ABSTRACT

TTV GUIDE IT - A RANDOMISED CONTROLLED TRIAL TO COMPARE THE SAFETY, TOLERABILITY AND PRELIMINARY EFFICACY BETWEEN STANDARD AND TORQUE TENO VIRUS-GUIDED IMMUNOSUPPRESSION IN STABLE ADULT KIDNEY TRANSPLANT RECIPIENTS WITH LOW IMMUNOLOGICAL RISK IN THE FIRST YEAR AFTER TRANSPLANTATION - A TRIAL PROTOCOL

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Background: Immunosuppression after kidney transplantation is mainly guided via calcineurin inhibitor (CNI) trough level, which is able to predict CNI toxicity, but neither allograft rejection nor infection sufficiently. The peripheral blood level of the apathogenic and highly prevalent Torque Teno virus (TTV) is associated with the immunosuppression of its host. Non-interventional studies suggest TTV load to predict allograft rejection and infection after kidney transplantation.
Aim: The aim of the TTV GUIDE IT trial is the assessment of safety, tolerability and preliminary efficacy of TTV-guided immunosuppression in stable adult kidney transplant patients with low immunological risk in the first year after transplantation.
Trial design: A randomised and controlled, interventional, two-arm, single-blinded, multinational, investigator driven phase II trial.
Primary end point: A composite of infection, allograft rejection, death and graft loss.
Main secondary end points: Estimated glomerular filtration rate, protocol biopsy at month 12 post-transplantation including molecular microscope, donor specific antibodies, health related quality of life (SF-36 and MTSOSD-59R questionnaire) and drug adherence (electronic drug monitoring, BAASIS questionnaire, claimed prescriptions, psychological evaluation).
Number of Subjects: 130 per treatment group, 260 in total.
Randomization, Concealment: 1:1 randomization, allocation concealment.
Main inclusion criteria: Recipient of a kidney allograft, adult (≥18 years of age), post day 93 post-transplantation, TAC-based immunosuppression.
Main exclusion criteria: HLA/ABO incompatible transplantation, cyclosporine, mTor inhibitor or co-stimulation blocker based immunosuppression, unstable graft function.
Intervention: TTV level-guided TAC dosing: TAC target range will be adapted according to TTV copies in the plasma quantified by quantitative real time PCR (TTV R-GENE; bioMérieux, France; CE-certificate 7th of June 2021). If TTV is not within the predefined optimal range, TAC target trough level will be adapted by one step up or down compared to current TAC trough level. If TTV is below the optimal TTV range, the target TAC trough level has to be increased by one step compared to current TAC trough level, if TTV is above the target TTV range, the target TAC trough level has to be decreased by one step compared to current TAC trough level.
Control group: TAC will be dosed according to TAC trough levels defined by the local centre standard and clinical standard assessment.
Blinding: The study will be conducted using a single-blind design (participants blinded). All episodes of infection and allograft rejection will be re-assessed by personnel blinded to the randomization code (infection: two infectious disease specialists; rejection: two kidney pathologist).
Trial Schedule: Recruitment time: 12 months, trial start: April 2022 (6 months roll out), trial end: April 2025; trial subject: 3 months screening, 9 months intervention (last 6 weeks follow-up).
Including Centres: Berlin, Dresden, Graz, Grenoble, Groningen, Innsbruck, Leiden, Linz, Prague, Regensburg, Strasbourg, Valencia and Vienna.
Impact: The trial has the potential to reduce infection and graft rejection by 20% thereby significantly improving graft and patient survival of kidney transplant patients. Healthcare costs may be reduced by ~€ 50 million in the EU per year. The project will serve as a proof-of-concept for TTV-based assessment of the immune system, with potential applications in solid organ transplantation, autoimmune and infectious disease and oncology.
EudraCT-number: 2021-002525-24.
Sponsor: Medical University of Vienna.
Funding: Horizon 2020, grant agreement ID: 896932; start: 1st May 2021, end: 30th April 2026; budget granted by the European Commission: € 6 099 831.
Web: https://www.ttv-guide.eu/ https://cordis.europa.eu/project/id/896932

ABSTRACT

PROTEINURIA IN DISEASED KIDNEY TRANSPLANT DONORS FOR PREDICTION OF CHRONIC LESIONS IN PRE-TRANSPLANT BIOPSIES - A PROSPECTIVE OBSERVATIONAL STUDY

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Background: Pre-transplant kidney graft biopsies have been suggested to assess donor organ quality. However such biopsies are not broadly implemented in clinical routine as a screening tool in Europe and most transplant centers use donor data to guide decision upon indication of pre-transplant biopsies. Currently, there is no data on the association of donor proteinuria and pre-transplant biopsies results.
Methods: This prospective observational study was designed to test the association between donor proteinuria and organ quality in the setting of kidney donation from deceased donors. We included all 256 diseased donor kidneys procured locally and transplanted at the Medical University Vienna (MUV), between July 2017 and May 2020 with sufficient data on pre-transplant biopsy and donor proteinuria. Each compartment of allograft kidneys obtained by pre-transplant punch biopsies was assessed on paraffin embedded material, processed by standard methodology for kidney biopsies (HE, PAS, Silver-Methenamin and SFOG staining) and scored from 0 to 5, with 1 point per 20% affected glomeruli/area/lumen stenosis to define the amount of glomerulosclerosis (gs), intima fibrosis (if), hyalinosis (h), interstitial fibrosis (f) and tubulus atrophia (ta). Data on urinary dip sticks were retrieved from the Eurotransplant donor report. Donor urinary protein to creatinine ratio (UPRR), urinary albumin to creatinine ratio (UACR) and electrophoresis were performed at the MUV.
Results: There was a positive correlation between donor UPCR and the extent of chronic lesions (sum of all chronic lesions: gs+if+h+f+ta) in the corresponding kidney biopsies ($P = 0.017$, Spearman's rank correlation coefficient). Biopsies with less than two chronic lesions (= median) had a median donor UPCR of 274 mg/dL (IQR 211–556) compared to a UPCR with a median of 486 mg/dL (IQR 251–717) in biopsies with two or more leasons ($P = 0.016$, Wilcoxon rank-sum test). The risk for the detection of two or more chronic lesions in pre-transplant biopsies rose by 18% for every log increase of donor UPCR (generalized linear model; RR 1.18, 95% CI 1.03–1.25, $P = 0.017$). Multivariate analysis including the most relevant factors for kidney donor organ quality (donor age, sex, weight and size, creatinine on admission and last creatinine obtained before organ procurement, history of peripheral arterial hypertension or diabetes and stroke as the cause of death) revealed an independent association between donor UPCR and the presence of chronic lesions in the pre-transplant biopsy. A high sensitivity of 90% was calculated for a UPCR $\geq$130 mg/dL and a high specificity of 90% was calculated for a UPCR $\geq$ 1040 mg/dL to detect two or more chronic lesions in pre-transplant biopsies (AUC 0.62, 95% CI 0.54–0.70, $P = 0.0496$). Multivariate logistic regression modeling demonstrated the value of donor UPCR addition to increase predictive performance of known donor risk factors for chronic lesions in pre-transplant biopsies.

UACR was also associated with donor organ quality, but not urinary dip sticks or electrophoresis.

There was no association between donor proteinuria and kidney function and proteinuria in the first year after transplantation.
Conclusion: Donor UPCR is associated with chronic histologic lesions in pre-transplant kidney graft biopsy and might be useful to guide decision upon indication of pre-transplant biopsy. Our data might trigger discussion concerning inclusion of UPCR in the Eurotransplant donor report. Further prospective studies are needed to determine the value of scores to predict organ quality and graft outcome including donor UPCR.
Additional Information: Institutional review board, approval number: 267/2011; German Clinical Trials Registry, register number: DRKS00025574; no funding source.

IMMUNE CELL PROFILING DURING NORMOTHERMIC MACHINE PERFUSION OF HUMAN LIVER ALLOGRAFTS

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Background: Liver transplantation (LT) remains the only effective therapy for end-stage liver disease. Normothermic machine perfusion (NMP) allows to maintain the liver ex vivo in a complete functional state at 37°C, under physiological conditions. The aim of this study was to perform an immune cell profiling and cytokine characterization in the perfusate during NMP.
Methods: 26 liver allografts were subjected to NMP. Perfusate samples were collected at 1, 4, 6, 12, 24 hours (h). Immune cell profiling was performed with flow cytometry for perfusate samples and with immunohistochemistry for liver parenchyma. Cytokine levels were measured using the Luminex technology. H/E staining was applied for assessment of fibrosis, steatosis and necrosis on liver tissue.
Results: Total amounting of granulocytes, lymphocytes and monocytes (all $P < 0.001$) in the perfusate, significantly decreased over time. The proportion of CD3+ T cells ($P = 0.007$), monocytes, CD19+ B cells, CD4+T-helper cells, CD8+ cytotoxic T cells and FoxP3+ regulatory T cells (all $P < 0.001$) varied significantly over NMP period, while granulocytes, CD56+ natural killer cells, CD3+CD56+ natural killer T cells, mucosal-associated invariant T cells, Kupffer cells and dendritic cells remained constant over time. Pro-inflammatory cytokines IL-1a, IL-2, IL-7, IL-9, IL-17a, IL-18, IL-27, Eotaxin, GROa, IL-8, IP-10, MCP-1, MIP-1a, MIP-1b, SDF-1a, IFNg, TNFa ($P < 0.001$) and IL1b ($P = 0.047$) significantly augmented in the perfusate during NMP. Anti-inflammatory cytokines levels of IL-4 and IL-13 increased ($P = 0.001$), while IL-10 levels drastically dropped ($P = 0.001$) during NMP. Immune cell infiltrate in liver tissue was composed by granulocytes (40–80%), T cells (40–80%) and B cells (<40%), without relevant changes over NMP time. Liver allografts showed an invariate mild fibrosis and steatosis, while necrosis minimally increased towards NMP end.
Conclusion: A detailed first phenotypization of liver specific immune cells and cytokine profiling during NMP was performed as basic approach for future analyses.

EVALUATION OF PRE-TRANSPLANT CHECK-UP PROCEDURES OF PATIENTS WITH END-STAGE RENAL DISEASE NOT ELIGIBLE FOR KIDNEY TRANSPLANTATION

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Background and Aims: Patients with end-stage renal disease (ESRD), who are candidates for kidney transplantation (KT), are subjected to a detailed evaluation before they can be waitlisted for KT. The evaluation procedure includes a series of immunological, surgical, radiological, cardiovascular and oncological examinations according to a consensus of Austrian transplant centres published in 2007. After performing all necessary examinations, a board of kidney transplant experts decides whether the candidate is eligible for KT or further examinations and/or interventions are necessary. In some cases, however, candidates do not fulfil the eligibility criteria primarily due to underlying severe co-morbidities. Data on evaluation strategies as well as the proportion of patients declined for KT in Austria is lacking. The aim of the current study was i) to perform an evaluation of candidates who underwent KT evaluation in our center, but finally were declined for KT, as well as ii) to investigate the reasons for the ineligibility.
Methods: In this retrospective monocentric chart review we included 203 adult KT candidates screened between 1995 and 2017 at the Medical University of Graz, who were found to be ineligible for a KT. We analyzed baseline characteristics, co-morbidities and causes/reasons of KT ineligibility, which were categorized as follows: immunological, cardiovascular, oncological or surgical reasons, chronic infection, death during evaluation, patient-related reasons (e.g.: non-adherence) or other reasons (e.g.: change of transplant center).
Results: The median age was 55 years, the youngest patient evaluated was 19 and the oldest 76 years. 66% were male and 90% were treated with hemodialysis as a renal replacement therapy; the rate of re-transplantation was 20%. The most common underlying renal diseases were diabetic nephropathy (26%) and vascular nephropathy (19%).
Interestingly, the most common reason for ineligibility were patient-related reasons/non-adherence (26%) followed by other reasons (19%) and surgical concerns (15%). No patient was rejected from wait listing due to immunological reasons, 2% had chronic infections and only 9% of candidates were rejected due to cardiovascular reasons.
Discussion: Surprisingly, a small number of patients were rejected due to cardiovascular reasons despite high cardiovascular morbidity and mortality in ESRD patients. In our single-center experience we found patient-related factors (e.g. not completing necessary pre-transplant check-up examinations) as the main cause of non-eligibility for KT. With a more focused patient education demonstrating all potential benefits of receiving a kidney allograft as well as simplifying pre-transplant (in particular cardiovascular) screening procedures the access to the KT waiting list in the future could be improved.

IGE RELATED CHANGES AFTER ACUTE HUMORAL REJECTION OF CARDIAC ALLOGRAFTS IN MICE

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Background: Transplantation is the gold standard for treatment of patients with end-stage organ failure. While the 1-year graft survival rate has increased significantly, long-term allograft survival is still limited, mainly due to the presence of donor-specific antibodies (DSA) leading to antibody mediated rejection (ABMR). We previously demonstrated the production of MHC-specific DSAs of the IgE subtype upon allograft rejection in mice and humans. The impact of donor-specific IgE and its effector cells in the pathology of ABMR remains to be delineated.
Methods: For further studies on MHC-specific IgE, we used CCR5KO recipients as a mouse model of acute ABMR. Fully mismatched BALB/c cardiac allografts were transplanted onto untreated CCR5KO recipients and wild type C57BL/6 mice (WT) as controls. MHC-specific antibodies of the IgE and IgG1 isotype were measured using a custom-made ELISA employing MHC class I and II monomers. Levels of IgE effector cells, such as basophils and eosinophils, and B cells were measured using flow cytometry in the periphery weekly before and after transplantation.
Results: Cardiac allograft survival in CCR5KO mice compared to WT mice was not significantly different. However, levels of DSAs of the IgG isotype were significantly higher in the CCR5KO group. Using our MHC-specific ELISA, we were able to demonstrate the production of MHC-specific IgE upon acute humoral rejection. Moreover, levels of MHC-specific IgE after rejection were slightly higher in CCR5KO recipients compared to controls. Basophil (CD49b+ FceRI+ IgE+) levels in the periphery measured via flow cytometry were significantly elevated at the time of rejection in CCR5KO mice and an increase in basophil-bound IgE was detected in both groups, CCR5KO and controls. Notably, IgE+ CD23+ B cells were present after cardiac allograft rejection with significantly higher levels in CCR5KO than in WT recipients.
Conclusion: We demonstrate the formation of MHC-specific IgE upon allograft rejection in a murine model of acute ABMR. Levels of basophils in periphery and basophil-bound IgE are elevated after rejection, indicating a possible role of IgE and its effector cells in the immune response of allograft rejection. The increase in IgE+ CD23+ B cells after rejection suggests a possible production of IgE immune complexes upon rejection.

ABSTRACT

SEX DIFFERENCES IN KIDNEY TRANSPLANTATION: AUSTRIA AND THE UNITED STATES, 1978 TO 2018

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Rationale and Objective: Systematic analyses about sex differences in wait-listing and kidney transplantation after dialysis initiation are scarce. We aimed at identifying sex-specific disparities along this path (including death), comparing two countries with distinctive health care systems, the US and Austria, over time.
Study Design: Observational cohort study.
Setting and Participants: Subjects who initiated dialysis, from the US Renal Data System and the Austrian Dialysis and Transplant Registry plus Eurotransplant datasets, 1978–2018.
Predictors: Sex, age at dialysis initiation.
Outcomes: Time to event from starting to end point of the consecutive states: dialysis initiation, wait-listing, kidney transplantation, death.
Analytical Approach: Cox regression, modelling male-to-female cause-specific hazard ratios (csHRs, 95% confidence intervals) for transitions along treatment states, adjusted for age and stratified by country and decade of dialysis initiation.
Results: Among 3 053 206 US and 36 608 Austrian patients starting dialysis, men had higher chances to enter the wait-list, which however decreased over time (male-to-female csHRs [95% CI] for wait-listing, 1978–1987: US 1.94 [1.71, 2.20], AUT 1.61 [1.20, 2.17]; 2008–2018: US 1.35 [1.32, 1.38], AUT 1.11 [0.94, 1.32]). Once wait-listed, the advantage for men became smaller, but persisted in the US (male-to-female csHR for transplantation after wait-listing, 2008–2018: 1.08 [1.05, 1.11]). The greatest disparity between men and women occurred in older age groups in both countries (male-to-female csHR for wait-listing after dialysis, adjusted to 75% age quantile, 2008–2018: US 1.83 [1.74, 1.92], AUT 1.48 [1.02, 2.13]). Male-to-female csHRs for death were close to one, but higher after transplantation than after dialysis.
Limitations: No differentiation between sex and gender; no data on comorbidities and socioeconomic factors.
Conclusions: We found evidence for gender disparities in both countries, although the gender gaps are decreasing over time. Historically, men in the US and Austria had 90%, respectively 60% higher chances of being wait-listed for kidney transplantation. Efforts should be continued to render kidney transplantation equally accessible for both sexes, especially for older women.

