Surgery I

V001

CLINICAL USE OF CONTROLLED OXYGENATED REWARMING OF KIDNEY GRAFTS PRIOR TO TRANSPLANTATION BY EX VIVO MACHINE PERFUSION: A PILOT STUDY

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Introduction: Controlled oxygenated rewarming of kidney grafts on ex vivo machine perfusion with acellular perfusion solution has been reported to be feasible for reconditioning of marginal grafts.
Methods: We conduced small pilot clinic trial with marginal kidney grafts form extended criteria donors. $N = 6$ ECD kidney grafts were included in the treatment arm. After cold-storage the kidney arteria was cannulated and all six treatment arm grafts we perfused with acellular 1:1 Steen/Ringer solution with COR protocol The perfusion was done for 2 hours and kidneys were gradually rewarmed to 35° C . $N = 6$ controls were included in the control arm and were directly transplanted after cold-storage.
Results: Primary end-point was Creatinine clearance at post-operative day 7. Secondary end-point were defined form delay graft function, graft-survival at 3th month and post-operative complication Clavien-Dindo > 3. The patient cohort was well balanced without significant differences. Age, cold-ischemic time, warm-ischemic time, kidney donor risk index were comparable between the both groups.
The creatinine clearance at POD 7 was significantly higher in the treatment arm with $P < 0.05$.
There were no significant differences in the secondary end-points between the treatment and control arm.
Conclusion: Acellular ex vivo machine perfusion with controlled oxygenated rewarming improves the functional outcome of marginal ECD kidney grafts.

V002

NORMOTHERMIC VERSUS SUBNORMOTHERMIC EX VIVO MACHINE PERFUSION RESULT IN SIMILAR OUTCOME AFTER DCD PORCINE KIDNEY TRANSPLANTATION

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Introduction: Ex-vivo machine perfusion is a novel preservation technique for the storage and assessment of marginal kidney grafts. Normothermic (NEVKP), subnormothermic (SEVKP), as well as hypothermic machine perfusion have been developed in the recent past. The optimal perfusion temperature remains controversial. In the current study, we compared the protective effects of SEVKP and NEVKP in a porcine kidney autotransplantation model.
Methods: All pig kidneys were exposed to 60min of warm ischemia followed by 8hrs of either SEVKP (22°C) or NEVKP (37°C) ($n = 5$ in each group). After contralateral nephrectomy, grafts were autotransplanted and animals were followed for 3 days. Kidney function and injury markers were compared between groups.
Results: All animals survived the follow-up period. Grafts preserved by NEVKP vs SEVKP showed comparable postoperative kidney function. Serum creatinine (SrCrea) peaked on postoperative (POD) day 2 (NEVKP vs SEVKP: 6.7±3 mg/dl vs 5.3±1.8 mg/dl) and started decreasing on POD3 in both groups. Differences in daily SrCrea levels did not reach significance. Tubular injury scores on POD3 were similar in both groups. Interestingly, grafts perfused with SEVKP showed significantly less metabolic function during ex vivo perfusion. Oxygen consumption ($(pO_2art\text{-}pO_2ven)$ *flow/weight)) was reduced during SEVKP (mean O_2 consumption NEVKP vs SEVKP: 805±103 ml/min/g vs 248±71ml/min/g). Lactate significantly decreased during NEVKP, while lactate remained constant during SEVKP. Despite identical arterial pressure, the arterial flow was significantly higher during NEVKP compared to SEVKP and the IRR was

significantly lower in grafts perfused with NEVKP over the course of perfusion (mean IRR NEVKP vs SEVKP–1hr: 0.37 ± 0.03 vs 0.55 ± 0.07, $P < 0.05$; 8hr: 0.33 ± 0.05 vs 0.47 ± 0.07, $P < 0.05$).

Conclusion: SEVKP vs NEVKP is associated with a reduced metabolic activity during the perfusion period. Both perfusion settings provide identical graft function post porcine DCD kidney transplantation. Further studies are warranted to explore which technology represents a better platform to assess and repair expanded criteria kidney grafts.

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Clinical management I

V004

HIGH RIPK3 EXPRESSION IS ASSOCIATED WITH HIGHER RISK FOR EARLY KIDNEY TRANSPLANT FAILURE

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Introduction: Receptor-interacting protein kinase 3 (RIPK3) is a key-effector of necroptosis, a regulated form of cell death [1]. Preclinical studies have shown that genetic loss of RIPK3 protects against IRI in the kidney [2,3]. Here we present the first-in-human study evaluating the role of Receptor-interacting protein kinase 3 (RIPK3) expression with respect to clinical outcome parameters for kidney allograft recipients.

Methods: The primary analysis included 374 allografts with a baseline biopsy 10 minutes after reperfusion, per-protocol. These kidney biopsies were immunohistochemically stained for RIPK3 and expression was semiquantitatively scored ranging from 0 to 3 in a blinded fashion, referred to as the "RIPK3 score". Clinical outcome parameters for all biopsied kidney allograft recipients were analyzed.

