitalization and dialysis after transplantation are shorter with fewer sessions of dialysis.

Discussion: Our results confirm the reduction in the incidence of the DGF of ECD kidneys preserved by machines, with 2.2 times less risk despite a population more at risk in this group, and a lower 5.2 times risk in the population of the kidneys "twins". It remains to assess the impact of the DGF in the long term survival and measure the cost effectiveness of this strategy.

O16 HYPOTHERMIC PULSATILE PERFUSION OF HUMAN PANCREAS: A TECHNICAL FEASIBILITY STUDY

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Background: There are currently two approaches of hypothermic preservation for most solid organs: static or dynamic. Cold storage is the main method for static storage (SS), while hypothermic pulsatile perfusion (HPP) and other machine perfusion-based methods such as normothermic machine perfusion and oxygen persufflation are the methods for dynamic preservation. HPP is currently approved for kidney transplantation.

Methods: We evaluated for the first time the feasibility of HPP on 11 human pancreases contraindicated for clinical transplantation because of advanced age and/or history of severe alcoholism and/or abnormal laboratory tests. Two pancreases served as controls in SS, 2 other were splitted for SS and HPP and 7 were tested in HPP. HPP preservation lasted 24 h at 25 mmHg. Resistance index was continuously monitored and histology of the pancreas, and the duodenum was evaluated every 6 h.

Results: The main observation was the complete absence of oedema of the pancreas and duodenum at all studied periods during HPP. Insulin, glucagon and somatostatin staining was normal. Resistance-index decreased during the first 12 h and remained stable thereafter.

Conclusion: 24-h hypothermic pulsatile perfusion of marginal human pancreas-duodenum organs was feasible and with no deleterious parenchymal effect. These observations encourage us to move further and evaluate safety of HPP after clinical transplantation.

O17 TARGETED ELIMINATION OF SENESCENT CELLS TO PROTECT KIDNEYS AGAINST ISCHEMIA REPERFUSION INJURY

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Accumulation of damage in an organ, and the inability to effectively cope with this, can result in subsets of cells entering a state called senescence. Senescence is characterized by a permanent cell cycle arrest and development of a chronic pro-inflammatory state; the so called senescence associated secretory phenotype (SASP), which negatively affects the surrounding tissue. Also, due to their permanent cell cycle arrest, senescent cells reduce the regenerative capacity of the tissue. Senescent cells accumulate with age, but can evolve in response to acute damage, such as high levels of oxidative damage, as well.

We have shown that 2 months after renal IRI, induced by bilateral kidney clamping, there is an increased expression of several validated senescence markers, such as $p16^{Ink4a}$, Interleukin-6 and chemokine (C-C motif) ligand 2 (CCL2), whilst downregulated expression of Lamin B1 which is lost in senescent cells. The increase in senescent cell levels 2 months after IRI is accompanied by worsened kidney function, compared to SHAM operated mice.

Through SASP and by reducing regenerative capacity, we argue senescent cells are a culprit of IRI-induced long-term damage in kidneys. We aim to remove these senescent cells and improve kidney transplant outcome by the targeted eradication of senescent cells. Recent developments in the understanding of the biology of aging have led to the development of a new class of therapeutic agents aimed at eliminating senescent cells. For the first time, we have a therapeutic compound (Proxofim) to target senescence in vivo and improve kidney function in mouse models for kidney aging (Baar et al, 2017). Proxofim was shown to improve kidney function of aged mice and reduce levels of pro-inflammatory factors such as IL-6 and CCL2, as a result of the targeted elimination of senescent cells. Therefore we are using Proxofim to overcome transplantation associated injury and improve kidney function.

O18 ADDITION OF DIFFERENT OXYGEN CONCENTRATIONS DURING LONG-TERM HYPOTHERMIC MACHINE PERFUSION IN A CLINICALLY RELEVANT PORCINE DONATION AFTER CIRCULATORY DEATH MODEL

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Background: To date, hypothermic machine perfusion (HMP) has become standard care in many centres preserving deceased donor kidneys. Despite a significant reduction of metabolism at low temperatures, remaining cellular activity in the organ still requires oxygen. However, oxygen supply during HMP is not standard yet, as its role and safety has not been fully clarified yet. This study investigates the effect of administering oxygen during HMP on renal function in a clinically relevant porcine donation after circulatory death model.
Methods: After 30 min of warm ischemia, porcine slaughterhouse kidneys were preserved for 24 h by static cold storage (CS), or HMP with Belzer Machine Perfusion Solution (UW- MPS) with the addition of 0%, 21% or 100% oxygen. Next, kidneys were reperfused for 4 h in a normothermic autologous blood machine perfusion (NMP) setup. Kidneys were assessed on their renal function, oxidative stress and injury markers.
Results: HMP resulted in significantly better kidney function during NMP in terms of creatinine clearance, fractional sodium excretion and proteinuria. The addition of 100% oxygen showed the highest creatinine clearance, but did not reach statistical significance. TBARS, markers of oxidative stress, were negligibly low in all preservation modalities. Urinary TBARS at the end of NMP were highest in the CS group with a mean of 11.2 μM compared to 7.7 μM in the 100% oxygen group (NS). HMP preserved kidneys showed significantly lower injury markers compared with those preserved by CS. No such differences were found between the HMP groups with different oxygen concentrations.
Conclusion: This study demonstrated that kidney preservation with HMP is superior to CS. Although the addition of oxygen to HMP did not result in significantly improved renal function during NMP, beneficial effects were found in terms of reduced oxidative stress. Oxygen addition during HMP did not result in detrimental effects in this model.

O19 EFFECT OF PREGABALIN ON KIDNEY TISSUE IN SPINAL CORD ISCHEMIA REPERFUSION INJURY-INDUCED RATS

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Aim: The aim of this study is to investigate the protective effects of low and high doses of pregabalin administered with ischemia on renal tissue in spinal cord ischemia-reperfusion (I/R) in rats.

Method: 24 Wistar rats were randomly divided into four groups (n = 6). Control (group C), I/R (group I/R), I/R-low dose pregabalin (group I/R-LP), I/R-high dose pregabalin (group I/R-HP). All groups underwent laparotomy under anesthesia. After laparotomy in group I/R, a cross clamp was placed in the abdominal aorta for 120 min to perform spinal cord ischemia injury. Following 120 min of ischemia, reperfusion was achieved by opening the vascular clamp. 30 mg/kg (group I/R-LP) and 200 mg/kg (group I/R-HP) pregabalin was administered intraperitoneally 15 min before the ischemia. At the end of the reperfusion period, kidney tissue was taken for biochemical and histopathologic examinations.
Results: It was determined that the amount of erythrocytes leaking out of the vessel wall in the Bowman capsule in the I/R group was higher than the control group in the general tissue evaluation with Hematoxylin Eosin staining, that is, there was edema in the Bowman capsule. In the low-dose group, there was no change in this histopathological findings, but the 200 mg/kg group showed significant improvement, suggesting a similar appearance to the control group. When there were no p53 expressing cells in the control group, p53 expression was detected in the I/R group very distinctly, especially at different intensities in some Bowman capsules. Interestingly, in the low-dose group, it was found that p53 expression was markedly faded but very prominent in the 200 mg/kg group. TOS and TAS enzyme activity was significantly higher in the I/R group than in C,I/R-LP and I/R-HP groups.
Conclusion: These results indicate that different doses of pregabalin administered before ischemia in spinal cord I/R injury has partial protective effects in rats.