FREQUENCY OF HLA-SPECIFIC IGE ANTIBODIES IN SOLID ORGAN TRANSPLANT RECIPIENTS

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Background: Whereas the one-year graft survival after organ transplantation improved over the last decades, the long-term outcome needs to be improved and is still on focus of recent research. Humoral immunity, e.g. antibody-mediated rejection (ABMR), is one of the leading causes of immune-mediated graft injury. Donor-specific antibodies (DSA), mainly anti-HLA of the IgG isotype, are known to affect the graft survival but the pathophysiological mechanisms are still incompletely understood. Our group previously described the occurrence of HLA-specific antibodies of the IgE isotype in a small cohort of highly sensitized kidney recipients. Here, we performed a prospective study to assess the frequency of HLA-specific IgE in recipients of various types of organ transplants.
Methods: HLA-specific IgE antibodies were prospectively measured in a cohort of 105 transplant recipients (60 kidneys, 15 heart, 15 lungs and 15 livers) at baseline, three- and 12 months post-transplant. We used a single antigen-beads based immunoassay to measure HLA-specific IgE class I and II based on multiplex technology in human serum (OneLambda, LabScreen Single Antigen HLA class I and II). Serum was centrifuged at 1500×*g* for 10 min at 10°C and stored at −80°C.
Results: 10% ($n = 6$ out of 60) of kidney transplant recipients showed detectable HLA-specific IgE antibodies before transplantation, 5% against HLA class I and 8.3% against HLA class II. Among those, a small cohort of 3.3% ($n = 2$ out of 60) had pre-existing IgE against donor HLA antigens (i.e. IgE-DSA). In total 16.7% ($n = 10$ out of 60) were pre-sensitized against HLA-specific IgG. Within the heart transplant cohort, 6.7% ($n = 1$ out of 15) had pre-existing HLA-specific IgE antibodies class I and 6.7% ($n = 1$ out of 15) of the liver recipients had pre-transplant HLA class II- specific IgE antibodies without any donor specificity. Three-month post-transplantation, 8.8% ($n = 5$ out of 57) from the kidney recipients had anti-HLA IgE antibodies, 5.3% HLA class I and 7% HLA class II and 1.8% ($n = 1$ out of 57) developed de novo anti-HLA-specific IgE for HLA class I and II. Furthermore, one heart and one liver transplant recipient also developed de novo anti-HLA-specific IgE after three months post-transplantation (TX). After one-year post-TX, 8.2% ($n = 4$ out of 49) of kidney recipients had detectable anti-HLA antibodies, 6.1 % ($n = 3$ out of 49) for HLA class I and 6.1% HLA class II. 7.7% ($n = 1$ out of 13) heart transplant recipients had HLA class I specific IgE antibodies after one-year post-TX. There were no positive HLA-specific IgE antibodies detected at any time point within the lung recipients. In the majority of cases, IgE positivity coincided with IgG positive immunization. However, anti-HLA IgE reactivities were detectable in eight patients without the presence of IgG against the same HLA specificity. All kidney recipients with positive anti-HLA IgE were already highly sensitized towards HLA-IgG and 83.3% ($n = 5$ out of 6) had pre-existing IgG-DSAs. Furthermore, the heart recipient that was positive for anti-HLA IgE, was highly sensitized for HLA-specific IgG before transplantation as well as the liver recipient who had additionally IgG-DSA.
Conclusion: We could successfully show that HLA-specific IgE antibodies exist in a low frequency before transplantation and also develop de novo in a small subset of kidney, heart and liver transplant recipients. The potential role of IgE DSA in humoral allo-immunity requires further investigation.

EFFECTS OF PROTEASOME INHIBITION IN LATE ANTIBODY-MEDIATED REJECTION ON ANTI-DONOR IGM REACTIVITY AND COMPLEMENT FIXATION—RESULTS FROM A RANDOMIZED CONTROLLED TRIAL (THE BORTEJECT TRIAL)

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Background: Late antibody-mediated rejection (ABMR) is a cardinal cause of kidney allograft failure. Currently, there is no proven effective treatment for this type of rejection. In a recent randomized controlled trial, we have reported no meaningful effect of plasma cell-directed treatment using the proteasome inhibitor bortezomib on the clinical course of late ABMR (BORTEJECT trial; ClinicalTrials.gov, NCT01873157; Eskandary et al., J Am Soc Nephrol, 2018, 29:591). Treatment had also no significant effect on IgG type donor-specific antibody (DSA) patterns, possibly due to rapid reconstitution of the allospecific plasma cell repertoire. To further dissect the impact of bortezomib on donor-specific B cell alloimmunity, we here sought to investigate whether and to which extent bortezomib impacts the dynamics of specific alloreactivity patterns, in particular, the course of IgM type DSA and complement-activating capability of detected DSA.
Methods: The BORTEJECT trial was a randomized, placebo-controlled parallel group trial designed to investigate whether two cycles of bortezomib (each cycle: 1.3 mg/m^2 intravenously on days 1, 4, 8 and 11) is able to halt the progression of IgG-DSA-positive late ABMR. Forty-four recipients were randomly assigned to receive bortezomib ($n = 21$) or placebo ($n = 23$). In the present re-analysis of biobanked serum samples obtained in the context of the trial, we applied modified single antigen bead assays to assess IgM-DSA and, in addition, complement (C1q) binding. To estimate antibody/complement binding, levels of mean fluorescence intensity (MFI) were recorded. IgM DSA were defined in relation to donor/recipient HLA typing, independently of IgG reactivity patterns.
Results: At baseline, the complement-fixing capability of the immunodominant IgG DSA did not differ between groups [C1q-MFI; bortezomib vs. placebo: 5924 (median; IQR 334 â€“ 22831 vs. 13218 (1567 â€“ 22728), $P = 0.095$]. Thirteen of 21 (bortezomib) and 17 of 23 recipients (placebo), respectively, had detectable IgM DSA, and we observed a considerable overlap of IgG and IgM DSA specificities (matching of DSA specificity among 10/21 (bortezomib) vs. 12/23 (placebo) patients, respectively). At baseline, IgM DSA levels were similar between groups [IgM-MFI; bortezomib vs. placebo: 753 (median; IQR 275 â€“ 4075) vs. 907 (216 â€“ 2383), $P = 0.57$]. Bortezomib treatment did not affect the complement-fixing capability of IgG DSA over time [C1q-MFI 5420 (median; IQR 553 â€“ 20141) at month 6 and 2963 (I306 â€“ 20604) at month 24 in the bortezomib arm vs. 11780 (3189 â€“ 21792) and 9769 (1318 â€“ 22528) in the placebo arm, $P = 0.65$]. Similarly, there was no effect on the course of IgM-DSA [-MFI: 1137 (median; IQR 319 â€“ 4914) at month 6 and 657 (276 â€“ 5427) at month 24 in the bortezomib group vs. 1034 (304 â€“ 2700) and 1 455 (318 â€“ 2791) in the placebo group, $P = 0.78$]. Also percentage changes of IgM DSA levels and C1q deposition did not reveal significant differences between study groups.
Conclusion: A limited course of bortezomib as a sole treatment strategy may not be sufficient to impact on DSA levels, including the evolution of IgM type DSA and the complement-binding capability of detected alloreactivity patterns. Our study results, which may mirror the lack of clinical efficacy, reinforce the need for the development of innovative new strategies to effectively target plasma cells and halt the progression of ABMR.

INVESTIGATION OF CHRONIC REJECTION IN A RAT MODEL OF ORTHOTOPIC HINDLIMB TRANSPLANTATION

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Background: Vascularized tissue allotransplantation (VCA) is a maturing field of transplantation. With increasing follow-up periods, chronic rejection is more frequently seen. Despite a growing number of reports on chronic rejection observed in clinical VCA, in depth investigation on underlying mechanisms and dynamics of progression is still lacking. This study aims to implement a model of chronic rejection in a rodent model of hindlimb transplantation to investigates patterns and progression of induced chronic changes in VCA.
Methods: In an orthotopic hindlimb transplantation model, male Fischer and Lewis rats served as donors and recipients, respectively. A total of five experimental groups were investigated (Group 1: naÃ¯ve Lewis rats, Group 2: Lewis isotransplants, Group 3: Fischer Lewis allotransplants, Group 4: Fischer Lewis allotransplants with 10 h cold static storage, Group 5: Fischer-Lewis allotransplants with induction therapy [anti lymphocyte serum]). Macroscopic and functional assessment (motion scale and Catwalk device) was carried out throughout the study period and protocol biopsies of skin and muscle were taken on days 150 and 250 for histopathological assessment.
Results: Weaning from tacrolimus led to acute rejection in all minor mismatched experimental groups. In the absence of induction therapy, acute rejection was seen in all recipients, which was reduced to 43% with the use of ALS as induction agent (Group 5). Additional tacrolimus treatment was necessary for 29 (15–34), 32 (13–33), and 0 (0–27) days (median, IQR) for Groups 3, 4 and 5 to overcome acute rejection, respectively. Chronic rejection was observed macroscopically in all minor mismatched grafts and mainly manifested as skin sclerosis, dyschromia and graft atrophy. Macroscopic changes occurred between POD 40 and 100 and were most pronounced in combination with cold static storage (Group 4), and least in Group 5. Histopathological alterations included sclerosis and degeneration in muscle tissue, as well as sclerosis, chronic immune cell infiltration and intima/media proliferation in skin. Graft survival was comparable between all experimental groups (log rank $P = 0.3$). In general, function of transplanted limbs was significantly inferior compared to naÃ¯ve limbs. Isotransplants demonstrated superior overall motion compared to allotransplanted animals ($P < 0.001$). No difference in limb function was observed between the three allogenic groups.
Conclusion: The Fischer-Lewis minor mismatch model is suitable to induce macroscopic and histopathologic changes consistent with chronic rejection in VCA and may serve as a model to investigate underlying mechanisms and potential treatment strategies.

ABSTRACT

BIOENERGETIC FUNCTION AND TISSUE VIABILITY IN HUMAN LIVERS DURING PROLONGED *EX VIVO* LIVER NORMOTHERMIC MACHINE PERFUSION

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Background: Liver transplantation (LT) is the only curative treatment option for end-stage liver disease but the demand for organs exceeds the offer. Extracorporeal regeneration of injured livers may help to overcome organ shortage. Thus, in-depth knowledge of cellular mechanisms occurring during normothermic machine perfusion (NMP) is required. Mitochondria are key players in tissue bioenergetics to cover the energy needs of the liver. Thus, we aimed for an in-depth assessment of mitochondrial respiration during prolonged liver NMP.
Methods: 14 human liver allografts declined for transplantation were machine perfused under normothermic conditions for up to 5 days. Liver quality was monitored by analyzing the perfusate lactate, AST and ALT levels every 6 hours (h). Mitochondrial respiration was assessed in tissue homogenates before the start of perfusion and later every 24 h by high-resolution respirometry (HRR) for the succinate-linked pathway. The coupling control states oxidative phosphorylation (OXPHOS), resting respiration (LEAK), and electron transfer (ET) capacity were determined. The respective coupling control ratios (*P-L* and *E-P* control efficiencies) were calculated to assess the efficacy of the bioenergetic function. To analyze the cell viability and tissue integrity in the biopsies, serial histology and real-time confocal microscopy (RTCM) was used and scored semiquantitatively.
Results: Machine perfusion of the livers could be performed with a mean duration of 92 $\pm$ 33 h (mean $\pm$ SD), the high standard deviations are due to the heterogeneity of the declined organs. Close to physiological perfusate lactate levels could be maintained for at least 72 h (16.22 $\pm$ 7.22 mg/dL). Still, OXPHOS capacity halved, but *P-L* control efficiency did not decrease significantly, which indicates a shift in mass-specific respiration, but not in its efficacy for 96 h of NMP. This observation was confirmed by a significant increase of the RTCM score (pre: 0.4 $\pm$ 0.8, 72 h: 1.6 $\pm$ 0.69). In the time course of the further prolongation of NMP, the proportion of LEAK respiration doubled, most probably due to oxidative damage to the mitochondrial inner membrane. This is also indicated by an exponential increase in lactate levels (117.28 $\pm$ 73.74 mg/dL). In line with this, the integrity of the outer mitochondrial membrane deteriorated, resulting in significantly elevated cytochrome *c* control efficiency ($p < 0.0001$, Wilcoxon matched pairs test). Moreover, the *E-P* control efficiency halved ($p < 0.0001$) as a result of damage to the ET machinery.
Conclusion: The in-depth, real-time analysis of coupling control in mitochondria is a sensitive tool for the assessment of organ function in livers undergoing NMP. According to our results, the loss of mitochondrial bioenergetic function seems not to be the early limiting factor during prolonged *ex vivo* machine perfusion.

IMPACT OF AWAKE VERSUS SEDATED ECMO BRIDGE-TO-TRANSPLANT STRATEGIES ON EARLY AND LONG-TERM OUTCOME AFTER LUNG TRANSPLANTATION

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Background: Bridge to transplant (BTT) using extracorporeal membrane oxygenation (ECMO) is a viable option in selected patients with end-stage lung disease. Traditionally, patients on BTT ECMO were kept sedated and intubated during the waiting time for lung transplantation, however, ambulatory or awake ECMO strategies have been developed during the last years. As these patients remain physically active, recovery after lung transplantation can be shortened. However, the published experience on awake versus sedated BTT is still sparse. This study aims to elaborate the differences in early postoperative and long-term outcomes after lung transplantation of awake and sedated BTT patients.
Material and Methods: All BTT patients receiving a LUTX at the Department of Thoracic Surgery, Medical University of Vienna, between January 2015 and March 2021 were retrospectively analyzed. Patients were considered awake if they had an equivalent of a Richmond Agitation-Sedation Scale (RASS) score of -1 or higher until at least 24h before transplantation. Demographic characteristics, perioperative variables and long-term outcome was analyzed and compared between awake and sedated patients.
Results: A total of 65 patients were included in the final analysis. 25 (38.5%) patients were in an awake BTT setting, whereas the remaining 40 (61.5%) patients were allocated to the sedated BTT group. There was no significant difference in age, gender, type of transplant (single lung vs. double lung), body mass index or underlying lung disease. However, sedated ECMO patients had a significantly longer BTT duration than awake patients (14 days vs. 7 days, $P = 0.040$). Preoperatively, 38.1% of awake BTT patients were able to perform active physiotherapy while lying in bed, 23.8% while sitting in bed and 38.1% were standing despite ECMO support. Mobilization to standing position after lung transplantation was achieved earlier in the awake BTT patients (9 vs. 19 days, $P = 0.001$). Moreover, postoperative ventilation time (347 vs. 143 hours, $P = <0.001$), postoperative intensive care unit (ICU) stay (36 vs. 18 days, $P = <0.001$) and length of hospital stay (55 vs. 40 days, $P = 0.047$) was significantly longer in the sedated BTT cohort compared to awake BTT patients.
No difference was found regarding the rate of ECMO associated complications (20.0% vs. 24.0%, $P = 0.762$) or the rate of severe complications (30.0% vs. 32.0%, $P = 1.000$). Also, one-year and three-year overall survival did not differ between cohorts (75.3% vs. 84.0%, $P = 0.679$; 71.5% vs. 69.3%, $P = 0.951$).
Conclusion: Despite the complexity in the perioperative management of BTT patients, excellent survival rates after lung transplantation can be achieved. Awake BTT concepts are associated with a significantly faster recovery and earlier discharge from hospital compared to sedated BTT patients. However, the long-term outcome was comparable between awake and sedated BTT patients. We conclude that even several weeks of sedated ECMO support should not be considered a contraindication for lung transplantation.