Results: RIPK3 expression varied widely between the 374 allografts. In allografts with detectable expression, RIPK3 was primarily detected in proximal tubular cells followed by distal tubular cells, both of which are critically affected by IRI. Kaplan-Meier-analysis revealed that a RIPK3 score greater than 2.0 ($n = 211$) was associated with significantly higher risk of one-year transplant failure (9.2 % vs. 1.9 %, $P = 0.003$ by the log-rank test). Moreover, in a Cox proportional hazards model, the RIPK3 score was prognostic for one-year transplant failure (hazard ratio for death-censored transplant failure, 2.09; 95% confidence interval {CI}, 1.13 to 3.87; $P = 0.019$), independent from donor and recipient associated risk factors in multivariate analyses. The RIPK3 score also correlated significantly with deceased donation, cold ischemia time, and the extent of IRI, histologically represented as degree of acute tubular injury.

Conclusion: This is the first indication that necroptosis represented by RIPK3 expression plays a critical role in human transplantation and identifies it as a potential target to extend the limited donor pool.

Acknowledgement: The project was realized in collaboration with the "RIPK3 in kidney transplantation" collaborators.

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Infections

V007

IMPAIRED IMMUNE RESPONSE TO SARS-COV-2 VACCINATION IN KIDNEY TRANSPLANT RECIPIENTS

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Introduction: Patients after renal transplantation have a high risk for severe COVID-19 infection and vaccination against SARS-CoV-2 is the only expedient prophylaxis. Generally, immune responses are attenuated in these patients, however, systematic analyses of immune responses to SARS-CoV-2 vaccination in kidney transplant recipients (KTR) are still missing.
Methods: In this prospective multicentric cohort study, antibody responses to COVID-19 mRNA vaccines (BNT162b2; BioNtech/Pfizer or mRNA-1273; Moderna) were measured in 149 KTRs. SARS-CoV-2-specific antibodies and neutralization capacity were evaluated and compared to controls ($n = 174$).
Results: After the first vaccination, SARS-CoV-2-specific antibodies were nearly undetectable in KTRs. After the second vaccination, 94% of the controls but only 23% of KTRs developed SARS-CoV-2-specific IgG above cut-off of 35.2 BAU/ml. Mean IgG levels were significantly lower in KTRs (53.60 ± 144.14 BAU/ml) compared to controls (2563.87 ± 3580.51 BAU/ml, $P < 0.001$). Importantly, compared to controls, neutralizing antibody titers were significantly lower in KTRs compared to controls. After the second vaccination, 86% of KTRs did not show any neutralization capacity against SARS-CoV-2 suggesting impaired seroprotection. Multivariate logistic regression analysis revealed a highly significant influence of the immunosuppressive therapy with mycophenolate mofetil and the kidney function on the immune response in KTRs.
Conclusion: KTRs show a significantly weaker antibody response compared to controls. Most strikingly, only one out seven KTRs developed neutralizing antibodies against SARS-CoV-2 after two doses of vaccine. These data suggest that vaccination strategies need modification in transplant patients.

V008

PREEXISTING SARS-COV-2 B AND T CELL IMMUNITY IN UNEXPOSED TRANSPLANT AND IMMUNOCOMPETENT ADULTS

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Introduction: Preexisting immune responses to seasonal endemic coronaviruses or other pathogens might have a pivotal role in protection against SARS-CoV-2. While pre-existing SARS-CoV-2-reactive T cells were previously described for immunocompetent populations, data on pre-existing humoral and cellular immunity in transplant population and more importantly its correlation with clinical outcomes is currently lacking.
Methods: In this study, we analyzed the preexisting antibody, B, and T cell immune responses against SARS-CoV-2in unexposed adult kidney transplant recipients (Tx, $n = 14$) and immunocompetent non-transplant individuals (non-Tx, $n = 12$) sampled before the pandemic in comparison to 22 convalescent COVID-19 patients by ELISA and flow cytometry.
Results: In both unexposed groups, SARS-CoV-2 IgG antibodies were not detectable. However, we detected spike protein SARS-CoV-2-binding memory B cells in 64% of unexposed Tx and 33% of unexposed non-Tx patients, whereas 62% of convalescent patients showed SARS-CoV-2-binding memory B cells. In comparison to convalescent patients, unexposed patients showed a lower magnitude of spike-reactive B cells, however, without statistical difference. Of interest, the magnitude of SARS-CoV-2-reactive T cell immunity in transplant patients was comparable to non-transplant patients.

Furthermore we detected SARS-CoV-2-reactive follicular T helper cells in 44% of the unexposed cohort without statistically significant differences between Tx and non-Tx. The patients of the unexposed cohort were not infected with SARS-CoV-2 during the observation period until January 2021.

Conclusion: We demonstrate memory B and T cells against SARS-CoV-2 in unexposed transplanted and immunocompetent adults suggesting pre-existing adaptive immunity. The magnitude of cellular immunity in transplant patients was comparable or even higher in transplant patients. Although first data demonstrate no clinical manifestation of COVID-19 in patients with pre-existing immunity, further larger studies are required to demonstrate the antiviral protection.