O20 HIGH OXYGEN PRESSURE DURING CONTINUOUS HYPOTHERMIC MACHINE PERFUSION IS ASSOCIATED WITH A BETTER *EX VIVO* RENAL BLOOD FLOW AND EARLY GRAFT FUNCTION IN A PORCINE DCD AUTO-TRANSPLANT MODEL

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Introduction: Continuous hypothermic machine perfusion (HMP) demonstrated improved early graft function compared to static cold storage (SCS) alone. The aim of this study was to evaluate the impact of different perfusate oxygen pressures during continuous hypothermic machine perfusion on physical machine perfusion parameters and early graft function in a porcine auto- transplant model.
Materials and methods: The left kidney of a ± 40 kg female Landrace pig was exposed to 30 min of warm ischemia by vascular clamping and randomized after standard procurement and *ex vivo* donor blood flush out to one of 4 studied preservation strategies: (i) 22 h SCS, (ii) 22 h (no active oxygen supply) HMP, (iii) 22 h oxygenated HMP ($HMPO_2$low) (pO_2 = 220–240 mmHg), and 4) 22 h oxygenated HMP ($HMPO_2$high) (pO_2 = 700–800 mmHg). The LifePort Kidney Transporter® (Organ Recovery Systems) was used for all machine perfusion strategies. The left kidney was auto-transplanted in a right orthotopic position.
Results: Twenty-four auto-transplants were performed with 6 pigs per study group. Renal blood flow (RBF) was significantly higher in both $HMPO_2$high and $HMPO_2$low groups compared to non oxygenated HMP. The RBF increase was faster in the $HMPO_2$high group (significant from 3 to 20 h compared to the HMP group) compared to the $HMPO_2$low group (significant from 8 to 19 h compared to HMP group). At the end of the HMP no difference was observed in RBF between the machine perfusion groups. No significant difference in RBF was observed between the $HMPO_2$low and the $HMPO_2$high group during the whole period of machine perfusion. Serum creatinine at day 1, 2, and 3 was significantly lower in the $HMPO_2$high group compared to non- oxygenated HMP ($p = 0.0126$; $p = 0.0013$ and $p = 0.0236$, respectively) and SCS ($p = 0.0001$; $p < 0.0001$; $p < 0.0001$, respectively). We observed a tendency toward better renal function during the first 3 days after transplantation in favor of the $HMPO_2$high group compared to the $HMPO_2$ low group, but the difference was not statistically significant. No difference in serum creatinine was observed between all the study groups at 7 and 13 days of follow-up.
Conclusions: The administration of high levels of perfusate oxygen concentration during HMP positively influence ex vivo renal blood flow and early graft function compared to low or no oxygen supply during HMP.

POSTERS

P1 SIGMA-1 RECEPTOR AGONISTS ARE RENOPROTECTIVE IN A RAT MODEL OF KIDNEY TRANSPLANTATION

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Introduction: Kidney transplantation (Tx) is associated with better quality of life and reduced costs compared to dialysis, but the shortage in donor organs is a limiting factor. Graft survival is highly dependent on the extent of ischemia/reperfusion injury (IRI) during Tx. We were the first to describe the renoprotective effects of Sigma-1 receptor (S1R) agonist treatment in renal IRI.
Aims: To develop a preservation solution, which minimizes ischemic graft damage in order to improve Tx outcomes and to increase the number of organs suitable for Tx.
Methods: Kidneys of male Wistar rats were perfused and placed in ice cold (i) Custodiol preservation solution; Custodiol containing S1R agonists (ii) fluvoxamine or (iii) SA-4503 for 2 h, then autotransplanted and sacrificed 24 h after reperfusion. Sham-operated rats served as controls. In a second experiment kidneys were perfused and placed in ice cold Custodiol or Custodiol containing various selective S1R agonists for 2/3/8/24 h and tissue samples were collected.
Results: S1R agonists mitigated renal functional impairment and tubular dilatation following Tx. Expression of early and sensitive tubular injury markers *Kim1* and *Ngal* were markedly less elevated in S1R agonist treated kidneys. S1R agonists alleviated renal apoptosis and increased anti-apoptotic *Bcl2* expression. Reduced number of CD45 + leukocytes and inflammatory cytokine (*Mcp1, Il1a, Il6, Tnf*) expression confirmed the anti-inflammatory effect of S1R agonists. All S1R agonists mitigated cold ischemic structural kidney damage at all time points.
Conclusion: The addition of S1R agonists to the preservation solution during Tx improves graft function and alleviates structural damage, thus improving long-term outcomes. S1R agonists reduce graft injury during cold storage, therefore the number of transplantable donor organs can be increased.

P2 PRE- AND POSTCONDITIONING EFFECTS OF METFORMIN IN NORMOTHERMIC MACHINE PERFUSED PORCINE AND RAT KIDNEYS

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Introduction: Metformin is the most widely used antihyperglycemic drug. It has been proposed that partial inhibition of the mitochondria by metformin can reduce ischemia-reperfusion injury.
Objectives: Investigate if metformin reduces preservation injury in two *ex-vivo* normothermic machine perfusion (NMP) models using porcine and rat donor kidneys.
Methods: Porcine kidneys were perfused for 4 h with oxygenated blood. As postconditioning, metformin was added in increasing doses to the perfusion fluid until approximately 300 mg/L was achieved. In the rat study, NMP was performed for 90 min. Metformin or saline was administered via oral gavage the day before nephrectomy as preconditioning. Metformin was added in two different concentrations (30 or 300 mg/L) to the perfusion fluid as reconditioning. The control group received no metformin, constituting six groups in total. The interaction between treatment and perfusion time was determined using mixed-effect models with repeated measures.
Results: Compared to controls, creatinine clearance was decreased in metformin-treated porcine kidneys, while no difference was observed in rat kidneys. Oxygen consumption of metformin-treated porcine kidneys was non-significantly decreased after 2 h ($p = 0.06$). Metformin reconditioning of rat kidneys resulted in decreased oxygen consumption with accompanying increase in lactate. Fractional sodium reabsorption was increased in preconditioned rat kidneys without postconditioning, possibly indicating improved tubular function. In both studies, perfusate ASAT and LDH concentration, and tissue ATP concentration did not differ between both treatment groups.
Conclusion: Creatinine clearance was decreased in porcine kidneys, while this was not observed in the rat study. No differences were found in any known marker of injury. In both studies, the oxygen consumption was decreased in the metformin treated group. Whether metformin affects donor quality has to be further elucidated.