MITOCHONDRIAL RESPIRATION OF DCD AND DBD LIVER ALLOGRAFTS DURING NORMOTHERMIC MACHINE PERFUSION

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Background: Liver transplantation (LT) is still the only curative option for end-stage liver disease. Due to organ shortage, also livers donated after cardiac death (DCD) are considered for transplantation. However, the use of marginal organs is often associated with inferior graft function, emphasizing the need for reliable assessment tools of organ function prior to LT. Normothermic machine perfusion (NMP) allows extended preservation and simultaneous functional analysis, and has recently been associated with enhanced bioenergetic parameters in DCD grafts, as assessed by metabolomics [1]. Since mitochondria are key players in cellular homeostasis, we aimed to compare the bioenergetic function of DCD organs with livers donated after brain death (DBD) and correlated those with the clinical outcome.
Methods: 71 liver allografts (DBD: n = 52, DCD: n = 19) were enrolled in this prospective clinical study and underwent NMP (metra, OrganOx) up to 24 h, whereas 47 livers (DBD: n = 38, DCD: n = 9) were transplanted subsequently. Wedge biopsies were taken prior to NMP, at 1 h, 6 h, 12 h, 20 h after NMP start, and upon reperfusion. Mitochondrial respiration was assessed by high-resolution respirometry (HRR) for the succinate-linked pathway to analyse the oxidative phosphorylation (OXPHOS capacity). *P-L* control efficiency and cytochrome *c* control efficiency were calculated to determine efficacy of OXPHOS coupling and damage to the outer mitochondrial membrane, respectively. Mitochondrial parameters were compared between DBD and DCD groups and their predictive value during NMP was assessed by correlation with early allograft dysfunction (EAD).
Results: Significant differences (p = 0.0038) in the OXPHOS capacities were observed between both groups in the cold-stored biopsies taken before NMP start (DBD: 33.9 (27.66–41.64), DCD: 56.2 (31.77–71.22), pmol s^{-1} mg wet $weight^{-1}$; median and 95% CI). This difference disappeared in the course of NMP. In line with this, DCD allografts showed a significant decrease in OXPHOS capacity after 20 hours (p = 0.0016), while *P-L* control efficiency increased. Further, cytochrome *c* control efficiency increased with prolonged perfusion in the DCD group, compared to DBD livers (p = 0.005). In both groups, the fold change of cytochrome *c* control efficiency from pre to 6 h after NMP start predicted EAD (p < 0.0001).
Conclusions: Mitochondrial respiration of DCD livers aligns with those of DBD livers during NMP, possibly indicating a change in the bioenergetic pattern and in turn, cellular function. Importantly, the mitochondrial outer membrane damage, calculated as cytochrome *c* control efficiency, may serve as a predictive marker for organ function after LT.
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ANTI-INTERLEUKIN-6 ANTIBODY CLAZAKIZUMAB IN LATE ANTIBODY-MEDIATED RENAL ALLOGRAFT REJECTION—MODULATION OF COMPLEMENT AT THE LEVEL OF C4 AND C3 AS A NOVEL MODE OF ACTION

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Background: Antibody-mediated rejection (ABMR) is a major cause of kidney allograft dysfunction and loss, and currently there is no proven effective treatment. Targeting the proinflammatory cytokine interleukin-6 (IL-6) involved in B cell activation and development, however, may represent a promising therapeutic approach. Recent trials have suggested stabilization of graft function and reduction of ABMR activity. It was also shown that prolonged anti-IL-6/IL-6 receptor antibody treatment might substantially impact intragraft complement activation, as reflected by reduced capillary C4d deposition. One may speculate that beyond moderate decreases in donor-specific antibody (DSA) levels, this may be due to direct modulation of complement strength. In this sub-analysis of a recently reported randomized controlled trial evaluating anti-IL-6 antibody clazakizumab in late ABMR, we sought to dissect the impact of IL-6 blockade on key components of the complement cascade.
Methods: We report a sub-study of a prospective bi-centric randomized controlled phase 2 pilot trial of anti-IL-6 antibody clazakizumab in late ABMR (Doberer *et al.*, J Am Soc Nephrol, 2021, 32:708; ClinicalTrials.gov, NCT03444103). Twenty DSA+ kidney transplant recipients diagnosed with active ABMR ≥365 days after transplantation were randomized to clazakizumab versus placebo (4-weekly doses; 12 weeks), followed by a 9-month extension where all recipients received clazakizumab. Protocol biopsies for morphologic and molecular analysis were performed after 11 and 51 weeks, respectively. Complement studies were performed on biobanked serum and plasma samples. C3 and C4 components were measured by nephelometry. Soluble complement split products C4a and C3a, alternative pathway (AP) and lectin pathway (LP) activity were quantified by commercially available ELISA kits. Classical pathway (CP) activity (CH50) was measured applying a hemolytic titration test based on Mayer's method. RNA transcripts were determined by real-time qPCR (peripheral blood) and microarray analysis (biopsies). For soluble immune complex measurement, an in-house assay based on C1q-coupled magnetic beads was utilized.
Results: In the overall cohort, complement component C4 levels significantly decreased at week 52, from a median of 24.5 (IQR: 20.1–34.0) at baseline to 11.2 (8.1–15.3) mg/dL ($P < 0.001$). In parallel, C3 decreased from 108 (90–129) to 73 (63–90) mg/dL ($P < 0.001$). Comparing clazakizumab vs. placebo at week 12, we found significantly different changes in C4 (median [IQR]: 52% [42–65%] vs. 99% [81–108%] of baseline [$P < 0.001$]) and C3 levels (79% [69–93%] vs. 100% [89–109%]; $P = 0.004$), as well as in CP (87% [71–100%] vs. 101% [91–130%]; $P = 0.043$), LP (43% [33–85%] vs. 98% [89–116%]; $P = 0.015$) and AP activities (62% [54–83%] vs. 103% [92–112%]; $P < 0.001$), respectively. Complement pathway activities were mostly in a normal range at baseline (CP in 95%, LP in 75%, AP in 90% of patients), but below normal in 50% of patients for CP, in 40% for LP and in 85% for AP at week 52. C4 and C3 RNA synthesis in peripheral cells and renal biopsies, however, was not different. Nevertheless, subsequent analyses argued against a pathogenic role of clazakizumab-triggered complement consumption: C4 and C3 reduction was not paralleled by activation-associated split product C4a and C3a release. Even though there was an accumulation of monomeric clazakizumab-IL-6 complexes, we did not observe any increase in circulating complement-binding immune complexes or ultrastructural immune complex deposits in renal biopsies.
Discussion: Our results suggest that IL-6 antagonism in ABMR may strongly affect complement protein levels and activity, presumably by modulation of hepatic C4 and C3 synthesis rather than complement consumption. We speculate that complement modulation could contribute to the observed effects of clazakizumab on the phenotypic presentation and clinical course of ABMR. However, one may also speculate that it could potentially contribute to an increased risk of infection.

ABSTRACT

GLOMERULAR C4D IN IGA NEPHROPATHY AFTER RENAL TRANSPLANTATION IS ASSOCIATED WITH DECREASED ALLOGRAFT SURVIVAL

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Post-transplant glomerulonephritis (GN), including post-Tx IgA Nephropathy (IgAN) is an important contributor to limited long-term allograft survival. The immunopathological detection of the complement degradation product C4d in glomeruli (C4dG) has been described as risk factor in native kidney IgAN, but only little is known about C4dG deposition in post-Tx IgAN. We hypothesized that glomerular C4d may indicate a more aggressive disease course and worse allograft survival in patients with post-Tx IgAN. In this retrospective study, we evaluated the presence and clinical relevance of C4dG in patients with post-transplant IgAN, biopsied at the Medical University of Vienna. We analyzed 885 renal allograft recipients, including 84 patients with post-transplant GN. All patients received their allograft between January 1999 and April 2006 and underwent at least one for-cause biopsy. Our primary endpoint was death-censored graft survival, within a median follow-up of 9.6 (IQR 3.8-13.2) years. The prevalence of post-Tx glomerulonephritis was 9.5%. Of those, 27 patients with post-Tx IgAN were included. C4dG positive patients ($N = 18$, 66.7%) had significantly worse allograft survival compared to C4dG negative post-Tx IgAN patients and patients without post-Tx IgAN [C4dG positive: 27.8% vs. 55.6% and 66.0%; log-rank: $P = 0.01$]. C4dG remained a significant risk factor (HR 2.22, 95% CI 1.27-3.87) for allograft loss even after adjustment for T cell mediated rejection (TCMR) and antibody mediated rejection (ABMR). In conclusion, glomerular C4d deposition represents an independent risk factor for worse graft-survival in patients with post-Tx IgAN, even after adjusting for other established risk factors such as antibody mediated rejection. Assessment of glomerular C4d deposition may provide a valuable prognostic risk assessment tool to identify patients with post-Tx IgAN and high risk for allograft loss.

ANTI-HLA ANTIBODIES FOR EARLY PREDICTION OF PREECLAMPSIA – A PROSPECTIVE COHORT STUDY

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Background: The significance of antibodies directed against human leukocyte antigen (HLA) epitopes in the context of obstetric disorders is discussed controversially. In our previous work, we described elevated anti-HLA reactivity detected by bead-based multiplex screening assay early in the course of pregnancy of women with subsequent diagnosis of preeclampsia. However, this approach does not allow for the identification of the targeted epitopes precluding the evaluation of antibody specificity patterns. The current study was designed to evaluate anti-HLA antibodies on a single antigen level to further characterize anti-HLA reactivity in preeclampsia and to improve diagnostic accuracy.
Methods: For the present analysis, we used bio-banked serum samples that were prospectively collected at 8 predefined time-points from pregnant women, starting as early as gestational week 11. We included all samples from women who developed preeclampsia ($n = 11$) and women with uneventful pregnancies ($n = 101$), yielding at a total sample size of 742 sera. The patients specimens were retrospectively tested for the presence of anti-HLA directed IgG antibodies using single antigen bead assays for both HLA class I and II reactivity. Sera were pretreated with EDTA to correct for the prozone effect.
Results: Baseline characteristics did not reveal differences between groups. The majority of the women ($n = 94\%$) showed detectable HLA class I and/or class II reactivity at any time point during pregnancy. Only seven women never showed HLA reactivity, all of them with uneventful pregnancies. While 86% had reactivity against HLA class I, 76% had HLA class II antibodies. Whereas only 41% of women without uneventful pregnancy had detectable antibodies against HLA-C, this percentage was higher in the group of women with subsequent preeclampsia (73%). Whereas women who developed preeclampsia showed a rapid rise of the leading anti-HLA antibody (the anti-HLA antibody with the highest binding strength as quantified by the mean fluorescence intensity, MFImax) levels early in the pregnancy, women with uneventful pregnancy had low to moderate levels that were relatively stable throughout the observation period (week 14 to 17: median MFImax: 12 356, IQR: 2994–21 026 vs. 2610, IQR: 1240–5539; $P = 0.02$). The difference was even more pronounced when assessing total anti-HLA reactivity (MFIsum) leading to significantly different results as early as gestational week 11–13 (median MFIsum 106 327, IQR 81 406–204 400 in preeclampsia vs. 59 836, IQR: 44 607–104 882 in uneventful pregnancies; $P = 0.009$]. Each log increase in MFIsum quantified in gestational week 14 to 17 increased the risk to develop preeclampsia throughout the course of the pregnancy by 85% (95% CI: 1.21–2.84, $P = 0.005$). The effect size of this association did not change after adjustment for the number of gravidities and age of the study subjects in a multivariable model (RR: 1.86, 95% CI: 1.08–3.19). The area under the curve for the detection of preeclampsia by MFI sum was 0.80 (95% CI: 0.68–0.92, $P = 0.002$). A high sensitivity of 90% was calculated for a MFIsum > 58 276 with a low specificity of 64% and a high specificity of 90% was calculated for MFIsum > 316 046 with a low sensitivity of 20%.
Conclusion: The current data describe a distinct longitudinal pattern of anti HLA antibodies allowing for robust prediction of the subsequent occurrence of preeclampsia. Moreover, the study provided clinically useful cut-off values for risk prediction, very early in the course of pregnancy, even before any other signs of preeclampsia become clinically overt. Such early risk stratification might offer a therapeutic window in a common disease with high disease burden for the affected women. The frequent presence of anti HLA-C antibodies - expressed on the endovascular trophoblast - in women who develop preeclampsia might point towards a causative role. However, subsequent studies providing data of the foetal HLA are necessary to support this hypothesis.

ANALYSIS OF MITOCHONDRIAL RESPIRATION DURING NORMOTHERMIC MACHINE PERFUSION OF THE PORCINE LIVER FOR VIABILITY TESTING

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Background: Normothermic machine perfusion (NMP) enables organ viability testing prior to transplantation. However, reliable parameters are lacking. Hepatic mitochondria play a central role in cellular bioenergetics. Thus, analysis of mitochondrial respiration using high-resolution respirometry (HRR) may provide direct information on the hepatic function during *ex vivo* NMP. We herein aimed at identifying sensitive parameters which assess mitochondrial function of the porcine liver.
Methods: Porcine livers were machine perfused under normothermic conditions. Wedge biopsies were taken every 24 h and analyzed for mitochondrial respiration. Oxidative phosphorylation (OXPHOS) for succinate, nicotineamide adenine dinucleotide (NADH), and fatty acid oxidation (FAO) pathways was assessed, and *P-L* capacity control efficiencies were calculated. Further, LEAK respiration, capacity of the electron transfer (ET) system and the outer membrane integrity were evaluated. Parallel to the mitochondrial measurements, perfusate samples were collected during NMP and analyzed for lactate levels.
Results: In general, the OXPHOS capacity of the succinate pathway was 4-times higher as OXPHOS capacity of NADH- and FAO pathways. In contrast, the calculated *P-L* control efficiencies were the highest for NADH- and FAO pathways. During NMP, an overall decline of mitochondrial respiration could be observed, indicating an impairment of the bioenergetic function. These changes were detectable at first in the *P-L* control efficiency of the succinate pathway, reflecting a decreasing efficacy of ATP production. In line, the perfusate levels of lactate, a marker which is known to be elevated in liver injury, increased during NMP. Concomitantly, the *E-P* control efficiency and cytochrome *c* control efficiency increased, indicating damage to the phosphorylation system and loss of the outer membrane integrity, respectively.
Conclusion: Analyzing the dynamics of the mitochondrial respiration provide reliable information about the quality of the *ex vivo* perfused porcine liver, whereby the succinate pathway seems to be the most sensitive.