Clinical management II

V009

STEERING OF IMMUNOSUPPRESSION BY VIRUS-SPECIFIC T CELLS AFTER PEDIATRIC KIDNEY TRANSPLANTATION IN THE RANDOMIZED CONTROLLED IVIST TRIAL

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Introduction: Pharmacokinetic monitoring alone is insufficient to estimate the intensity of immunosuppression after kidney transplantation. Levels of virus-specific CD4 T cells (CD4Tvis) have been shown to identify overimmunosuppression. The IVIST trial has demonstrated that additional steering of immunosuppressive therapy by CD4Tvis levels is safe and reduces exposure to immunosuppressants with significantly lower trough levels but without increasing the risk of acute rejections.

Methods: In the multicenter, randomized controlled IVIST trial, 64 pediatric kidney recipients were randomized 1:1 to a control group with trough level monitoring of immunosuppressants or to an intervention group with additional steering by CD4Tvis levels against ADV, CMV and HSV. The immunosuppression consisted of cyclosporine A, everolimus and glucocorticoids. CD4Tvis were quantified by cytokine flow cytometry in 20 visits during the two-year study period.

Results: At time of transplantation, ADV-CD4Tvis were detectable in 30/31 patients from the intervention group, CMV-CD4Tvis and HSV-CD4Tvis only in 12/31. No significant ADV- or HSV-DNAemia was found; only two patients showed transient CMV-DNAemia based on CMV-reactivation. Five primary CMV-infections with seroconversion and boost of CMV-CD4Tvis were observed without significant CMV-DNAemia. The mean level of ADV-CD4Tvis was 1.63 (SD 1.25), 2.03 (SD 1.8), 2.18 (SD 2.51) and 1.97 cells/µl (SD 1.34) 1,6,12 and 24 months after transplantation. In case of CD4Tvis < 2-cells/µl 125 dose reductions of immunosuppressants (96% based on ADV-CD4Tvis) were performed in 28/31 children with a median of 4 Tvis-based dose reductions (range 0-10) per patient. 48% of the Tvis-based dose reductions were carried out in month 2-6.

Conclusion: Under the intensified immunosuppression during the initial post-transplant period low ADV-CD4Tvis levels were observed with subsequent increase after dose reduction of immunosuppression. ADV-CD4Tvis are most suitable for immune monitoring because of their high prevalence (even in children) and stability combined with absence of ADV-DNAemia. Routine monitoring of ADV-CD4Tvis is recommendable especially in the first post-transplant year to identify overimmunosuppression.

V010

IMPACT OF VPRA ON WAITING TIME PRIOR TO KIDNEY TRANSPLANTATION IN ETKAS AND ESP IN GERMANY

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Introduction: Assignment of unacceptable HLA mismatches (UAM) in sensitized patients prevents transplantation of incompatible grafts but potentially prolongs waiting time. Whether this is true under the circumstances of the Eurotransplant Kidney Allocation Scheme (ETKAS) and the Eurotransplant Senior Program (ESP) in Germany is a matter of much debate and is relevant for UAM assignment strategies.

Methods: The proportion of the donor pool excluded due to UAM was expressed as virtual panel reactive antibodies (vPRA). We used simple and multiple linear regression analyses to investigate the impact of vPRA at the time of KTX on waiting time (1st day of hemodialysis until KTX) for all patients receiving a kidney-only graft between 01.02.2019 and 31.01.2021 via ETKAS ($n = 1548$) or ESP ($n = 566$) in Germany. Patients transplanted under special circumstances (AM, HU, multi-organ transplants, rescue allocation) were excluded from analyses.

Results: In ETKAS, there was a modest effect of vPRA levels on median waiting time in univariate analysis: 0% vPRA: 8.72 years ($n = 1072$), >0–50%: 8.72 years ($n = 212$), >50–85%: 9.25 years ($n = 162$), >85%: 9.4 years ($n = 95$), $P = 0.003$. In a multiple linear regression model, adjusting for HLA-matching, recipient blood group, mismatch probability score, DSO region and the proportion of active days on the waiting list, vPRA showed a significant association with waiting time (B = 4.59 (95% CI 3.06–6.12) $P < 0.001$). Thus, a patient had an average additional waiting time of 4.59 days per 1% increase in vPRA. In ESP, waiting time was also longer in patients with higher vPRA levels: 0%: 3.76 years ($n = 476$), >0–50%: 4.24 years ($n = 59$), >50–85%: 4.74 years ($n = 29$), >85%: 5.27 years ($n = 2$), $P = 0.023$. In a multiple linear regression model, a 1% increase in vPRA translated in a prolongation of waiting time of 5.4 days (B = 5.4 (95%-CI 1.60–9.21) $P\% = 0.005$).

Conclusion: For both ETKAS and ESP, the effect of vPRA on waiting time is modest. Given the difficulties in finding robust criteria for UAM for the individual patient, these results support an approach favoring optimal HLA compatibility.