P3 MODULATION OF SIMULATED REPERFUSION INJURY THROUGH INSTIGATION OF MACHINE PERFUSION PARAMETERS ON A PROXIMAL TUBULE CELL LINE MODEL OF KIDNEY STORAGE

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Introduction: Although hypothermic machine perfusion (HMP) of cadaveric kidneys prior to transplantation has been reported to improve graft outcome, optimal conditions for HMP remain undefined and under evaluation in both laboratory and clinical studies. To facilitate a high throughput analysis of the effect of HMP parameters, we describe a cell line model in which conditions such as fluid shear stress and hypoxia can be simulated both during storage and after simulated reperfusion.
Methods: Human proximal tubule cell line (RPTEC/TERT1) was cultured until confluence and submerged in Kidney Perfusion Solution (KPS-1). Cells were subject to combinations of conditions: fluid shear stress (FSS) in the region of 1 dyne/cm^2, static storage (SS), Hypoxia (-H) and Normoxia (-N). Hypoxia chambers purged with nitrogen were used to create hypoxic conditions whilst normoxia was instigated by leaving plates open to atmospheric oxygen. Samples were stored in hypothermic conditions for 24 h, after which viability was assessed using the SRB assay. Replicate plates stored in parallel were re-incubated with fresh media, simulating reperfusion. Percentage differences in cell viability were detected using an unpaired *t*-test.
Results: Following 24 h of static hypothermic storage, cells stored under hypoxia (SS-H) were less viable (89%) than those stored under normoxia (SS-N), ($p < 0.01$). Furthermore, under hypoxia, the presence of fluid shear stress (FSS-H) improved cell viability by 14.8% when compared to static storage (SS-H). When plates run in parallel were re-immersed in media, simulating 24-h reperfusion, plates that had been stored under hypoxia and FSS (FSS-H) had 14.4% improved viability over those that had been stored statically (SS-H) ($p \leq 0.01$).
Conclusions: Using a cell viability assay, our preliminary results supports the usage of perfusate oxygenation during storage. Modulation of fluid shear stress during storage may also confer a therapeutic effect.

P4 SHORT-TERM OUTCOMES FOLLOWING A MODEST PROLONGATION OF COLD ISCHAEMIC TIME (CIT) WITH HYPOTHERMIC MACHINE PERFUSION (HMP) USAGE

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Background: The role of Hypothermic Machine Perfusion (HMP) as a bridging tool to safely prolong the CIT in kidneys prior to transplantation remains contentious. The aim of the current study was to compare outcomes for HMP kidneys with a modest prolongation of CIT with static cold stored (SCS) kidneys with a shorter CIT.
Methods: A population based cohort study was performed using prospectively collected data from the National Health Service Blood and Transplant service in the United Kingdom. All adult recipients of single organ DCD kidneys transplanted between 2007 and 2015 were included. Outcomes for SCS kidneys with CIT 10–12 h were compared with HMP kidneys with CIT 12–18 h with delayed graft function and 1-year death censored graft survival used as the primary outcome measures.
Results: A total of 4529 DCD kidneys were included for analysis of which 817 met the inclusion criteria. For the kidneys stored in SCS conditions with CIT 10–12 h ($n = 505$), the rate of delayed graft function was 41.2%. Whilst this was higher than for the HMP groups with more prolonged CIT 12–18 h (35.3%), this was not significant ($p = 0.104$). There was no difference in 1 year death censored graft survival between the two groups (SCS CIT 10–12 h 94.7%, versus HMP 12–18 h of 93.8%, $p = 0.533$).
Discussion: Our UK population-based study demonstrates equivalent short term outcomes for HMP kidneys with a moderately prolonged CIT compared to SCS kidneys with shorter CIT. This would appear to support the practice of safe prolongation of CIT using HMP such as during overnight perfusion.

P5 VISUALIZATION OF GLYCOCALYX BY ELECTRON MICROSCOPY

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For the past decade, several studies have described the endothelium glycocalyx, as an important factor in vascular physio- and pathology [1], giving rise to increase knowledge about its composition to have a better understanding of its structure and function relationships [2]. Settled as a real vascular barrier, the most relevant involvement of endothelium glycocalyx is its role in the mechano-transduction regulation and the associated "shear-stress" effects [3]. Relationships between shear-stress and morphological changes of endothelium are now well advanced restoring an interest for the investigations of the glycocalyx ultrastructure by electron microscopy [4,3]. Such approaches require reliable techniques to preserve the glycocalyx in a native state and to image it correctly in term of contrast. The appearance of the glycocalyx (and its thickness) depends really of the procedure employed in the electron microscopy preparation. A dynamic but fragile structure, the glycocalyx is rapidly degraded when it is removed from its natural environment . The aim of this work is to report a simple methodology to investigate intestinal and vascular endothelium glycocalyx by electron microscopy. Perfusion fixation is applied using aldehydes fixatives enriched with lanthanum ions. Additional lanthanum nitrate in fixing solution allows to enhance the staining of the glycocalyx in transmission electron microscopy bright field and improves its visualization by detecting the elastic scattered electrons providing a chemical contrast. Fixation and staining procedures are discussed in regard of ultra-structural glycocalyx findings [5].

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P6 MULTIPARAMETRIC, NONINVASIVE MONITORING OF TEMPERATURE IN THE PRESENCE OF THERMAL GRADIENTS – RESULTS OF ^{1}H MRS DEVELOPMENTS TOWARD APPLICATIONS IN ORGAN PRESERVATION

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Monitoring and controlling organ temperature in cold storage is an active domain of transplantation research. During both cooling and re-warming, considerable temperature gradients occur within the organ, and change significantly over time. This highlights the necessity to not only measure average temperature values, but to quantify thermal heterogeneity within the organ.

Although noninvasive temperature mapping for individual selected slices of body tissue has recently been used in clinical environments [1], this approach does not provide parameters that characterize thermal heterogeneity in a *quantitative* fashion. To address this challenge, we have recently developed a new paradigm allowing, for the first time, examination of the statistical distribution of temperature values within a given volume of (semi)-aqueous material, including also organs [2]. Our novel technique results in at least eight different, quantitative parameters characterizing the thermal heterogeneity within the object of choice.

This is achieved experimentally by multiparametric analysis of the proton magnetic resonance spectroscopic (^{1}H MRS) signal of water, an intrinsic "physicochemical temperature probe" for (semi-)aqueous materials. We present here this new method, including results obtained on test objects such as well-defined hydrogels and excised muscle tissue. Our technique can easily be combined with MRI (magnetic resonance imaging) and phosphorus (^{31}P) MRS. The potential of our approach in temperature monitoring during organ cooling and re-warming is also discussed.