MEDICATION ADHERENCE OF IMMUNOSUPPRESSIVE DRUGS IN THE FIRST YEAR AFTER RENAL TRANSPLANTATION - A PROSPECTIVE NON-INTERVENTIONAL TRIAL

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Background: Kidney transplantation is the treatment of choice for chronic end stage renal disease. Potent immunosuppressive treatment has led to substantial prolongation in short-term allograft survival in recent decades, but long-term performance has not improved in the same way. Decline of allo-graft function is mainly cause by chronic graft damage due to allo-recognition and subsequent immunologic response. Thus, optimization of immunosuppressive regimens represents the key for maximisation of allo-graft survival. Besides personalization of the amount and type of immuno-suppression, improvement of medication adherence is the second pillar of intervention. However, detailed granular data on medical adherence following kidney transplantation is still expandable, while the implementation of assessments in routine care remained limited. Therefore we designed the prospective ADTORQUE trial including a state of the art monitoring of adherence behaviours in the first year after renal transplantation. The trial was approved by the ethical committee of the Medical University of Vienna (EK-Nr.: 2100/2017) ad registered at the DRKS.
Methods: All 284 consecutive adult renal recipients of a kidney graft transplanted at the Medical University Vienna between January 2018 and December 2019 were invited at the day of transplantation. Two hundred twenty six recipients were enrolled and monitored for up to 12 months with five study visits (V1-V5). The first visit was scheduled at the first out-patient contact after discharge from the ward post transplantation. Subsequent visits (V2-V5) followed in a three month interval. The adherence monitoring was compiled on a multi-component assessment. Medication adherence was assessed by self-report, electronic drug monitoring (MEMS®Cap and MEMS®Button), pharmacy refill records, tacrolimus trough level, individual evaluation by a transplant psychologist and Torque Teno virus level in the blood (a non-pathogenic highly prevalent virus reflecting the immune function of its host). Herein we present preliminary analysis of the data on the adherence self-reporting using the BAASIS© questionnaire. The BAASIS questionnaire includes 5 items referring to dose-taking regularity and punctuality, regimen aberrations by the patient themselves and the potential discontinuation of dose taking. In a dichotomized rating any sign of non-adherence in any item rates the recipient as non-adherent.
Results: Median follow up of the 226 included kidney transplant recipients was 52 weeks (median; IQR: 31–56). Mean recipient age at transplantation was 57 years and 33% were female. Graft and patient survival was 98% and 97%. A total of 845 BAASIS self-reports were performed, which accounted for a response rate of 88%. Non-adherence was detected in 52% of recipients across all time-points: 60 (27%) of the transplant recipients reported non-adherence at least once within the first year post transplantation, while 57 recipients (25%) revealed non-adherence at multiple times. The proportion of non-adherence increased within the first 3 months post transplantation, from 11% at V1 to 31% at V2. At following visits, non-adherence rates of 27% at V3, 27% at V4 and 32% at V5 were recorded. Deviations from dose-timings were indicated in 41% of all recipients and constitutes for the most frequent cause for non-adherence ratings. Patient revealed non-adherence once had a significantly higher rate of biopsy proven rejections than adherent patients (18% vs. 6%, $P = 0.015$).
Conclusion: The current trial reveals substantial and continuing self-reported non-adherence already in early phases within the first year post transplantation, whereby taking doses on time was the main barrier.
Outlook: Subsequent analysis of the ADTORQUE trial includes results from electronic drug monitoring, pharmacy refill record, tacrolimus trough level, evaluation by a transplant psychologist and Torque Teno virus level. The granular data from this unique monitoring might improve the understanding of behavioural patterns and accurate assessments of non-adherence following kidney-transplantation. Linked with clinical outcome our data might help to define the optimal diagnostic setting for clinical meaningful non-adherence. Ultimately improved handling of non-adherence might lead to prolonged long-term graft survival.

ABSTRACT

CLINICAL RELEVANCE OF ABSOLUTE BK POLYOMA VIRAL LOAD KINETICS IN PATIENTS WITH BIOPSY PROVEN BK POLYOMAVIRUS ASSOCIATED NEPHROPATHY

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The absolute BK viral load is an important diagnostic surrogate for BK polyomavirus associated nephropathy (PyVAN) after renal transplant (KTX). The hallmarks of the diagnosis of PyVAN are the quantitative detection of BKPyV DNA via PCR in plasma and urine, as well as distinct histological and immunohistochemical findings in the renal biopsy as a gold standard for organ invasive infection. While serial assessment of BKPyV viremia after kidney transplantation (KTX) is recommended, there is no data indicating which particular viral load change, i.e., absolute vs. relative viral load changes (changes in copies/ml; percentage of the preceding viremia) is associated with worse renal graft outcomes. Currently the optimal treatment strategy of PyVAN is unknown. The main recommended pillar of treatment remains the reduction of the immunosuppression (IS), however several treatments with antiviral agents such as leflunomide, cidofovir, fluoroquinolones, and immunoglobulin therapy (IVIG) with variable results were attempted.

In this retrospective study of 91 biopsy proven PyVAN we analyzed the interplay of exposure time, absolute and relative viral load kinetics, baseline risk, and treatment strategies as risk factors for graft loss after 2 years using a multivariate Poisson-Model.

Ninety-one patients were included in the final analysis with a mean follow up after diagnosis of 646 ± 193 days. Fifty-three (58%) patients underwent standardized reduction of IS, thirty (33%) patients were switched from MMF/Azathioprine to Leflunomide. Five (6%) patients received IVIG and three (3%) patients Cidofovir as rescue medications. The median viral load at the index biopsy was 2.15E + 04 copies/ml (IQR: 1.70E + 03–1.77E + 05) and median peak viremia was 3.6E + 04 (IQR: 2.7E + 03–3.3E + 05). Treatment strategies and IS-levels were not related to graft loss. Patients without graft loss showed a highly significant decline in absolute viremia between baseline and viremia at months 3 ($p < 0.001$), 6 ($p < 0.001$), and 12 ($p < 0.001$) in contrast to patients with graft loss (month 3; $p = 0.9$, month 6; $p = 0.5$ and 12; $p = 0.25$.) After correction for baseline viral load and eGFR, absolute viral load decrease/unit remained an independent risk factor for graft loss [IRR = 0.77, (95% CI: 0.61–0.96), $p = 0.02$].

This study provides evidence for the prognostic importance of absolute BK viremia kinetics as a dynamic parameter indicating graft survival independently of other established risk factors. Our findings support serial measurement of absolute BK viremia load changes to early identify patients with persistent viremia levels and consequently higher risk for graft loss.

CD38 ANTIBODY DARATUMUMAB – NOVEL TREATMENT FOR ANTIBODY-MEDIATED GRAFT REJECTION SHORTLY AFTER ORTHOTOPIC HEART TRANSPLANTATION IN HIGHLY SENSITIZED RECIPIENTS

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Background: We report the successful treatment of an early antibody-mediated allograft rejection (AMR) in a 39-year-old, highly sensitized woman after orthotropic heart transplantation with Daratumumab. Daratumumab is a human monoclonal antibody against CD38 highly expressed on plasma cells and natural killer cells. Primarily approved for multiple myeloma therapy its mechanisms on complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity/phagocytosis and apoptotic signaling also show effect on AMR in solid organ transplantation.

Methods: Next to centre-standardized immunosuppression protocol (r-ATG, MMF, prednisolone, tacrolimus) 10 doses of Daratumumab were administered over 6 months whereof the first six were provided intravenously and the last four subcutaneously.

Results: After the first administrations cytotoxic titers of donor specific antibodies (DSAs) (MFI of $2*10^6$) decreased radically and declined within 160 days to an MFI level of $<2*20^3$. Besides, right ventricular dysfunction improved and vast deposit of C4d and C3d in endomyocardial biopsies was reduced until no signs of antibody-mediated allograft rejection could be found.

Discussion: Highly sensitized transplant candidates (calculated panel-reactive antibody >80%) have a significant longer waiting period and higher risk for death on wait list. Therefore, in high urgent patient reduction of unacceptable human leukocytes antigens (uAGs) is essential, accepting the increased possibility of positive crossmatch and a subsequent AMR. This 12 months-follow up not only reveals the efficacious reduction of cytotoxic DSAs with Daratumumab but also its rapid onset and durable effect.

COUNTRY-SPECIFIC SEX DISPARITIES IN LIVING KIDNEY DONATION

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When on dialysis, women are less likely to be waitlisted for kidney transplantation and to receive a deceased or living donor organ, while in reverse, more women than men are living kidney donors. Country-specific analysis might reveal further inequalities in the process of kidney donation that place women at a disadvantage. We examined 14 studies reporting the sex distribution in living kidney donation. Based on the observed sex distribution of kidney donors in each study, we calculated intervals for the sex distribution of the 'expected donor pool', representing ranges of sex distributions within which men and women are equally likely to donate. If the sex distribution of the country's general population was within the intervals of the expected donor pool, we interpreted this result as unbiased kidney donation.

Among 36 666 living kidney donations from 14 countries, 45.4% of donors were men and 54.6% were women, while for recipients, 59.7% were men and 40.3% were women. When weighted with the population size of each country, the donor distribution consisted of 36.0% men and 64.0% women, and for the recipients, of 78.3% men and 21.7% women, although not all studies were likely to be population representative. Six out of 14 studies reported a women donor proportion above 60%. In 10 out of 14 studies, women were over-represented in the expected donor pool, compared with the proportion of women within the general population. Oman was the only country where the expected donor pool was in line with the countries sex distribution, thus men and women were equally likely to donate. In only 3 countries (Iran, South Korea and Thailand), we observed donation rates which were shifted towards men.

It is still unknown what causes the gender disparity and the process of decision-making is complex, including themes of compelled altruism, inherent responsibility, accepting risks, family expectation, personal benefit, and spiritual confirmation. It is possible that gender roles, empathy and altruistic behavior of women contribute to greater living kidney donation in women.

Our analysis is limited by the possibility that the sex distribution within a country might not accurately depict the distribution within the true donor pool, since we did not have data on sex-specific comorbidity burden. Further we could not include influential variables such as age, health and lifestyle factors.

In conclusion, the female-to-male donor rate was disproportionately high in relation to the sex distribution of the respective countries. We assume a great impact of socio-economic, -cultural and psychological factors, as we could identify varying proportions of woman kidney donors compared to men. These differences may influence country-specific donation processes, which need to be researched further.

TORQUE TENO VIRUS LOAD IN KIDNEY TRANSPLANTATION: ASSOCIATION WITH DONOR AND RECIPIENT CHARACTERISTICS AND CLINICAL FOLLOW-UP DATA

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Background: The apathogenic and torque teno virus (TTV) is associated with the state of immunosuppression in solid organ transplant recipients. After kidney transplantation, quantification of TTV viral load may serve as a risk stratification tool for allograft rejection and infectious events. Besides a robust and independent association between recipient age, sex, and TTV load, respectively, limited data exists on other potential determinants of TTV load. With the release of a CE certificate for the clinical application of a TTV quantification assay and growing evidence for a potential clinical use, in depth analysis of potential determinants of TTV load is essential. This trial was designed to analyse the association of TTV and detailed recipient and donor baseline characteristics and clinical follow-up parameters, respectively.

Methods: This retrospective analysis included all 386 consecutive adult recipients of a kidney allograft transplanted between January 1, 2016 and June 30, 2018 at the Medical University Vienna from the prospective observational TTV-POET trial (institutional review board approval number: 1785/2016; German Clinical Trials Registry number: DRKS00012335). For the present analysis, we included TTV measures before transplantation (d0), at month 1 (m1), at month 3 (m3) and at month 12 (m12) after transplantation. TTV DNA was extracted from patient plasma and assessed by real-time PCR with laboratory developed primers, as described previously. TTV load was associated with recipient and donor baseline characteristics and follow-up data.

Results: Median recipient and donor age at transplantation was 55 years. 135 (35%) kidney allograft recipients were female, 74 patients (19%) had a history of prior kidney transplantation, 318 patients (82%) received a deceased donor kidney transplant. 34 patients (9%) had pre-formed donor-specific antibodies (DSA); 19 patients (5%) received an AB0-incompatbile transplant. For the whole cohort, one-year patient survival was 95.6% and one-year death censored graft survival was 94.8%. Baseline TTV load (d0) was lower in female recipients ($p = 0.003$) and higher in older recipients (β 0.033, 95% CI 0.002–0.066; $p = 0.039$). Baseline TTV load was not associated with recipient body mass index and history of immunological cause of end-stage renal disease and diabetes mellitus, respectively. TTV load within the first year after kidney transplantation did not show an association with the prevalence of preformed DSA, induction treatment, AB0-incompatibility, and HLA-mismatch, respectively. Our trial detected an association between donor age and TTV load. Recipients from older donors showed higher TTV loads (m1, β 0.037, 95% CI 0.008–0.065, $p = 0.01$; m3, β 0.04, 95% CI 0.012–0.068, $p = 0.005$; m12, β 0.065, 95% CI 0.028–0.01, $p = 0.001$). These findings might indicate introduction of increased TTV load or genotype number from older donors. There was no association between TTV load during the first year after transplantation and donor sex.

When investigating the association between TTV load and clinical follow-up data, TTV levels showed a negative correlation with peripheral blood leukocyte and lymphocyte counts during the first year after transplantation (lymphocytes: m1, β −0.867, 95% CI −1.367 to −0.364, $p = 0.01$; m3, β −0.985, 95% CI −1.709 to −0.260, $p = 0.008$; m12, β −1.304, 95% CI −2.156 to −0.452, $p = 0.003$; data on leukocytes not shown). These findings might reflect the role of lymphocytes for TTV control. Additionally, TTV viral load negatively correlated with the estimated glomerular filtration rate (MDRD) within the first year after kidney transplantation (m1, β −0.043, 95% CI −0.066 to −0.02, $p < 0.001$; m3, β −0.024, 95% CI −0.049 to −0.001, $p = 0.06$; m12, β −0.078, 95% CI −0.111 to −0.045, $p < 0.001$). One might speculate that a higher eGFR might be a proxy for a healthier donor being more capable of controlling TTV infection. TTV viral load was associated with tacrolimus trough levels in month 12 after transplantation (β 1.262, 95% CI 0.0221–0.250, $p = 0.019$), but not with the amount of mycophenolic acid dosage. Finally we found higher TTV levels in recipients with prophylactic CMV treatment in month 3 after transplantation ($p < 0.001$). This finding might reflect an optimized environment for TTV replication due to CMV control.

Conclusion: This study revealed an association between TTV viral load and distinct baseline donor and recipient characteristics and clinical follow-up data, including kidney function. Further analysis, especially longitudinal assessment of TTV levels, may help to further dissect the interplay between TTV viral load and the state of immunosuppression in patients undergoing kidney transplantation.