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P7 EFFICACY OF THE NOVEL PRESERVATION SOLUTION ECOSOL IN A RAT LIVER TRANSPLANTATION MODEL

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Background: Despite recent advances in organ preservation techniques and development of novel immunosuppressants, remarkable progress in graft preservation quality has not been achieved for over several decades. Ecosol, an extracellular-type, colloid-based preservation solution, has recently been developed for washout, cold storage, and machine perfusion preservation of kidney grafts. The aim of this study was to assess the efficacy of Ecosol compared to the widely used University of Wisconsin (UW) solution for cold storage preservation of liver grafts.
Material and Methods: Liver grafts (n = 12 per group) were retrieved from male Lewis rats weighing 250–300 g and transplanted into male Lewis (Syn) or Brown Norway (Allo) rats after 8 h cold storage using Ecosol or UW. Recipients were sacrificed 24 h or 168 h posttransplant and blood and tissue samples were collected for biochemical, hematological and histopathological analyses. Additionally, livers retrieved from Luc-Lew rats (n = 4 per group) were cold stored for 8 h in Ecosol or UW for graft viability assessment using the Lumina XR II In Vivo Imaging System (IVIS).
Results: Graft and recipient survival was 100% in all groups. ALT and LDH values at 24 h posttransplant were significantly higher in the UW groups compared to the Ecosol groups. Portal venous flow rates after reperfusion were significantly higher in the Ecosol groups (8.16 $\pm$ 1.52 vs 5.24 $\pm$ 2.86 and 15.86 $\pm$ 0.95 vs 12.31 $\pm$ 3.51 mL/min, Eco-Syn versus UW-Syn and Eco-Allo versus UW-Allo groups resp.). Cholangitis did not occur in the Ecosol groups, contrary to severe cholangitis in the UW groups (UW-Syn 43%, UW-Allo 14%). IVIS assessment of liver tissue after preservation showed a higher photon flux in the Ecosol group compared to the UW group indicating better graft viability.
Conclusion: Ecosol solution improved the preservation quality of cold stored liver grafts and better maintained the microcirculation compared to UW in a rat liver transplantation model.

P8 RESULTS OF KIDNEY TRANSPLANTS FROM MAASTRICHT II CIRCULATORY DEATH DONORS: MONOCENTRIC EXPERIENCE AFTER 100 TRANSPLANTATIONS

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Introduction: From 2006, Maastricht type II non-heart beating donation program has been introduced to relieve the donor shortage. Limited to few centers, the procedure is restrictive and the results are controversial.

The aim of this study was to establish the results after 100 transplantation from circulatory death donor (DCD) and to compare them to the results from brain death donors (BDD).
Material and Methods: We retrospectively analyzed data which were collected prospectively. Transplantations were realized between August 2007 and May 2016. We studied donor characteristics, perfusion type, ischemia time, rates of delayed graft function (DGF), creatinine level, graft and global survivals at 1 and 5 years.
Results: Over Mastricht II donor, mean age was 45.2 years, 75% were men. Mean warm and ischemia time were 11 min et 515 min respectively. 61% of preservation have been made by normothermic regional perfusion. Mean age among recipients was 52.1 years. 65% presented a DGF with a mean hemodialysis sessions of 3.4. Diuresis started with a mean time of 4 days and creatinine nadir was obtained after 23 days. Mean Creatinine was à 146 mmol/L and 185 mmol/L at 1 and 5 years.

Global and graft survival were respectively 99% et 96 à 1 an et de 93 et 89% à 5 ans. In our center, these results are comparable to the results with standard criteria DBD donors and better that those with extended criteria.
Conclusion: DCD procedure with Maastricht II donors is currently forsaken in favor to the Maastricht III procedure. The latter seems to be easier to develop and the results appear to be better. However our study showed that results with Maastricht II donor are better that those from extended criteria BDD and that the procedure should not been abandoned.

P9 NORMOTHERMIC MACHINE PERFUSION OF ISCHAEMICALLY DAMAGED PORCINE KIDNEYS WITH AUTOLOGOUS, ALLOGENEIC AND HUMAN RED BLOOD CELLS

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In porcine kidney auto-transplant models red blood cells (RBCs) are required for ex-vivo normothermic machine perfusion (NMP). As large quantities of

RBCs are needed, utilising autologous RBCs would imply lethal exsanguination of the pig that is donor and recipient-to-be in the same experiment. The purpose of this study was to determine if an isolated porcine kidney can also be perfused with allogeneic or human RBCs instead.

Porcine kidneys, autologous and allogeneic blood were obtained from a local slaughterhouse. Human RBCs (Opos), were provided by our transfusion laboratory. Warm ischaemia time was standardised at 20 min and subsequent hypothermic machine perfusion with UW-MP lasted 1.5–2.5 h. Next, kidneys underwent NMP at 37°C during 7 h in a recirculating circuit with either washed, leukocyte depleted autologous, allogeneic, or human RBCs (n = 5 per group). Other components of the perfusate were Williams' Medium E, albumin and creatinine.

During perfusion kidneys were functional and produced urine. No macroscopic adverse reactions were observed. ASAT was significantly higher in the xeno group ($p = 0.01$). LDH release, peripheral renal resistance and fractional excretion of sodium did not differ significantly between groups. Creatinine clearance was significantly higher in the xeno group in comparison with the other groups ($p = 0.02$), but not in comparison with the autologous group alone. The concentration of albumin in the urine was significantly higher in the xeno group ($p < 0.005$). Renal histology revealed acute tubular necrosis in all groups. There were signs of glomerular hyperfiltration in the xeno group.

In conclusion, perfusion of porcine kidneys with RBCs of different origin proved feasible. However, laboratory analysis and histology revealed more damage in the xeno group compared to the other two groups. These results indicate that the use of allogeneic RBCs is preferable to human RBCs in a situation where autologous RBCs cannot be used for NMP.

P10 EXTRACORPOREAL REGIONAL PERFUSION FROM DONORS AFTER CONTROLLED CIRCULATORY DEATH: CLINICAL AND BIOLOGIC RETROSPECTIVE ANALYSIS IN 3 CENTERS IN FRANCE

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Background: Donation after circulatory death (DCD) is particularly susceptible to the effects of warm ischemia injury. Normothermic regional perfusion (RP) by extracorporeal membrane oxygenation (ECMO) offers the possibility of minimizing the effects of ischemia/reperfusion injury. In France, RP use in controlled DCD is required for hepatic transplant and highly suggested for renal transplant. However, proper assessment of RP, optimal methods during ECMO remain key issues. We describe a retrospective experience of selective use of RP in 3 centers (Nantes, Tours, La Roche-sur-Yon).
Methods: From April 2015 to October 2017, we performed 54 RP for all controlled DCD. The national protocol of RP was used in each case. The pH was kept between 7.3 and 7.4 by continuous adjustments with bicarbonate and setting of ECMO. Blood samples were withdrawn 10 min after RP was started and every 60 min to determine biochemical and hematological parameters.
Results: 8 adverse events occurred during RP: 4 accidental decannulations, 2 arterial dissections, 2 balloons inflated in the abdominal aorta. Organs were lost for 6 donors. Cannulas were introduced into femoral vessels via percutaneous technic in 54% cases, via chirurgical technic in 16% or both in 30%. Medians donor warm ischemia time (minutes) with chirurgical technic or percutaneous technic or both were respectively 37.5 (quartile: 30; 39) 33 (quartile: 29.5; 49), 23 (quartile: 20; 30) (p value <0.0001). Means vascular filling were respectively 1535 mL at 30 min, 1000 mL between 30 and 60 min ($p < 0.05$). For the same periods, means bicarbonate volume (bicarbonate 8.4%) were 182 and 122 mL ($p < 0.05$), means pHv were 7.05 and 7.30 ($p < 0.01$), means pvCO2 were 74.6 et 50.5 ($p < 0.01$).
Conclusion: RP is a safe technic but a learning curve is necessary. To implant cannula, percutaneous procedure is the faster, reduces the warm ischemia time and thereby could increased the number of hepatic transplant.

P11 DISCARDED STEATOTIC LIVERS EVALUATED BY NORMOTHERMIC MACHINE PERFUSION: WILL MODERATE – SEVERE FATTY LIVERS CONTINUE TO BE A CONTRAINDICATION FOR TRANSPLANTATION?

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Background: The scarcity of liver grafts and the enlargement of indications for liver transplantation (LT) have a negative impact on the waiting list mortality. Moderate to severe steatosis is a contraindication in LT; mechanisms why some steatotic livers failed to function remain unclear. Our aim is to evaluate the functionality of this type of grafts by the "*ex vivo*" normothermic machine perfusion (NMP).
Methods: 10 steatotic livers declined for transplantation prior to a frozen section biopsy (>50% macrosteatosis), were procured and perfused during 6 h by NMP. Perfusate samples were taken to measure transaminases, lactate, electrolytes and glucose. Quantification of bile production and vascular resistance and flow were analysed at different moments. Complete anatomopathological examination was done after perfusion.
Results: Non-alcoholic fatty liver disease (NAFLD) was found in 50% of the cohort and the rest had Non-alcoholic steatohepatitis (NASH). The median percentage (range) of macrosteatosis (MaS) and microsteatosis (MiS) was of 40% (10–90%) and 40% (20–50%), respectively. Significantly higher bile production was seen in NALFD grafts ($p < 0.05$) as well as lower vascular resistance. Lactate levels were lower at the end of perfusion in NALFD grafts ($p < 0.05$) however only 3 (30%) were under normal values. Transaminases were significantly higher in the group of NASH ($p < 0.05$). No difference was found concerning electrolytes and glucose.
Conclusion: NMP enables assessment of hepatic function and suggest a possible route for the recovery of fatty livers discarded on conventional criteria. We demonstrate that NAFLD grafts could be used potentially in the future to address the shortage of organs for transplantation. Further research should be done.

P12 RIVAROXABAN, A DIRECT INHIBITOR OF FACTOR XA, ATTENUATES MYOCARDIAL ISCHEMIA-REPERFUSION INJURY IN RATS AT THERAPEUTIC CONCENTRATIONS

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Introduction: Acute myocardial infarction is a leading cause of death worldwide. Although highly beneficial, reperfusion of myocardium is associated with reperfusion injury. Indirect pharmacologic inhibition of factor Xa by fondaparinux has been shown to attenuate myocardial ischemia-reperfusion (I/R) injury *via* the activation of the SAFE pathway. The link between the inhibition of factor Xa and the activation of this cardioprotective pathway remains unclear.
Objective: To study the effect of a direct inhibitor of factor Xa, rivaroxaban (RIV), on myocardial I/R injury.
Methods: We investigated the ability of RIV to prevent I/R injury in a model of transient coronary ligation in rats. 40-min of myocardial ischemia was followed by 120-min of reperfusion. RIV (3 mg/kg) was injected intraperitoneally (IP) 10-min before reperfusion or given *per os* (PO) 1-h before reperfusion. Infarct size was assessed after 120-min of reperfusion. RIV concentrations were measured after both administration protocols. Myocardial tissues were collected at 30-min reperfusion for western-blots analysis.
Results: RIV decreased infarct size by 19% when administrated IP (44.1% vs 54.2% in RIV-treated rats and controls respectively, $p < 0.05$) and by 21% when administrated PO (42.9% vs 54.2% in RIV-treated rats and controls respectively, $p < 0.05$). After IP administration, concentrations of RIV where higher than after PO administration (7244.3 ± 1853 ng/mL vs 387.7 ± 152.3 ng/mL respectively). There was no effect of RIV on the phosphorylation of STAT-3, GSK-3β, AKT and ERK1/2.
Conclusion: RIV decreased myocardial I/R injury in rats at concentrations similar to those known as antithrombotic in human therapeutics. Unlike FDX, this protective effect was not mediated through the activation of the cardioprotective pathways RISK and SAFE.

P13 P66SHC: A DRUGGABLE TARGET IN THE PREVENTION OF ISCHEMIA-REPERFUSION INJURY (IRI)?

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Excessive production of reactive oxygen species (ROS) has been causally linked to the development of ischemia/reperfusion injury (IRI) during solid organ transplantation. Since antioxidants failed in the clinic for the prevention of IRI, our work focuses on the mechanisms that control ROS production. To this end we have shown in the past the signaling through RAF-MEK-ERK prevented excessive mitochondrial ROS levels and cell death, while activation of p38 kinase had the opposite effect. The oxidoreductase p66Shc is unique among ROS producing systems as its knockout did not affect normal survival while it prevented pathophysiological conditions caused by excessive ROS production including IRI. It thus may present a preferred target for therapeutic interventions. Since no inhibitors of p66Shc are available, we set out to identify the signaling proteins controlling its physiological activation.

Previous work had suggested that the activation of the pro-oxidant and pro-death function of p66Shc required phosphorylation on serine 36 (S36) followed by mitochondrial import. In our work we could confirm the requirement of PKCβ for ROS production and cell death but not for p66ShcS36 phosphorylation. Our search for a bona fide S36 kinase lead to JNK1/2, whose involvement was confirmed through the use of inhibitors and JNK1/2-deficient cells. Moreover, expression of a S36E mutant in p66Shc-deficient cells restored ROS

production under the stress conditions tested here. Additionally, we identified S139, T206 and S213 as critical PKCβ target sites regulating the pro-oxidant and pro-death function of p66Shc. Activation of both kinases, which is also observed during ischemia/reperfusion, is necessary for the full activation of p66Shc.

In summary in our work we established the conditions for future therapeutic inhibition of the oxidoreductase p66Shc, a main contributor to pro-oxidant damage during ischemia/reperfusion.