ABSTRACT

HYPOTHERMIC AND CRYOPRESERVATION OF SMALL LIVER BIOPSIES FOR HIGH-RESOLUTION RESPIROMETRY

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Background: Normothermic machine perfusion (NMP) enables assessment of liver function prior to transplantation (LT), but reliable parameters are not fully established. Mitochondria play a central role in cellular bioenergetics and are also sensitive for damage due to ischemia/reperfusion injury occurring during hypothermic preservation, NMP and LT. Thus, in-depth analysis of the bioenergetic function using high-resolution respirometry (HRR) could be utilized to assess organ function. As per gold standard, HRR is performed in fresh samples immediately after tissue biopsy collection. However, due to logistics or if samples need to be stored in biobanks, such measurements can only be carried out several hours, days, or even weeks later. Therefore, we aimed at establishing and at critical testing protocols for hypothermic and cryopreservation of tissue samples, which could bridge time to analysis.
Methods: Wedge biopsies of porcine (n = 10) and human livers (n = 5) were taken during NMP. Mitochondrial function was assessed in tissue homogenates of fresh biopsies, after 4 h storage at 4 ˚C in Custodiol organ preservation solution (Köhler Chemie GmbH), and after cryopreservation (1 ˚C/min cooling rate in a specific preservation solution; samples stored subsequently at −80 ˚C). Using HRR (O2k, Oroboros Instruments), succinate-linked oxidative phosphorylation (OXPHOS capacity) was measured and the respective control ratios were calculated: *P-L* control efficiency and cytochrome *c* control efficiency to determine efficacy of OXPHOS coupling and damage to the outer mitochondrial membrane, respectively. Electron transfer capacity and *E-P* control efficiency were also determined. Fold-change of fresh compared to preserved measurements was calculated and Wilcoxon matched-pairs test was applied to evaluate the two distinct preservation methods.
Results: With hypothermic preservation for 4 hours, OXPHOS capacity did not significantly decrease in hypothermic stored tissues compared to fresh measurements. In line with these results, the *P-L* control efficiency could be maintained, indicating that bioenergetic efficacy could be preserved. Moreover, hypothermic storage did not cause damage to the outer mitochondrial membrane. However, *E-P* control efficiency decreased significantly ($p = 0.004$), although somewhat less in the human cohort ($p = 0.034$). OXPHOS capacity was impaired in cryopreserved tissue, reaching only 60% of the initial respiration, and the respective control efficiencies showed also a significant decline. The *P-L* control efficiency was preserved up to 75% with the present protocol.
Conclusion: Hypothermic preservation may be a suitable method to preserve functionality and coupling of mitochondria until analysis. Thus, reliable information of the bioenergetic function and in turn the organ quality can be still assessed. In contrast, with cryopreservation protocols for tissue homogenate, the mitochondrial function and intactness could not be maintained. Still, this procedure may be sufficient to address specific questions related to bioenergetic analysis.
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LONGITUDINAL MONITORING OF TTV IN PEDIATRIC KIDNEY TRANSPLANT RECIPIENTS MAY PREDICT OPPORTUNISTIC VIRAL INFECTIONS

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Background: The dominant cause of late graft failure after kidney transplantation is clinical and subclinical rejection by alloimmunity.1 However, immunosuppression must be carefully balanced with risk of infectious disease such as viral infections. In patients with solid-organ transplantation, Torque-Teno virus (TTV) plasma loads have been identified as possible marker of immune status and were shown to correlate with dosages of different immunosuppressive medications, as for example in pediatric KTX patients. 2 The aim of this study was to investigate associations of TTV loads with the occurrence of other viral infections, such as Epstein-Barr virus (EBV), cytomegaly virus (CMV), BK polyomavirus (BKV), and JC polyomavirus (JCV) in pediatric patients with KTX during follow-up.
Methods: All pediatric patients with KTX at the Department for Pediatrics at the Medical University of Vienna, were screened for inclusion. Only patients with a post-transplant time shorter than 3 months were excluded from this study. TTV levels were routinely measured in our KTX patients on a monthly to bimonthly basis. Quantitatively PCR assessed log10 transformed TTV loads were investigated for possible prediction of quantitatively assessed log10 transformed virus loads (EBV, CMV, BKV, JCV) on the same or the next visit, utilizing a generalized poisson mixed model analysis accounting for temporal autocorrelation with random slopes and intercepts for each patient over time.
Results: A total of 72 patients with KTX were included in this study. 65% (47/72) patients were male. Median age at inclusion was 12.2 years (IQR 8.0-15.8) with a median age at KTX of 8.1 years (IQR 3.4-13.0) resulting in a median time post KTX of 569 days (IQR 98-1896) at baseline. Donor types were predominantly living donors (63%, 45/72), median HLA mismatch was 3 (IQR 2-3), median creatinine at baseline was 0.89 (IQR 0.54-1.31), resulting in a median kidney graft function of 96.1 ml/min/1.73mA^2 (IQR 75.9-134.3). Donor-specific antibodies were present in 21% of patients (15/72) at baseline. All patients received basiliximab as induction, and maintenance immunosuppression consisted of 89% (64/72) tacrolimus, 6% (4/72) cyclosporin A, 4% (3/72) sirolimus, 1% (1/72) without a calcineurin- or mTOR-inhibitor, 85% (61/72) mycophenolate mofetil, 7% (5/72) azathioprine, 8% (6/72) without an antiproliferative substance, 99% (71/72) with steroids, 1% (1/72) without steroids. Patients were followed for median of 2399 days (range 338-7069). Higher TTV loads significantly predict higher CMV counts ($p=0.02$), BKV counts in plasma ($p=0.0458$), BKV counts in urine ($p=0.011$), JCV counts ($p=0.000008$) on the same visit, and higher CMV counts ($p=0.004$), BKV counts in plasma ($p=0.04$) and BKV counts in urine ($p=0.01$) on the next visit. Associations with EBV counts were non-significant.
Conclusion: This is the first study investigating correlations between TTV and other clinically relevant viral loads in kidney transplanted children. The novelty of the study is that higher TTV levels can significantly predict higher levels of other virus loads (CMV, BKV, JCV) in these patients on the same or the next upcoming visit (monthly to bimonthly). The clinical relevance of this finding is under current analysis.
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MITOCHONDRIAL RESPIRATION ANALYSIS REVEALS DONOR-SPECIFIC ALTERATIONS OF LIVER ALLOGRAFTS PRIOR TO TRANSPLANTATION

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Introduction: Mitochondria play an important role in cell metabolism. The liver is a metabolically highly active organ, strongly relying on an adequate mitochondrial function to maintain cellular energy levels. Although the involvement of mitochondria in the pathogenesis of several donor risk factors and ischemia-reperfusion injury itself is well-described, data on the impact of donor characteristics on hepatic mitochondrial function after cold ischemia is scarce. Particularly with the increasing usage of extended criteria donor organs, the development of new reliable biomarkers for organ quality is all the more important. Therefore, the aim of this study was to characterize the impact of different donor organ parameters on mitochondrial bioenergetics.
Methods: In a prospective single-center trial, 35 adult patients (34.3 % female) who underwent deceased donor liver transplantation were included. Upon organ arrival in our center, a liver wedge biopsy was taken. Mitochondrial respiration was evaluated in tissue homogenates using high-resolution respirometry (HRR, O2k, Oroboros Instruments). We focused on the assessment of OXPHOS capacities of the succinate-, NADH- and fatty acid oxidation (FAO) pathways. Further, the *E-P* control efficiency, *P-L* control efficiency and cytochrome *c* control efficiency of the succinate pathway were calculated. These allowed the evaluation of different electron transfer pathways and coupling control states and their impact on the distinct donor organ parameters including sex, steatosis, cytomegalovirus infection, body mass index, cold ischemia time and age.
Results: Specific mitochondrial respiration patterns for donor parameters could be observed. If calculated the relative contribution of the respective substrate pathways, mitochondrial function showed a significantly lower fatty acid oxidation in allografts derived from female donors (female 0.22 ± 0.05 *vs* male 0.26 ± 0.06, $p = 0.023$; mean $\pm$ SD). Furthermore, histologically proven mild steatosis resulted in a significant elevation of FAO (0.28 ± 0.06 *vs* lean 0.23 ± 0.06, $p = 0.031$). Interestingly, mitochondrial adaptation with increased fatty acid oxidation of mildly steatotic allografts was lost in moderate steatosis (0.28 ± 0.06 *vs* moderate 0.23 ± 0.03; $p = 0.179$). Additionally, cytochrome *c* control efficiency in liver tissue homogenate was significantly higher in donors infected with CMV compared to non-infected donors (0.14 ± 0.09 *vs* 0.08 ± 0.07; $p = 0.027$).
Conclusion: In this study, we could demonstrate that donor characteristics strongly influence the mitochondrial respiration in liver grafts. Specific mitochondrial respiration patterns for various donor characteristics could be determined by high-resolution respirometry and the influence of those factors on the clinical outcome requires further investigation. Mitochondrial respiration analysis could serve as a new tool in organ quality assessment of liver allografts.

SARS-COV-2 ANTIBODY RESPONSE IN RELATION TO TTV LEVELS IN PEDIATRIC PATIENTS WITH KIDNEY TRANSPLANTATION

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Background: After two doses of SARS-CoV-2 messenger RNA vaccination, adult kidney transplanted (KTX) patients exhibit considerably lower antibody levels than healthy controls. In addition to the critically diminished humoral response, cellular response also seems to be almost absent. Torque-Teno virus (TTV) has been identified in a number of studies as a possible marker of the functional immune status in transplanted patients including kidney transplanted children. The aim of this study was to investigate humoral response to mRNA vaccination in pediatric KTX patients in the context of immune function represented by TTV counts.
Methods: All pediatric patients with KTX at the Department for Pediatrics at the Medical University of Vienna, eligible for SARS-CoV-2 vaccination (BNT162b2, Biontech/Pfizer, age above 16 years), were screened for inclusion. SARS-CoV-2 vaccine antibody levels and serological proof of previous SARS-CoV-2 infection was prospectively collected at the date of the 2nd vaccine, as well as 4–8 weeks after the 2nd vaccine. TTV levels are routinely measured in our KTX patients on a monthly to bimonthly basis.\r \nCorrelation (I) between median log10 TTV counts during the last year before SARS-CoV-2 vaccination and vaccine antibody levels (II) as well as between last TTV plasma count (on the day of the 2nd vaccination) and vaccine antibody levels were analyzed.
Results: 19 patients were screened for inclusion, in total 15 (79%) were included in this study. Patients were predominantly male (12/15, 80%), median age at inclusion was 16.9 years (IQR 16.5–19.1), median time after KTX was 71 months (IQR 32–143). In all but one patient, steroids were part of their maintenance immunosuppression. 87% received tacrolimus (13/15), 7% sirolimus (1/15) and 7% belatacept (1/15). Antiproliferative immunosuppression consisted of mycophenolate mofetil in 87% (13/15) and azathioprine in 7% (1/15). 67% (10/15) of patients did not have serological evidence of previous SARS-CoV-2 infection at the time of the 2nd dose of their SARS-CoV-2 vaccination. Median SARS-CoV-2 vaccine antibody titers were significantly higher in patients with serological evidence of previous SARS-CoV-2 infection four weeks after the first and four to eight weeks after the second vaccination ($p = 0.002$, $p = 0.005$). However, no correlation between log10 TTV levels, median value during the last year or last level at the time of the 2nd dose, and SARS-CoV-2 vaccine antibody titers could be identified. Interestingly, SARS-CoV-2 antibody titers were significantly higher in patients with lower HLA recipient-donor mismatch, both four weeks after the first and four to eight weeks after the second vaccination ($p = 0.03$, $p = 0.01$). However, median log10 TTV counts during the last year showed no significant correlation with HLA mismatch, but patients with previous SARS-CoV-2 infection also had significantly lower HLA mismatch ($p = 0.03$).
Conclusion: Overall, serological response after two doses of mRNA vaccine was low, except for patients with serological evidence of previous SARS-CoV-2 infection, where high titer boosts were observed. Patients with lower HLA mismatch had higher rates of previous SARS-CoV-2 infection and overall higher antibody levels, however, the significance of this finding is not clear. TTV loads did not relate to SARS-CoV-2 vaccine antibody response or to previous infection, however, it is important to emphasize that the number of patients is very low. Further analyses are planned to assess antibody response in correlation with TTV titers after the 3rd vaccination dose as well as in additional patients (aged 12–16 years, as BNT162b2 was recently approved for this age group) which is currently in the rollout phase at our center.

ABSTRACT

PHENOTYPES IN CHRONIC LUNG ALLOGRAFT DYSFUNCTION

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Background: Despite the improvement of short-time outcomes, lung transplantation still records a worse survival rate compared to other solid organ transplantations. One of the most relevant causes of morbidity and mortality is chronic rejection, called chronic lung allograft dysfunction (CLAD). Three main clinical phenotypes can be distinguished: bronchiolitis obliterans syndrome (BOS), restrictive allograft syndrome (RAS) and a mixed phenotype. BOS is the most frequent manifestation of CLAD and shows an obstructive pattern whereas RAS shows a restrictive one. CLAD phenotypization is essential during follow-up since long-term outcomes differ considerably. Namely, RAS patients have the worse survival, approaching 90% mortality and the worse response to available therapies.
Objectives: The main aim of the current study was to characterize CLAD phenotypes in lung transplant recipients who received alemtuzumab as induction therapy followed by a low-dose maintenance immunosuppression and compare the phenotypes regarding overall patients' and graft survival. Risk factors for CLAD occurrence were investigated in the same cohort.
Methods: The current study is a retrospective single-center data analysis including patients transplanted between 2008 and 2019 at the Division of Thoracic Surgery at the Medical University of Vienna. Inclusion criteria were induction therapy with alemtuzumab and age over 18 at time of transplantation. Excluded were patients receiving another induction therapy agent or had multiorgan transplantation. The cohort consisted of 721 patients. Patients with CLAD were included in the current analysis.
Results: One-hundred eighty-nine (26.2%) patients developed CLAD during the follow-up, 128 (67.7%) developed BOS, 18 (9.5%) RAS and 43 (22.8%) a mixed phenotype.

Overall survival of the cohort was 2850 days with survival rates of 85%, 66% and 51% at 1, 5 and 10 years, respectively. Significantly, worse overall survival was observed in RAS and mixed phenotypes ($P = 0.006$). At 5-years patients suffering from BOS, RAS and mixed phenotype showed a survival of 70%, 37% and 75%, respectively. At 10-years patients suffering from BOS, RAS and mixed phenotype showed a survival of 53%, 18% and 30%, respectively. Graft survival rates for BOS at 1, 5 and 10 years were 97%, 68% and 53%, for RAS 94%, 34% and 17%, and for the mixed phenotype 91%, 57% and 30%, respectively. 13 patients underwent re-transplantation due to CLAD. Patients' characteristics did not significantly differ between the three phenotypes. Independent risk factors for the occurrence of CLAD were antibody-mediated rejection (AMR) (HR: 3.319 CI: 2.008–5.485, $P < 0.001$), de novo donor-specific antibodies (DSAs) (HR: 1.520 CI: 1.065–2.169, $P = 0.021$) and infections requiring either inpatient or ICU care (HR: 1.184 CI: 1.062–1.321, $P = 0.002$ and HR: 1.642 CI: 1.153–2.340, $P = 0.006$). Female gender was a protective factor against CLAD (HR: 0.636 CI: 0.463–0.872, $P = 0.005$).
Conclusion: Our findings confirmed that RAS is associated with the worse outcome among the CLAD phenotypes. AMR, DSAs and infections were identified risk factors for CLAD in our cohort. Further studies are necessary to identify risk factors for the development of a specific phenotype.

INVESTIGATION OF CYTOKINE RELEASE FOR ALEMTUZUMAB TREATED LTX PATIENTS

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Fundamental developments of the immunosuppression protocols in the last 20 years contributed to the improvement of long-term outcome in the present era. Currently, immunosuppression protocols include an induction therapy and a triple-drug maintenance immunosuppression, con-stituted by a calcineurin-inhibitor, an anti-proliferative agent and steroids. Alemtuzumab is a monoclonal antibody, recently used as induction agent in solid organ transplantation. Based on the published experience, this agent shows promising results in terms of rejection rates and sur-vival. Alemtuzumab is targeting CD52 antigen, expressed on mononuclear cells including T, B and Natural Killer (NK) cells. The primary use of induction therapy at the time of transplant, is to avoid early acute rejection. However, the mechanisms of action of alemtuzumab as induction therapy in lung transplant recipients have not been investigated yet.

The aim of the current study was to investigate the expression of a panel of cytokines in lung transplant recipients who received alemtuzumab induction therapy and compare it with a matched group of LTx recipients who did not receive any induction.

By flow cytometry, a panel of cytokines and chemokines, including IL-5, IL-13, IL-2, IL-6, IL-9, IL-10, IFN-γ, TNF-α, IL-17A, IL-17F, IL-4, IL-22, IL-4, TNF-ß, IL-12p70, APRIL, BAFF, sCD40L, was measured in prospectively collected serum samples of lung transplant (LTx) recipients, who received either alemtuzumab or no induction therapy at two time points (time of transplantation and 1 year thereafter).