P14 EFFECTS OF IRISIN ON SKELETAL MUSCLE IN MICE WITH LOWER LIMB ISCHEMIA REPERFUSION INJURY

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Aims: Acute ischemia-reperfusion (I/R) injury can be seen in the lower extremities during aortic surgery when a temporary cross-clamp is placed in the abdominal aorta. In the present study, we aimed to investigate the effects of irisin on reperfusion in mice with lower limb I/R model.
Methods: Mice were divided into 4 group; Control, Irisin, I/R and I/R+Irisin. Irisin was administered intraperitoneally (0.5 μg/g) 30 min before the procedure. An atraumatic microvascular clamp was placed across the infrarenal abdominal aorta in I/R groups. Following 120 min of ischemia, the clamp was removed and reperfusion was continued for 120 min. At the end of reperfusion period, skeletal muscle samples of lower extremity were taken from all groups for biochemical and histopathological examinations.
Results: Total oxidant status enzyme activities is significantly higher in I/R group according to control, Irisin and I/R+Irisin group ($p = 0.007$, $p = 0.008$, $p = 0.009$, respectively). Total antioxidant status enzyme activities is significantly low in in I/R group according to control, Irisin and I/R+Irisin group ($p = 0.045$, $p = 0.015$, $p = 0.011$, respectively). Myositis caspase 3 and 8 enzyme activities are high especially in I/R and also in control , Irisin group. Inflammation is significantly high in I/R group according to control, Irisin and I/R+Irisin ($p = 0.007$, $p = 0.037$, $p = 0.037$, respectively). Myositis injury is also significantly high in I/R group according to control, Irisin and I/R+Irisin ($p < 0.0001$, $p = 0.022$, $p < 0.0001$, respectively).
Conclusion: Our results confirm that, irisin has protective effects against the skeletal muscle damage resulting from I/R in mice. Future studies conducted to evaluate the effects of irisin on damage to various organs following different I/R durations may help understanding possible protective effects of irisin and underlying mechanisms in tissue damage related to I/R injury.
Keywords: Ischemia reperfusion, Irisin, Caspase 3 and 8, TOS, TAS, Mice.

P15 MODULATION OF THE PULMONARY ARTERIAL ENDOTHELIAL DYSFUNCTION BY HUMAN SERUM ALBUMIN IN A RAT ISCHEMIA/REPERFUSION MODEL WITH CARDIOPULMONARY BY-PASS

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Introduction: Ischemia/reperfusion (I/R) causes endothelial dysfunction (ED) and systemic inflammatory response syndrome (SIRS) during lung transplantation (LT) which can lead to primary graft dysfunction. Use of cardiopulmonary by-pass (CPBP) during LT remains controversial, it would increase I/R damages. Human Serum Albumin (HA) would limit ED and SIRS induced by CPBP. The aim of this study was to evaluate the impact on HA on ED and glycocalyx (GX) lesions induced by CPBP and I/R on rats' pulmonary artery.
Material and Method: There were 3 groups of 8 rats; SHAM in which rats were scarified without any intervention, GELO-Group and HA-Group with a 45 min CPBP; in the first group the priming was made by Gelofusine and in the second it was made by HA, during CPBP there was 15 min of left lung ischemia and 30 min of reperfusion. We recorded clinical outputs (mean arterial pressure, cardiac frequencies and amount of vascular filling). At the end of experimentation, PA was isolated and ED was studied by Mulvany myography (3 PA per rat). We recorded the contraction level after injection of phenylephrine, and the percentage of dilatation after acetylcholine injection. The GX were studied by electron microscopy to evaluate the impact on its structure.
Results: Vascular filling was significantly decreased by HA during CPB (9.5 ± 3.46 mL vs 15.75 ± 6.2, $p < 0.05$) without effect on the other clinical outputs. In HA and SHAM group vasodilatation capacity were significantly higher (60% vs 20% GELO-group $p < 0.01$) despite an equal contraction level in HA and Gelo-group which was significantly increased versus SHAM (1.4 mN vs 0.8, $p < 0.05$). GX analyses showed a comparative preserved and homogenous structure between SHAM and HA-group unlike the GELO-Group in which GX were rare and not attached to endothelial cells.
Conclusion: HA as CPBP priming solution seems to decrease the ED of PA in our study and also decrease the amount of vascular filling, and preserved GX.

P16 HEMO2LIFE® IN ORGAN PRESERVATION

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Organ transplantation is the elective treatment in case of end-stage organ failure but its success is still limited by the ischemia-reperfusion injuries (IRI). Early function recovery of transplanted organ is a predictor of short and long-term outcomes after transplantation. The medical device $HEMO_2life^®$ is a breakthrough innovation using an extracellular hemoglobin (M101) extracted from *Arenicola Marina* used as an additive to organ preservation solutions. Featuring high oxygen carrying capabilities and unique anti-oxidant properties, $HEMO_2life^®$ limits the impact of IRI with benefits for graft function and survival. We report several studies in animal models for kidney, heart, liver and lung transplantation with M101. In all studies, following the addition of M101, organ functional parameters and IRI biological markers improved when compared to control groups. Preclinical data is being confirmed by the recent clinical trial in human kidney transplantation. In a kidney pig transplantation study, serum creatinine during first 2 weeks post-transplant decreased in M101 group showing an earlier functional recovery confirmed after 3 months of follow-up. In a pig lung transplantation model, hemodynamic and functional parameters improved in M101 treated group: reduction of graft vascular resistance and increase in graft oxygenation ratio. In isolated perfused rat hearts, coronary flow was significantly higher in M101 group with LVEDP and HR similar recovery compared to control. In pig liver transplantation model, M101 reduced cytolysis and showed less inflammatory cells activation on histological analysis. First clinical study with M101 as add-on to preservation solution was safe and showed promising efficacy data on 57 kidney grafts. No immunological, allergic or pro-thrombotic effects were reported. The existing body of evidence with $HEMO_2life^®$ pleads for a significant therapeutic benefit on reducing IRI during organ preservation.

P17 IGL-1 PRESERVATION SOLUTION AND LIVER GRAFT FUNCTION, A RETROSPECTIVE STUDY

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High risk organs are increasingly used for Liver Transplantation (LT), but they are more sensitive to ischemia-reperfusion injury and adequate preservation during cold storage is pivotal. We investigated the effects of Institute George Lopez 1 preservation solution (IGL-1) on short-term outcomes compared to University of Wisconsin (UW) and Histidine-Tryptophan-Ketoglutarate (HTK). After propensity score matching, 246 LT performed between 1/2000 and 1/2016 were considered. Donor, recipient demographic, transplant data, and short-term outcomes were compared between LT performed with IGL-1 ($n = 82$), UW ($n = 82$), or HTK ($n = 82$). Bonferroni correction for multiple testing was applied. A multivariable logistic regression, adjusted for LT era, assessed the effect of preservation solutions on Early Allograft Dysfunction (EAD). Data are expressed as median (IQR). Donor demographics was similar, but donors after circulatory death were more frequently used in IGL-1 (47.6%) than in UW (18.3%, $p = 0.02$) and HTK (23.2%, $p = 0.04$). Donor hepatectomy time was shorter in IGL-1 than in HTK [32 min (23.5–41) vs 39 min (28.5–52), $p = 0.01$]. Recipient demographics did not differ; however, none of them underwent LT due to acute liver failure in IGL-1, in contrast to both UW (2.4%, $p < 0.0001$) and HTK (7.3%, $p < 0.0001$). The duration of LT was longer for IGL-1 [6.57 h (5.49–8.27)] than UW [5.53 h (4.43–6.92), $p < 0.0001$] and HTK [6 h (4.49–6.74), $p = 0.0003$]. Cold ischemia was shorter in IGL-1 [5.51 h (4.51–8.17)] than in HTK [7.28 h (5.83–8.55), $p < 0.001$]. A peak AST >2000 IU/L within 7 days occurred less frequently in IGL-1 (9.8%) than in UW (13.6%, $p < 0.0001$) and HTK (24.4%, $p < 0.0001$), but the incidence of EAD did not differ. The rate of biliary strictures within 1 year was similar. IGL-1 was the only solution protecting against EAD at univariate regression [OR: 0.36, 95% CI: 0.17–0.74, $p = 0.01$], but not at multivariate analysis. IGL-1 might preserve liver grafts better than UW and HTK, but further investigations are needed.

P18 AGEING IN LIVER TRANSPLANTATION: DOES THE INTERACTION OF DONOR AND RECIPIENT AGE MATTER?

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The average age of both donor and recipient of Liver Transplantation (LT) have increased steadily. The effect of donor and recipient age interaction on outcomes after LT is poorly understood. We explore the impact of the age interaction on patient and graft survival after LT. The effect of donor, recipient demographics, transplant data on patient and graft survival were explored at multivariable Cox regression in 849 LT (2000–2015). The variable "age" was introduced as continuous and categorical. Median (IQR) is given.

Donor age was 52 years (41–62) and 12% were Donor after Circulatory Death with 19 min (15–25) Warm Ischemia Time. Recipients aged 57 years (49–64) were transplanted with a labMELD of 15 (11–23) mostly for HCC (30%) and ethylic cirrhosis (21%). Patient and graft survival at 5 years was 76% and 72%, respectively. Donor age had no impact on patient and graft survival after LT, in particular donor >70 years did not influence outcomes (HR: 0.87, 95% CI: 0.54–1.39, $p = 0.55$ for patient survival; HR: 0.78, 95% CI: 0.50–1.19, $p = 0.25$ for graft survival). Recipient age independently increased the risk of death post-LT (HR: 1.03, 95% CI 1.02–1.05, $p = 0.0001$), but the interaction with donor age was irrelevant (HR: 1; 95% CI: 0.99–1.001, $p = 0.88$). The adjusted effect of recipient age on patient survival was significant at 6 months (HR: 1.06, 95% CI: 1.03–1.09, $p = 0.0001$) and 1 year post-LT (HR: 1.05, 95% CI: 1.03–1.08; $p < 0.0001$). After stratification of recipient age, the adjusted risk became significant for patients aged 61–70 years (HR: 1.84, 95% CI: 1.24–2.74, $p = 0.003$) and maximal for >70 years (HR: 1.96, 95% CI: 1.03–3.72, $p = 0.04$). Matching donors >70 years to recipients >70 years did not vary the risk of death (HR: 0.49, 95% CI: 0.17–1.39, $p = 0.18$). Graft survival was not affected by recipient age (HR: 1, 95% CI: 0.93–1.05, $p = 0.89$).

Older donors are safe but recipients >70 years have a risk of death post-LT almost doubled. Strategies matching young donors to older recipients do not reduce their increased risk of death.

P19 ASSESSMENT OF THE EFFECTS OF LEVOSIMENDAN AND NIGELLA SATIVA ON MYOCARDIAL ISCHEMIA REPERFUSION INJURY IN RATS

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Ischemia-reperfusion injury is a chain of events put in place by tissue ischemia. Reperfusion following the cellular damage causes an active inflammatory response. In this study we aimed to evaluate the protective effect of levosimendan and nigella sativa on myocardial ischemia-reperfusion injury in rats.

Methods: Twenty-four Wistar albino rats were included in the study. The animals were randomly divided into four experimental groups. The coronary arteries of rats in Group C (control group) were not occluded or reperfused. Myocardial IR was performed by ligating the left anterior descending coronary artery for 30 min, followed by 2 h of reperfusion in the IR (IR), IR-levosimendan (24 μg/kg) (IRL) group and IR-nigella sativa (0.2 mL/kg) (IRNS) group.

Results: Inflammation findings were significantly higher in the IR group compared with the C, IR-NS, and IR-L groups ($p = 0.001$, $p = 0.019$, $p = 0.019$, respectively). Compared with the C, IR-NS, and IR-L groups, the microscopic myocardial disorganization was significantly higher among the IR group ($p < 0.0001$, $p = 0.007$, $p = 0.001$, respectively). The light microscopic myocardial tissue interstitial fibrosis levels were significantly higher in the IR group than in the C, IR-NS, and IR-L groups ($p < 0.0001$, $p = 0.044$, $p = 0.003$, respectively).

Conclusion: Levosimendan and NS administration at the beginning of myocardial ischemia can provide varying degrees of protection against negative effects of variations in light microscopic inflammation findings, myocardial disorganization degrees and myocardial tissue interstitial fibrosis levels.

Keywords: Ischemia reperfusion, levosimendan, nigella sativa, heart.

P20 ACUTE KIDNEY INJURY (AKI) AFTER SOLID ORGAN TRANSPLANTATION – CIRCULATING FREE HEME AS A CONTRIBUTING RISK FACTOR

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Acute kidney injury (AKI) is a severe complication after solid organ transplantation (tx). Especially, lung-, and heart- tx are associated with the need of blood transfusion. Packed red blood cells (pRBC) are beneficial and in many cases lifesaving. However, ageing can cause release of toxic extracellular hemoglobin and heme, which may contribute to AKI. In this study, systemic heme release after orthotopic heart tx was compared to kidney tx in patients.

Patients undergoing htx ($n = 10$) or ktx ($n = 13$) at our institution were enrolled in a prospective clinical study and blood sampling was done prior to surgery, at 6 h, 24 h, 1 and 7 days after surgery. Free heme quantification was measured by peroxidase enzyme activity and compared to clinical outcome parameters such as s-creatinine, LDH increase.