Twenty-six LTx recipients who received alemtuzumab and twenty-seven LTx recipients without induction therapy were included in the analysis. Median age was 58 years in the control group compared to 52 years in the alemtuzumab group. The most represented diagnosis was chronic obstructive lung disease. In no-induction group, IFN-γ ($P = 0.048$), IL-17A ($P = 0.001$) and BAFF ($P = 0.009$) were found to be upregulated one year after transplantation. On the contrary IL-5 ($P = 0.032$), IL-13 ($P = 0.005$), IL-2 ($P = 0.007$), IL-9 ($P = 0.006$), IL-10 ($P = 0.001$), IL-17F ($P = 0.001$), IL-4 ($P = 0.001$) and IL-22 ($P = 0.034$) were all downregulated. In alemtuzumab group only BAFF ($P = 0.016$) was found to be upregulated one year after transplantation. Instead IL-13 ($P = 0.023$), IL-9 ($P = 0.056$), IL-10 ($P = 0.011$), IL-17A ($P = 0.043$), IL-4 ($P = 0.006$) and IL-22 ($P = 0.015$) were all downregulated. Interestingly, in the same group, IL-2 was not downregulated.

One year after transplantation, no induction group showed a skewed cytokine signature towards humoral and type 17 immunity. On the contrary, alemtuzumab showed a strong secretion of BAFF, underlining B-cell commitment and proliferation.

MITOCHONDRIAL RESPIRATION DURING NORMOTHERMIC MACHINE PERFUSION OF THE LIVER PREDICTS CLINICAL OUTCOME AFTER TRANSPLANTATION

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Background: The growing demand for liver grafts promotes the use of extended criteria organs. To assure good clinical outcome, pre-transplantational evaluation of organ quality is needed. We hypothesize that mitochondrial quality and performance during normothermic machine perfusion (NMP) correlate with organ function after liver transplantation (LTx). Thus, our aim was to determine mitochondrial function (MF) after cold ischemia and during NMP in order to investigate its use for organ quality assessment and as a predictive marker for the clinical outcome.
Methods: In a prospective clinical trial, livers underwent NMP (metra, OrganOx) for up to 24 h before transplantation (n = 35). Biopsy and perfusate samples were collected at the end of cold storage, at 1 h, 6 h and end of NMP, and at 1 h after transplantation. Serial histology and real-time confocal imaging were performed in biopsies. MF was characterized in tissue homogenates by high-resolution respirometry (HRR; O2k, Oroboros Instruments) and correlated with clinical outcome (MEAF score). Succinate-linked coupling control was assessed, and the damage of the outer mitochondrial membrane was monitored by cytochrome *c* addition.
Results: We observed a considerable variability in mitochondrial respiration between grafts during cold storage, irrespective of the coupling states: OXPHOS (P) 40.8 $\pm$ 14.5, LEAK (L) 7.9 $\pm$ 2.7, ET (E) 71.5 $\pm$ 28.4, (mean $\pm$ SD; pmol s^{-1} mg wet $weight^{-1}$). MF correlated with the clinical outcome, specifically, a higher *P-L* coupling efficiency and lower *E-P* control efficiency at 1 h NMP predicts a lower MEAF score. AUCs calculated for the above efficiencies during the first 6 h of perfusion show the same trend. Similarly, a higher AUC for cytochrome *c* control efficiency correlated with higher MEAF. Findings were compared in their predictive capacity with conventional perfusate biomarkers and the predictive power of MF exceeded that of other findings.
Conclusions: Improved indices of ATP production efficiency (*P-L* coupling efficiency) and mitochondrial outer membrane integrity (cytochrome *c* control efficiency) during NMP predicts the clinical outcome upon liver transplantation. Mitochondrial respiration assessed by HRR is therefore a promising tool to select optimal grafts.

TISSUE VIABILITY AND MITOCHONDRIAL RESPIRATION DURING STATIC COLD STORAGE OF THE LIVER PREDICTS OUTCOME OF THE TRANSPLANTATION

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Background: Donor and organ parameters with strong predictive value in liver transplantation are lacking. We herein evaluate the potential of in-depth liver viability and bioenergetics testing in static cold stored (SCS) grafts for their predictive value towards the outcome in liver transplantation.
Methods: In a prospective, single arm trial, we enrolled 43 patients undergoing liver transplantation. Liver wedge biopsy samples were taken upon arrival. Histology and real-time confocal imaging (RTCA) of SYTO 16/PI and WGA were employed. Mitochondrial respiration was assessed by high-resolution respirometry (HRR; O2k, Oroboros Instruments) with a focus on OXPHOS capacity and coupling efficiency (*P-L* control efficiency) of the succinate-linked respiration. Early allograft dysfunction (EAD) served as primary endpoint, MEAF and L-GrAFT, graft and patient survival, length of stay and biliary complications served as secondary endpoints. Data were analysed using parametric and non-parametric tests (including Spearman rank correlation). RTCA score and *P-L* control efficiency were evaluated in uni- and multivariate logistic regression analyses.
Results: Twenty-two recipients (22/43, 51.2 %) experienced EAD. Pre-transplant histology results were not significantly different between EAD and non-EAD. EAD correlated well with MEAF score ($p < 0.01$) and L-GrAFT ($p = 0.02$, Spearman's ρ 0.574 for MEAF and 0.357 for L-GrAFT). The mean RTCA score was predictive for EAD (-0.75 ± 2.27) *vs* non-EAD; (0.70 ± 2.08; $p = 0.01$). The *P-L* control efficiency correlated with EAD (0.8 in non-EAD compared to 0.7 in EAD-livers; $p = 0.02$) and was congruent with the RTCA result ($p = 0.005$, Spearman's ρ 0.493). The MEAF score correlated negatively with the RTCA readout ($p = 0.01$, Spearman's ρ -0.407). The occurrence of biliary complications and the overall length of stay were comparable between recipients with EAD and non-EAD.
Conclusions: Both RTCA and HRR are valuable tools for tissue viability and bioenergetics function assessment with predictive value towards EAD in liver transplantation.

ABSTRACT

LONG-TERM OUTCOMES OF ALEMTUZUMAB INDUCTION THERAPY AFTER LUNG TRANSPLANTATION

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Alemtuzumab is a monoclonal antibody targeting CD52, increasingly used as induction therapy after transplantation. The aim of this study is to analyze the outcomes of alemtuzumab induction therapy followed by a low-dose maintenance immunosuppression in a large single center cohort of lung transplant recipients.

All patients, who received alemtuzumab induction followed by a low-dose maintenance immunosuppression were included in the analysis. Short- and long-term outcomes were analyzed. 721 lung transplant recipients, transplanted between January 2008 and June 2019, were included in this retrospective study. Freedom from higher grade ACR at 1, 5 and 10 years was 98%, 96% and 96%, respectively. Thirty-nine patients (5%) developed clinical AMR. Twenty-one percent of patients developed high grade CKD. A total of 1488 infections were recorded. Sixteen percent were diagnosed within the first 3 months. Sixty-two patients (9%) developed a malignancy during follow-up. Freedom from CLAD at 1, 5 and 10 years was 94%, 72% and 53%, respectively. Overall survival rates at 1, 5 and 10 years were 85%, 71% and 61%, respectively. Alemtuzumab induction combined with a low-dose tacrolimus protocol is safe and associated with low rates of acute and chronic rejection as well as an excellent long-term survival.

LOW-DOSE IMMUNOSUPPRESSION WITH EXTRACORPOREAL PHOTOPHERESIS IN HIGH-RISK HEART TRANSPLANT PATIENTS

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Background: Current immunosuppressive protocols are associated with high risk of infection, malignancy and death in severely ill patients undergoing heart transplant (HTX) under urgent conditions. Low-dose immunosuppressive protocols are affected by higher rejection rates. Extracorporeal photopheresis (ECP) is an established therapy in the treatment of acute rejection. We therefore combined ECP with a reduced intensity immunosuppressive protocol.
Methods: We report on 28 high risk patients treated according to a novel reduced intensity immunosuppression ECP protocol. Immunosuppression consisted of low dose tacrolimus (target threshold range 8–10 ng/mL month 1–6; 5–8 ng/mL >6 months) with delayed start (median start on postoperative day (POD) 3), mycophenolate mofetil (MMF) (2 g/day), and low maintenance steroids (0.2 mg/kg/day prednisolone). ECP was applied according to a 6-month protocol (24 procedures).
Results: Indications were high risk of infection due to infection at the time of transplant ($n = 13$) or bridging to transplant via extracorporeal membrane oxygenation (ECMO) ($n = 7$), and high risk of malignancy ($n = 8$). One-year survival was 88.5%. Cause of death was infection in three (POD 12, 51, 351), recurrence of cancer (POD 400) in one patient. Incidence of severe infection was 17.9% ($n = 5$, respiratory tract). Within the first year, antibody mediated rejection (AMR) (1H) was detected in one (3.6%), acute cellular rejection (ACR) ≥ (à≥2R) in four patients (14.3%).
Conclusions: This is the first report on prophylactic use of ECP and reduced intensity immunosuppression in HTX recipients. This novel protocol is safe and effective to avoid allograft rejection in patients with risk of severe infection or cancer re-occurrence.

CARDIAC SURGERY AFTER HEART TRANSPLANTATION – AN EXPLORATORY, RETROSPECTIVE, MULTICENTER STUDY (CASH-STUDY)

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Background: Cardiac transplantation is the gold-standard for patients with end-stage heart failure. Due to excellent long-term survival (50% 20-years survival) and a growing rate of marginal donor hearts used for transplantation, cardiac disease can show up early and late after transplantation. Cardiac surgery for valvular disease, coronary artery disease (CAD), aortic pathologies or other is an option. There only exist single center experiences with low numbers. The aim of our analysis was to evaluate cardiac surgery after heart transplantation in a multicenter study.
Methods: This is a retrospective, exploratory, multicenter analysis of heart transplant recipients with a cardiac surgery after heart transplantation between January 1984 and October 2020. In total, 60 high-volume cardiac transplant centers were asked for participation.
Results: Data were available from 19 centers including a total of 113 patients. Participating centers were from North America (Canada 1, USA 6), South America (Argentina 1) and Europe (Austria 2, Croatia 1, France 1, Germany 2, Italy 1, Slovakia 1, Spain 3). 82.3% (n = 93) were male. A median of three cardiac operations after HTX were performed in each center (2-9.5), five centers included ten patients or more. Indication for surgery was valvular disease (n = 61), coronary artery disease (n = 17), constrictive pericarditis (n = 17), aortic pathology (n = 10), infection (n = 4), myxoma (n = 2) and other (n = 2). Median age at time of reoperation was 58 (47.8-63.1) with a median time to surgery of 4.3 years (1-9.3). Ten patients (8.8%) had the cardiac reoperation in the first 30 days after HTX, all with urgent indication for surgery. Twenty eight patients (24.8%) underwent reoperation in the first year after HTX. 1-year survival was 77% (n = 87) and overall survival 50.4% (n = 57) with a median follow-up of 3.85 years (1.17-8.08). In-hospital mortality was 9.7% (n = 11).

In-hospital and 1-year mortality was higher in emergency procedures (18.4% and 28.9% vs 5.3% and 12% in elective procedures).
Conclusion: Incidence and type of cardiac surgery after HTX varies significantly from center to center. Elective cardiac surgery after HTX has good outcome in selected patients.

FIRST EXPERIENCE WITH DONATION AFTER CIRCULATORY DEATH (DCD) HEART TRANSPLANTATION IN AUSTRIA

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Background: In an effort to address the increasing demand for heart transplantation, we established a clinical program of heart transplantation from donation after circulatory-determined death (DCD) donors in 2019. However, Austria has a longer observation (no touch) period compared to other countries, potentially harming the heart procured in DCD condition. We report the first cases performed in our center.
Methods: Hereby we describe our experience on Donation after Circulatory Death (DCD) heart transplantation with Direct Procurement and ex-vivo normothermic machine Perfusion (DPP) with Organ Care System (OCS, Transmedics). All donors were Maastricht category III.
Results: Five organs were resuscitated on OCS after direct procurement, two of them were rejected for transplantation (late administration of heparin at procurement and dilated heart in one and therapy-resistant high potassium leading to bradycardia in the other organ). All but one donor (donor for patient 3) and all recipients were male. Recipient age was 61.5, 68 and 33.8 years, donor age 42, 30 and 37, respectively. Total warm ischaemic times (time from withdrawal of treatment until cardioplegia) were 23, 23 and 27 minutes, back table times Perfusion times on OCS were 223, 236 and 316 minutes. Lactate levels (arterial/venous) were decreasing in all patients with levels at the beginning of perfusion of 5.72/5.45 mmol/L in patient 1, 5.7/5.62 in patient 2 and 6.19/6.20 in patient 3 and at the end of perfusion 1.34/1.26, 3.64/3.37, and 2.58/2.72, respectively. All were weaned successfully from heart lung machine with moderate inotropic support and normal cardiac function. Weaning from respirator was on day of transplant in all patients, length of intensive care unit stay was 7, 7 and 8 days, respectively. All had excellent postoperative recovery and ejection fraction is normal 480, 439 and 101 days after transplantation.
Conclusion: Early results show that excellent results can be achieved with DCD heart transplantation, even with prolonged observation times.

ABSTRACT

LEVELS OF TORQUE TENO VIRUS (TTV) IN THE EARLY PHASE AFTER LIVER TRANSPLANTATION

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Background: Torque Teno Virus (TTV), an apathogenic DNA virus, is highly prevalent in solid organ recipients and has been used to monitor the immunocompetence following transplantation. Limited data are available for TTV levels in the early postoperative phase after liver transplantation (LT).
Methods: 40 recipients were monitored for TTV levels prior and after LT (=day 1), day 4, 7, and 14. Patients received induction with antithymocyte globulin (1.5 mg per Kg) over 3 days following a tacrolimus and steroid based immunosuppression. Missing values and parameters were imputed without knowledge of the dependent variables. Multiple regression analyses were performed including subject and time point "if appropriate" information as correcting factors and using log10 TTV copies/ml as the independent variable.
Results: TTV could be detected in 87% (median 5.0e5 copies/ml) prior and in 97% after liver transplantation. TTV levels remain stable in the first days after LT without significant changes (day 1: 8.2e5, day 4: 3.1e5, day 7: 3.2e5, and day 14: 5.7e5 copies/ml, $P = 0.33$). Doses of ATG received blood transfusion volumes (PRBS), and length of stay in hospital (LOS) correlated significantly with TTV, all positively (*P*-values 0.005, 0.002, and <0.001, respectively). Length of stay in ICU did not correlate significantly.
Interestingly, Rhesus factor positive patients showed significant higher levels of TTV, corrected "in addition" for blood groups ($P < 0.001$).
Conclusion: Levels of TTV remained stable in the perioperative phase of a liver transplant cohort but correlated significantly with administered doses of ATG, PRBC volumes, LOS, and Rhesus factor, however, not with length of ICU stay. The role of TTV as a biomarker needs to be evaluated in further studies.

TORQUE TENO VIRUS (TTV) AS A MONITOR OF IMMUNOCOMPETENCE FOLLOWING LIVER TRANSPLANTATION

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Background: The apathogenic Torque Teno Virus (TTV) is highly prevalent in patients after solid organ transplantation. In liver transplant (LT) recipients TTV viremia may serve as a potential biomarker of immune status after transplant.
Methods: We included 40 patients after LT and monitored TTV levels within the first year after transplantation. Levels of TTV were measured prior and after LT (=day 1), day7, day 14 (early), after 3, 9, and 12 month (late). Patients received induction with antithymocyte globulin (ATG; 1.5 mg per Kg) over 3 days following a tacrolimus and steroid-based immunosuppression, after 3 month tacrolimus monotherapy if feasible. Multiple regression analyses were performed including subject and time point–if appropriate–information as correcting factors and using log10 TTV copies/ml as the independent variable.
Results: TTV load peaked at the end of month 3 post-transplant (8.1 log copies/ml) and reached steady state thereafter. In the late phase after LT levels of TTV correlated positively with tacrolimus levels (p=0.008), positively with LDH ($P < 0.001$) and negatively with BMI ($P < 0.001$), however, it was not associated with the level of liver enzymes.
Conclusion: Levels of TTV significantly correlated with trough levels of tacrolimus. The role of TTV as a tool to detect and/or predict immune-related events in the post-transplant period after LT needs to be evaluated in further trials.

PASSENGER LEUCOCYTES – IMPACT IN TREG-MEDIATED ALLOGRAFT SURVIVAL

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Background: Whether passenger leucocytes are friend or foe when it comes to allograft tolerance is still controversial, thus we aim to shed some light on this issue. We are utilizing a murine model of fully mismatched skin transplantation in which graft survival is mediated by regulatory T cells (Tregs) via combined treatment of IL-2 cplx, mTOR inhibition and IL-6 blockade. Since interleukin-2 (IL-2) coupled to a specific antibody against IL-2 (IL-2 cplx) has shown to selectively expand and activate Tregs in vivo, our group combined IL-2 cplx with rapamycin and short term IL-6 neutralization, which leads to significant prolongation of skin allograft survival. How passenger leucocytes affect the survival of fully mismatched skin grafts in this setting will be addressed by irradiation of the donor, which results a significant reduction of graft resident T cells.
Methods: Recipient C57BL/6 mice received fully mismatched BALB/c skin grafts in combination with IL-2 cplx protocol (4 weeks of IL-2 cplx, rapamycin and a short course of anti-IL-6 mAb). Donor mice were subjected to irradiation 8 days prior to transplantation, which results in profound depletion graft-resident T cells. To study the immunological mechanisms, graft infiltrating (recipient) leucocytes, differentiation of memory T cells as well as development of donor-specific antibodies were investigated.
Results: Depletion of passenger leucocyte has no impact on graft survival in untreated recipients, however, if recipients receive IL-2 cplx protocol survival of passenger leucocyte-depleted grafts was significantly prolonged (MST = 31 vs 53 days; $P = 0.0115$). Furthermore, analysis of graft infiltrating cells at day 20 post transplantation showed reduced amounts of recipient leucocytes within previously irradiated skin grafts ($P = 0.0095$). In addition, flow-cytometric immune phenotyping indicated reduced levels of CD4+ and CD8+ central memory T cells within secondary lymphoid organs of mice receiving passenger leucocyte-depleted grafts. Analysis of sera post graft rejection revealed a significant decrease of donor reactive IgG1 ($P < 0.05$) and IgG2a/b ($P < 0.01$), suggesting impaired humoral immune response.
Conclusion: We could show that passenger leucocyte depletion leads to significant prolongation of skin allograft survival, due to decreased infiltration of recipient leucocytes and reduced T cell memory differentiation, strengthening the hypothesis that graft resident leucocytes have a negative impact on allograft survival. As this effect was observed in IL-2 cplx protocol treated but not untreated mice, the impact of passenger leucocytes is suggested to be rather important in chronic than acute rejection. Importantly, although lasting tolerance could not be achieved in this setting, sensitization of mice was successfully prevented on both T cell and humoral level.

BONE MARROW RESIDENT LONG-LIVED PLASMA CELLS AS THERAPEUTIC TARGET IN ANTIBODY-MEDIATED REJECTION

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Background: Donor-specific antibodies (DSA) represent a decisive immunological obstacle in solid organ transplantation. Despite their impact on the field, our understanding of the exact mechanisms responsible for initiating and upholding a sustained humoral response against (donor)-HLA-antigens remains limited. Experimental models for allergy, infection and vaccination point towards bone marrow resident plasma cells as key contributors for long-lasting antibody secretion. We therefore hypothesize that in the transplant setting, those bone marrow plasma cells are the source of DSA on a cellular level. We aim to identify and characterize DSA-secreting plasma cells and to provide insight into their underlying biology.
Methods: C57BL/6 mice were grafted with a fully mismatched balb/c heart without any immunosuppressive treatment applied. Serum DSA levels were quantified via flow crossmatch at week 4 and 20 post-transplant. Splenic and bone marrow plasma cells were quantified and characterized using flow cytometry in transplant recipients and age-matched naïve individuals. Spleen and bone marrow cells of each individual heart allograft recipient were cultured separately for 48h. DSA within the cell culture supernatants were quantified via flow crossmatch to assess the contribution of each compartment in terms of DSA section.
Results: Fully mismatched Balb/c heart allografts were rapidly rejected within 12 days in untreated BL6 recipients. All recipients (13/13) developed high levels of donor-specific IgG within 4 weeks after transplantation (Mean DSA MFI: 5240, 95%-CI: 3260–7221). DSA levels remained stable until the end of follow up 20 weeks post-transplant (Mean DSA MFI: 4503, 95%-CI: 3587–5419). This chronic DSA response, 20 weeks post transplantation, was almost exclusively carried by the bone marrow rather than the spleen (Mean in-vitro DSA MFI; Bone Marrow: 441 vs. Spleen: 59; p = 0.00015).
While there was no difference in overall plasma cell counts between transplant recipients and age matched naïve controls, HTX recipients carried a substantially larger fraction of long-lived plasma cells (LLPC; B220low, CD138high, TACIhigh, Blimp1high, CD19 low) within their bone marrow (% of LLPC within all Plasma cells; naïve ($n = 7$): mean = 15.26%, 95%-CI = 10.38–20.14 vs. HTX recipients ($n = 12$): mean = 33.60%, 95%-CI = 28.56–38.64; $P = 0.0018$). Furthermore, bone marrow LLPC also increased in absolute cell numbers upon sensitization through cardiac transplantation (LLPC absolute cell numbers x10wenn möglich bitte als Hochzahl darstellen; naïve ($n = 7$): mean = 0.18, 95%-CI = 0.11–0.25 vs. HTX recipients ($n = 12$): mean = 0.46, 95%-CI = 0.41–0.50; p = 0.0004).
Conclusion: We demonstrated that chronic humoral anti-donor responses are sustained by the bone marrow and identified long-lived plasma cells as potential novel therapeutic target of interest.

ABSTRACT

ATG AND SHORT-TERM INTERLEUKIN-6 BLOCKADE SYNERGIZE TO PREVENT ALLOGRAFT REJECTION UNDER COSTIMULATION BLOCKADE

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Background: Specific disruption of the CD28-B7 costimulatory pathway using CTLA4-Ig (Belatacept) provides an efficient and non-nephrotoxic alternative to calcineurin-inhibitors (CNI). Clinically, Belatacept has demonstrated superior patient and graft survival in kidney transplantation compared to CNIs. In experimental models, CTLA4-Ig also provided exceptional control of humoral allo-immunity. Those benefits come at the cost of a markedly increased incidence of acute T-cell mediated rejection (TCMR) episodes under Belatacept. The decreased levels of regulatory T-cells observed under CTLA4-Ig may offer a potential explanation for the rise in TCMR. A rationally designed induction regimen might overcome this obstacle.
Methods: C57BL/6 mice were grafted with a fully mismatched Balb/c heart and received anti-thymocyte globulin (ATG; 0.15mg day 0, 5), anti-interleukin-6 monoclonal antibodies (αIL6; 0.6mg day -1; 0.3mg day 4, 6) and CTLA4-Ig (0.25mg day 0, 4, 14, 28, 56, 84) as indicated. Heart allograft survival was followed by palpation for up to 100 days. Flow cytometry of peripheral blood, spleen and graft-infiltrating lymphocytes was performed. Serum donor-specific antibodies were quantified via flow crossmatch at the time of rejection or day 100 post HTX.
Results: CTLA4-Ig monotherapy prolongs graft survival, but nearly all grafts are eventually rejected by day 45 (MST = 36days). Additional ATG induction, stretches the median survival time to 80 days. Combined induction therapy with ATG and anti-IL6 under CTLA4-Ig facilitated long-term (100 days) survival of fully mismatched cardiac allografts in all individuals (ATG+αIL6+CTLA4-Ig 8/8 vs. ATG+CTLA4-Ig 4/9; p = 0.0152). Mechanistically, the additional blockade of interleukin-6 further increases the frequency of regulatory T-cells and thereby compensates for the unfavorable effects of CTLA4-Ig on Tregs (peripheral blood day 8 post HTX; ATG+αIL6+CTLA4-Ig: mean = 12.81%, 95%-CI = 16.21–9.40 vs. ATG+CTLA4-Ig: mean = 6.98%, 95%-CI = 8.42–5.36; p = 0.0015). Notably, the short term blockade of IL-6 signaling results in a long-term disruption of CD8 effector memory T-cell formation (Spleen, day 100 post HTX) and markedly reduces CD8 T-cell infiltration in the cardiac allograft at day 100 post HTX.
Furthermore, the refined induction regimen provides superior control of humoral alloimmunity and significantly reduced DSA development (DSA positive heart allograft recipients; CTLA4-Ig: 5/5 vs. ATG+CTLA4-Ig: 8/8 vs. ATG+αIL6+CTLA4-Ig: 3/8).
Conclusion: ATG and short-term interleukin-6 blockade synergize as induction to prevent rejection under CTLA4-Ig.

DIAGNOSTIC VALUE OF DONOR-DERIVED CELL-FREE DNA TO PREDICT ANTIBODY-MEDIATED REJECTION IN DONOR-SPECIFIC ANTIBODY-POSITIVE RENAL ALLOGRAFT RECIPIENTS

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Circulating donor-specific antibodies (DSA) do not necessarily associate with ongoing antibody-mediated rejection (ABMR). Here, we evaluated the diagnostic value of donor-derived cell-free DNA (dd-cfDNA), as an add-on to DSA detection. The study included two independent cohorts of DSA+ kidney allograft recipients, 45 subclinical cases identified by systematic antibody screening (cohort 1), and 30 recipients subjected to indication biopsies (cohort 2). About 50% of the DSA+ recipients had ABMR and displayed higher dd-cfDNA levels than DSA+ ABMR- recipients (cohort 1: 1.90% [median; IQR: 0.78–3.90] vs. 0.52% [0.35–0.72]; $P < 0.001$); cohort 2: 1.20% [0.82–2.50%] vs. 0.59% [0.28–2.05%]; $P = 0.086$). Receiver operating characteristic (ROC) analysis revealed an area under the curve (AUC) of 0.89 and 0.69 for dd-cfDNA, and 0.88 and 0.77 for DSA mean fluorescence intensity (MFI), respectively. Combined analysis of dd-cfDNA and DSA-MFI did not enhance diagnostic accuracy. The modest diagnostic performance of dd-cfDNA in cohort 2 was related to the frequent finding of other types of graft injury among ABMR- recipients, like T cell-mediated rejection or glomerulonephritis. For dd-cfDNA in relation to injury of any cause an AUC of 0.97 was calculated. Monitoring of dd-cfDNA in DSA+ patients may be a useful surveillance tool to detect ABMR and other types of injury.

CUSTODIOL® SUPPLEMENTED WITH SYNTHETIC HUMAN RELAXIN DECREASES ISCHEMIA-REPERFUSION INJURY AFTER PORCINE KIDNEY TRANSPLANTATION

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Objective: Ischemia-reperfusion injury (IRI) is inevitable after kidney transplantation (KT) impairing outcomes. Relaxin-2 (RLX) is a promising insulin-related peptide hormone, that protects against renal IRI in rodents. Although, large animal models are needed before RLX could be tested in human setting.
Methods: In this blinded, randomized, and placebo-controlled experimental study kidneys from 19 donor pigs were retrieved after perfusion with Custodiol®±RLX (5 or 20 nmol/l) and underwent static cold storage (SCS) for 12 and 24 hours, respectively. Subsequently, KT was performed after unilateral right nephrectomy. Study outcomes included markers for kidney function, oxidative stress, lipid peroxidation, and endothelial cell damage. PCR analysis for oxidative stress and apoptosis-related gene panels as well as immunohistochemistry were performed.
Results: RLX upregulated SOD2 and NFKB expression to 135 % ($P = 0.042$) and 125 % ($P = 0.019$), respectively, while RIPK1 expression was downregulated to 82 % ($P = 0.016$) of corresponding controls. Further RLX significantly downregulated RIPK1 and MLKL expression and decreased the number of Caspase 3- and MPO-positive cells in grafts after SCS.
Conclusions: RLX supplemented Custodiol® significantly decreased IRI via both antioxidant and anti-apoptotic mechanisms. Clinical trials are warranted to implement synthetic human RLX as a novel additive to preservation solutions against IRI.

ORGAN DONATION DEMAND IN AUSTRIA

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Background: Organ donation is the prerequisite for successful organ transplantation programs; however, the true demand of organ donation has not been quantified yet, neither worldwide nor for Austria. The aim of this study was to assess the true need for donor livers in Austria, with specific focus on the "region south" of Austria.
Methods: The "liver donation gap" defined as difference between the number of available donor livers and the number that would be needed to avoid death on the waiting list and removal from the waiting list because of deterioration of the disease was assessed for Austria between 2010 and 2019 from Eurotransplant statistics. A pilot electronic survey was designed and distributed to members of the Austrian Society of Transplantation and the Austrian Society of Gastroenterology and Hepatology, to assess whether perceived organ shortage influences the decision to refer or select patients for the liver transplantation waiting list. To assess the additional need in oncologic indications, data from patients with the diagnosis of hepatocellular carcinoma (HCC) at their initial presentation in the tumor-board of the University Hospital Graz between 01.01.2017 and 31.12.2019 were analyzed.
Results: The analysis of the liver donation gap in Austria showed that in the last 10 years there was a shortage of 5.7 liver donors per million inhabitants. The survey on donor organ shortage showed that up to 10% of the doctors who make decisions about whether patients should be placed on the liver waiting list would include the organ shortage in their decisions and would recommend more patients for the waiting list if there were more organs available. Another 8.4 liver donors per million inhabitants would be necessary to meet the needs in the area of oncological liver transplant indications.
Discussion: Demographic changes and changes in the epidemiology of chronic liver diseases will further increase the demand for liver transplantation in the near future.

Overall, there is a well-functioning organ donation system in Austria. Self-sufficiency—as required by the World Health Organisation (WHO)—is not met yet for liver transplantations at the expense of deaths on the waiting list as well as strict policies for listing patients with oncological indications. 40-45 brain dead organ donors per million population will be necessary to meet the current and near future demand in Austria. Additional measures to optimize the organ donation process, surgical and logistic advances, machine perfusion and the development of donation after cardiac death programs will be necessary to meet the demand. Educational work and continuous evaluation and optimization of organizational structures are relevant management measures to achieve this goal.

ABSTRACT

ALLELE STUDY: A MULTICENTER, OPEN LABEL, PHASE 3 STUDY OF TABELECLEUCEL FOR SOLID ORGAN OR ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANT SUBJECTS WITH EPSTEIN-BARR VIRUS-DRIVEN POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE (EBV+ PTLD) AFTER FAILURE OF RITUXIMAB OR RITUXIMAB AND CHEMOTHERAPY

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Background: Epstein-Barr virus-driven post-transplant lymphoproliferative disease (EBV+ PTLD) is a rare and potentially life-threatening disease in the setting of immunosuppression following solid organ (SOT) or allogeneic hematopoietic cell transplant (HCT). Treatment of relapsed/refractory EBV+ PLTD is inadequate and has poor overall survival, highlighting a clear unmet medical need. Challenges with current therapies include limited efficacy, potential for graft rejection, or graft-vs-host disease (GvHD), and high mortality.

Tabelecleucel is an off-the-shelf, allogeneic EBV-specific T cell therapy. Here we describe the design of an ongoing multicenter study of tabelecleucel for subjects with EBV+ PTLD after failure of rituximab (R) + chemotherapy (CT) which is open for enrolment in Europe.

Trial design: ALLELE is evaluating the benefit/risk of tabelecleucel in SOT patients following treatment with R or R plus CT (N=33) and in HCT patients following treatment with R (n = 33) (NCT03394365).

Key inclusion criteria: biopsy-proven EBV+ PTLD and failure of R + CT.