The patients after htx had severe increase of free heme within 30 min after surgery baseline: 9 ± 7.6 to $17{,}255 \pm 3418$ fmol/μL after surgery. LDH increase ranged from 278 ± 60 up to 834 ± 4539–834 U/L. AKI rate was 60% and most prominent in patients with complicated surgeries and large amount of pRBC transfusion (up to 24 pRBC). In contrast, after ktx only minor systemic heme release without the need of pRBC transfusion was recorded.

pRBC are stored up to 42 days in Germany. Free hemoglobin, free heme and iron are released over time due to ageing and are potential harmful. Here, we show that in htx excessive heme release is measurable and correlates with LDH increase and AKI. After ktx only minor heme release was measured despite cold ischemia times of up to 20 h. Free heme is vasoactive and can cause microcirculation disturbances and is a strong inductor of complement activation and inflammation.

Conclusion: Transfusion of pRBC in the context of major surgery and htx can lead to increased systemic heme and might be a risk factor for AKI. Strategies to measure free heme in pRBC prior to transfusion could enhance patient safety.

P21 DIETRAY FOOD SUPPLEMENTATION WITH OMEGA-3 IN RENAL ISCHEMIA REPERFUSION INJURY ATTENUATES ACUTE KIDNEY INJURY (AKI)

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Background: Renal ischemia reperfusion injury (IRI) is an important comorbidity in the context of solid organ transplantation and causes AKI. Here we present a dietary omega-3 poly unsaturated fatty acid (PUFA) food supplementation study to investigate whether pre-treatment can reduce IRI.

Methods: Male 12–14 week old C57BL/6J mice received omega-3 food supplementation (2 % in the chow containing 10% fat) and a control group had chow with low omega-3 FA for 2 weeks prior to IRI. Bilateral 30 min IRI was done and mice were sacrificed at 24 h. S-creatinine and BUN elevation were measured. Kidney damage was analyzed by histology, immunohistochemistry. Pro-inflammatory cytokines (IL-6, MCP1) and FA and oxylipin pattern were quantified in blood and kidneys.

Results: The feeding massively increased the levels of omega 3-PUFA. Consistently eicosanoids and others oxylipins from omega 3 PUFA were elevated while omega 6 PUFA derived mediators such a proinflammatory prostaglandins were decreased. Omega-3 feeding attenuated s-creatinine increase significantly. Similar effects were seen for BUN. PAS stain revealed similar degrees of AKI and tubular NGAL elevation. However, the tubular transport marker A1M was significantly higher expressed in omega-3 compared to vehicle treated mice indicating better integrity of proximal tubular epithelial cells. IL-6 and MCP-1 elevation due to IRI in renal tissue was not affected by omega-3 treatment.

Discussion: There are various reports on treatment strategies with omega-3 FA in the context of renal diseases. Here, we showed that omega-3 pre-treatment attenuated worsening of renal function after IRI and that tubular transport was protected as well. However, inflammation was similar in the vehicle treated and the omega-3 treated groups.

Dietary omega-3 food supplementation resulted in beneficial effects on renal function impairment in experimental renal IRI in mice but did not attenuate tissue inflammation.

P22

FUROSEMIDE AND A CHLORID-RESTRICTIVE FILLING SOLUTION TO REDUCE ISCHEMIA REPERFUSION INJURIES IN RENAL TRANSPLANTATION

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Introduction: The choice of supportive treatment after renal transplantation as using furosemide or Chlorid-poor filling solution could have an interest to limit lesions of ischemia reperfusion. The theoretical benefit of furosemide is to improve oxygenation of the proximal tubule and rich chlorid filling solution has been shown to be nephrotoxic and could worsen ischemia-reperfusion injuries.
Methods: We conducted a prospective study in the Hospital Lapeyronie in Montpellier, France to evaluate the interest of furosemide and a chlorid-restrictive solution to limit ischemia-reperfusion injuries in renal transplantation. Kidney transplant recipients with an immediate resumption of diuresis were included. We defined 2 distinct periods of inclusion. During the first study period, all patients received furosemide. During the second study period, furosemide was discontinued. Patients were randomized to receive either NaCl 0.9% (Chlorine: 154 mmoL/L) or a balanced solution: Ringer Lactate (RL) (111 mmol/L). 4 groups were defined: NaCl 0.9% with Furosemide (Cl+/F+), RL with furosemide(Cl−/F+), NaCl 0.9% w/o furosemide (Cl+/F−), RL w/o furosemide (Cl−/F−). The main objective was the comparison of plasmatic creatinine level (μmol/L) during hospitalization and until day 30 post-transplantation.
Results: 45 patients were included: 8 patients in the Cl+/F+ group, 16 Cl−/F+, 8 Cl+/F− and 13 F−/Cl−. There was no significant difference in plasmatic creatinine level during hospitalization and at day 30. At day 7, mean creatinine level in the groups (Cl+/F+), (Cl−/F+), (Cl+/F−), (Cl−/F−) was respectively 171.6, 184.4, 176.9 and 238.7 μmoL/L ($p > 0.05$).
Conclusion: Among kidney transplant recipients with immediate resumption of diuresis, the use of furosemide and a chlorid-restrictive filling solution have no influence on recovery of graft function. It would be interesting to evaluate their impact in patients at risk of delayed graft function.

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USE OF STEM CELLS ISOLATED FROM URINE (USCS) AND USC-DERIVED EXOSOMES TO REDUCE ISCHEMIA-REPERFUSION LESIONS DURING KIDNEY TRANSPLANTATION: DEVELOPMENT OF THE *IN VITRO* PROOF OF CONCEPT

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Organ transplantation is the only option to solve many end-stage organ deficiencies but is impaired by organ shortage. This situation has huge economical and societal costs and it is crucial to extend the organ pool using "sub-optimal" organs coming from "marginal" donors. However this option increases transplantation failure risk since those are very sensitive to ischemia/reperfusion (IR) process accompanying transplantation. We propose a cell therapy strategy using urine as a source of stem cells to limit IR damages. We isolated urine stem cells (USCs) from voided urine of healthy individuals and diabetic nephropathy patients and showed that success rate was higher for patients than controls (71% vs 29%). USCs expressed renal markers (KSP, AQP2 etc.), stemness-related markers (SSEA4, TRA-1-80) and co-expressed CD24 and CD133, markers of renal progenitors. Yet, their expression profile was heterogeneous inter- and intra-individually, with some clones also displaying higher proliferation capacity. Then, we submitted renal endothelial cells (hRGECs) to 24 h of hypothermic-hypoxia in UW solution followed by normothermic-reoxygenation to mimic IR process *in vitro*. We showed that using USC-conditioned medium (USC-CM) during reoxygenation significantly protects hRGECs from IR-induced necrosis. Furthermore, we isolated and characterized exosomes from USC-CM using PEG-based enrichment, to mimic USC-CM effects. We are testing USC-exosomes using different renal cell types (hRGECs, tubular cells and pericytes) as well as in a 3D model of induced pluripotent stem cells (iPSCs)-derived kidney organoids that we are currently developing. If successful *in vitro*, our strategy will be tested in a preclinical porcine model of kidney transplantation available at the MOPICT facility. Overall, we intend to convert a non-invasive and unlimited substrate, urine, into a cell-free product compatible with clinical applications to extend the number of successful kidney transplantations.