Tabelecleucel is selected for each patient from an inventory based on an EBV HLA-restricting allele and a second shared allele. During each treatment cycle, tabelecleucel is administered intravenously at a dose of 2x10e6 cells/kg on days 1, 8, 15, followed by observation through day 35. Responses per radiographic and clinical assessment are evaluated at the end of each cycle using modified Lugano criteria. Patients receive additional treatment cycles until they meet end of treatment criteria. A restriction switch (tabelecleucel with different HLA-restriction) is permitted for patients with stable or progressive disease. After ending treatment, subjects are assessed quarterly up to 24 months for disease status, and biannually thereafter, up to 5 years, for survival status. The primary endpoint of the study is overall response rate (ORR), assessed by independent review, following the administration of tabelecleucel.

EPSTEIN-BARR VIRUS-DRIVEN DISEASES: A MULTINATIONAL, MULTICOHORT, OPEN-LABEL PHASE 2 STUDY TO ASSESS THE EFFICACY AND SAFETY OF TABELECLEUCEL USING AN ADAPTIVE STUDY DESIGN

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Introduction: Epstein-Barr virus (EBV) infection is associated with a variety of life-threatening malignant and non-malignant diseases. In immunocompromised individuals, impaired immunosurveillance can result in uncontrolled EBV infection. This may lead to EBV+ immunodeficiency-associated lymphoproliferative disorders (IA-LPD), EBV+ sarcomas, or EBV-associated end-organ damage. EBV-driven LPDs are a heterogeneous group of diseases with variable clinical and pathologic features, including primary IA-LPD (EBV+ PID-LPD), acquired IA-LPD (EBV+ AID-LPD) often associated with HIV infection, and post-transplant lymphoproliferative disorder (EBV+ PTLD). In addition, EBV infection of mesenchymal cells can cause sarcomas, including leiomyosarcoma (EBV+ sarcoma). In rare instances, EBV viremia (ie persistent EBV infection) is associated with life-threatening complications such as chronic active EBV (CAEBV) and hemophagocytic lymphohistiocytosis (HLH).

Tabelecleucel is an investigational, off-the-shelf, allogeneic T-cell immunotherapy without genetic modification. Initial safety and efficacy data showed that tabelecleucel was well tolerated and displayed clinical activity in patients with each of the EBV-driven diseases being proposed in this study (ATA129-EBV-205; Kurlander *et al.* Ann Oncol 2018; Nikiforow *et al* Blood 2020; Torno *et al.* Blood 2020; Prockop *et al.* J Clin Invest 2020). A Phase III study of tabelecleucel for solid-organ or allogeneic hematopoietic cell transplant patients with EBV+ PTLD after failure of rituximab or rituximab plus chemotherapy (NCT03394365) is ongoing.

Methods: This is a multinational, multicohort, open-label, single arm per cohort, Phase II study to assess the efficacy and safety of tabelecleucel in patients with EBV-associated diseases (ATA129-EBV-205; NCT04554914).

Participants with Epstein-Barr virus (EBV)-driven diseases where standard first-line therapy is not appropriate or who are relapsed/refractory to standard first-line therapy will be enrolled in the following cohorts: EBV+ AID-LPD, EBV+ PID-LPD, EBV+ sarcoma (including leiomyosarcoma [LMS]), CAEBV/HLH, EBV+ PTLD with central nervous system (CNS) involvement and first-line inappropriate EBV+ PTLD.

Prior to screening, the availability of tabelecleucel from an existing inventory based on an appropriate HLA restriction and allele profile will be confirmed. Tabelecleucel will be administered in cycles lasting 35 days. At the end of each cycle, responses will be assessed per disease-specific criteria.

An adaptive two-stage design will be used for each cohort in this study, and for each, a maximum of 8 patients will be enrolled in stage 1. The decision to move to stage 2 enrollment for any given cohort will be based on an analysis of the first 8 evaluable patients in the cohort using investigator's assessment (per disease-specific response criteria).

MUSEKAL – ASSESSING TOLERABILITY AND EFFECTIVENESS OF EXTENDED-RELEASE TACROLIMUS (LCPT) IN KIDNEY TRANSPLANT PATIENTS CONVERTED FROM OTHER TACROLIMUS FORMULATIONS

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Available tacrolimus (tac) formulations exhibit substantial inter- and intra-individual variability in absorption and metabolism leading to various difficulties in long-term maintenance of immunosuppression. LCPT was shown to maintain stable target trough levels (TL) at reduced total daily dose (TDD) compared to other tac formulations. This study assessed tolerability and effectiveness of LCPT in kidney transplant (Tx) patients (pts) converted from other tac formulations in real-life.

Methods: The study was conducted in Austria and the Czech Republic. Adults received LCPT per approved label and local clinical routine. Written informed consent was obtained. Safety and efficacy were assessed during 6 months of observation. Variables relevant to conversion pts are presented, i.e. tac TL, tac TDD and C/D ratio over time, number of dose adjustments and kidney function; reported timepoints are medians across individual patients' schedules. Adverse drug reactions (ADRs) were summarized using the MedDRA dictionary. Data collection period was 07/2016–08/2019.

Results: A total of 42 pts were analysed before and after conversion; pts were predominantly male (66.7%, $n = 28$) and <65 years old (78.6%, $n = 33$); 31 pts had no pre-existing abnormalities, 6 experienced tremors. After conversion to LCPT, TDD decreased over time from 4 mg at baseline to 2.25 mg at day 55 and beyond (median 54.0% decrease between initiation and day 180, $p = 0.002$, $n = 14$) while TL remained stable and consistently above 5 ng/mL (−10.0% between initiation and day 180, $p = 0.346$, $n = 14$). C/D ratio improved from 1.8 ng/mL*1/mg at day 7–2.8 ng/mL*1/mg at day 88 and 117 (25.7% median increase between initiation and day 180, $p < 0.198$, $n = 14$). Kidney function remained stable with eGFR at 50 ml/min/1.73 m^2 throughout the study (0.0% change, $p = 0.508$). LCPT was well tolerated with 6 ADRs in 4 pts (leukocytopenia, otitis media, diarrhoea [$n = 2$], CMV reactivation, febrile pulmonary infection); one serious ADR was reported (mild febrile pulmonary infection, no discontinuation of LCPT required).

Conclusions: After conversion to LCPT, target TL were maintained while the initial TDD could be significantly reduced during the first 3 weeks with few dose adaptations and remained stable thereafter. Improvements in C/D ratio also indicate a higher bioavailability of LCPT. Excellent tolerance, minimal dose adjustments and robust kidney function after conversion to LCPT indicate both practicability and safety of this strategy in renal Tx pts.

MUSEKAL – A MULTI-CENTRE NON-INTERVENTIONAL STUDY TO ASSESS THE TOLERABILITY AND EFFECTIVENESS OF EXTENDED-RELEASE TACROLIMUS (LCPT) IN DE NOVO LIVER TRANSPLANT PATIENTS

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Background: Available formulations of tacrolimus (tac) exhibit substantial inter- and intra-individual variability in absorption and metabolism. In clinical trials, LCPT achieved target trough levels faster and reduced the total daily dose needed to maintain therapeutic levels compared to other tac formulations. The present non-interventional study aimed to assess tolerability and effectiveness of LCPT in de novo hepatic allograft recipients in real-life.

Methods: The study was conducted in Austria and the Czech Republic. Patients ≥18 years received LCPT per approved label and local daily clinical routine. All participants signed informed consent. Tolerability and effectiveness were assessed in seven clinical visits during six months observational period. De novo patients are presented here with relevant clinical variables, i.e. tac trough level (TL), tac total daily dose (TDD) from day 1 to 6 months, number of dose adjustments; time (days) to standard reference range; kidney and liver function. Adverse drug reactions (ADRs) were summarized using the MedDRA dictionary. The data collection period was 07/2016–08/2019.

Results: A total of 70 de novo liver transplant patients were analysed; patients were predominantly male (72.9%, $n = 51$) and <65 years old (85.7%, $n = 60$). The mean time to achieve tac target TL was 6.4 days (SD 4.6) after a mean of 4 (SD 3.9) dose adjustments; thereafter, TL remained stable at around 8 ng/ml throughout observation. TDD was at 9 mg at day 7, remained stable at around 8 mg up to day 60, then dropped to 5 mg by day 180. Liver function continuously improved with AST rapidly decreasing until day 21, then stable at around 25 U/L, ALT rapidly decreasing until day 14, then stable at around 20–25 U/L, and GGT increasing between day 1 and 7 then steadily decreasing to around 40–50 U/L. Kidney function remained stable at around eGFR 50 ml/min/1.73 m^2 until the end of the study period. LCPT was well tolerated with 21 adverse events (14 drug related) in 5 patients (mostly mild renal insufficiency and haematological adverse events); two serious unrelated adverse events were reported (moderate atrial flutter, severe abnormal hepatic function).

Conclusions: After LCPT initiation in de novo liver transplant-patients, tac target TL were rapidly achieved and initial TDD was reduced after day 60 with few dose adaptations. Liver function rapidly improved, while kidney function remained normal. LCPT was well tolerated in this population.

ABSTRACT

TORQUE-TENO VIRUS LEVEL IN KIDNEY TRANSPLANT RECIPIENTS RECEIVING EXTENDED RELEASE AND TWICE-DAILY TACROLIMUS BASED IMMUNOSUPPRESSION – A SECONDARY ANALYSIS OF THE PROSPECTIVE TTV-POET TRIAL

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Background: Kidney transplantation is the treatment of choice for chronic end stage renal disease. Potent immunosuppressive treatment has led to substantial prolongation in short-term allograft survival in recent decades, but long-term performance has not improved in the same way. Decline of allograft function is mainly cause by chronic graft damage due to allo-recognition and subsequent immunologic response. Thus, optimization of immunosuppressive regimens represents the key for maximization of allograft survival. Besides personalization of the amount and type of immunosuppression, improvement of medication adherence is the second pillar of intervention. Recent non-interventional studies have linked extended-release tacrolimus (ER-TAC) formulations and reduced biopsy-proven acute rejection (BPAR) in kidney transplant recipients (KTR) compared to immediate-release formulation (IR-TAC). Moreover, ER-TAC has been reported to increase convenience and self-reported adherence among KTRs when compared to IR-TAC. This study was designed to analyze the association between TAC formulations and the level of Torque Teno virus (TTV – a non-pathogen and ubiquitous virus, which reflects the strength of the immune system of its host) in the peripheral blood of kidney transplant recipients.
Hypothesis: Immunosuppression is optimized by ER-TAC. Thus patients with ER-TAC based immunosuppression following kidney transplantation have higher levels of TTV.
Methods: In this secondary analysis of a prospective trial (TTV-POET, local institutional review board approval number: 1785/2016, German Clinical Trials Registry number: DRKS00012335) all 320 consecutive recipients of a kidney allograft transplanted between January 1, 2016 and December 31, 2017 at the Medical University of Vienna (MUV) were screened. Inclusion criteria for the present analysis were TAC-based immunosuppression and follow up at the outpatient clinic of the MUV at least until day 94 post-transplant. Exclusion criteria were no TTV measurement and data concerning TAC formulation at any monitoring time point, respectively. TTV was quantified prospectively per protocol in the peripheral blood at the following predefined time points: before transplantation and after transplantation once per week until discharge from the ward, on the first visit at the outpatient clinic, on month 3 after transplantation, and every 3 months thereafter. Treating physicians were unaware of the TTV results. TTV levels were quantified by RT-PCR in the plasma. Patients were followed for 2 years after transplantation. For the actual analysis only time-points after TTV reached stable levels (>day 93 post-transplant) were included. Patents were grouped according to ER-TAC and IR-TAC based immunosuppression. Generalized linear models were used to estimate the effect size of the association between TAC formation and TTV.
Results: For this study 282 patients with TAC based immunosuppression and data on TAC formulation and TTV were still followed at our outpatient center after the end of month 3 post-transplant and were enrolled in the current analysis. Median recipient age at the time of transplantation was 51 years and 42% were female. In total 2392 TTV measures were available. Forty-eight (17%) patients received an ER-TAC formulation based immunosuppression. TTV was quantified at a median of 300 days post-transplant (A± SD 235 days) and median levels in the plasma were 1.1 × 106 c/ml. Patients receiving ER-TAC showed lower TTV levels compared to patients receiving IR-TAC (8.8 × 105 c/ml, IQR 2.7 × 104–5 × 107 c/ml vs. 3.4 × 106 c/ml, IQR 1.4 × 105–2.8 × 108 c/ml; $p < 0.001$). The odds for receiving IR-TAC increased by 5% per log level of TTV c/mL (OR 1.05 95% CI 1.03–1.07, $p < 0.001$). However, patients on IR-TAC had higher TAC trough levels at the day of TTV assessment and TTV levels were quantified earlier post-transplant. As both of these factors are known to associate with higher TTV levels multivariate modeling was applied to test whether TTV was associated independently with TAC formulation. Indeed most of the association between TAC formulation and TTV level was explained by the timing of TTV assessment and TAC through level at the day of TTV quantification. Multivariate modeling suggests no association between TTV levels and TAC formulation: the odds ratio adjusted for recipient sex and age, days post-transplant of TTV measurement and TAC through level at the day of TTV assessment was 1.0 (95% CI 0.97–1.03).
Conclusion: ER-TAC dosing is not associated with higher TTV levels in renal transplant recipients. Our data do not support a more intense immunosuppression in patients with ER-TAC based immunosuppression compared to IR-TAC.

LAPAROSCOPIC LIVING KIDNEY DONATION AT A SINGLE CENTER: LONG-TERM FOLLOW-UP

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Laparoscopic living donor nephrectomy replaced open donor nephrectomy across the world.
Its advantages in patient recovery are well known. This longitudinal single center study was carried out to evaluate the safety of nephrectomy as well as further surgical outcomes among donors.
Patients and Methods: We collected and analyzed living donor nephrectomis from July 2015 till June 2021 at the OKL Elisabethinen in Linz. 79 donors were included, criteria of exclusion were lost to follow-up or lack of complete data. Prenephrektomy measures were body mass index, hypertonia, kidney height, number of arteries, GFR, scintigraphy of left and right side. Operative times as time of anastomosis and operation, warm and cold ischaemia, arteria reconstructions, unusual venous anatomy, conversions and postoperative complications were analyzed. The mean age at the time of nephrectomy, relationship between donor and recipient, laparoscopic versus open nephrectomy, median duration of hospital stay, postoperative kreatinine, pain and complications, reoperations, interventions and long term follow-up of donors was listed.
Results: Of all 79 donors were 69.6% female and 30.4% male, median age at the time of nephrectomy was 53.3 years, 9.48% had a mild hypertonia, kidney height was normal. The median body mass index was 26.22, the median initial glomerular filtration rate was 91 ml/min/1.73 mA². 10.27% right open versus 89.73% left laparoscopic kidneys were removed. Warm ischaemia was median 135 seconds, cold ischaemia 64 minutes. Operation time in right open kidneys was 117 minutes, in left laparoscopic kidneys 167 minutes. Anastomosis time median was 26 minutes. In 7.11% an arterial reconstruction because of pol arteries or two or three arteries was necessary. 3.95% had a left renal vein behind the aorta. Postoperative mortality was zero, 4.74% needed a reoperation because of haematoma subcutan (3), haematoma intraabdominal (2), or splenectomy (1). 13.43% were ABO incompatible donors, in 7.11% it was the second transplantation for the recipients. During 5 years follow-up, 89% attended all recommended visits. All of them had a stable renal excretory function, 1 donor died in 2020 because of SAB, she became an organ donor. 2 donors had SARS-Cov-2 in 2020 without hospital stay.
Conclusions: There are no really long-term health risks associated with living kidney donation when they are carefully selected.

LAPAROSCOPIC LIVING DONOR KIDNEY TRANSPLANTATION IN LINZ DURING THE PANDEMIC OF SARS-COV-2

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Introduction: Transplant patients are more susceptible to viral and bacterial infects because of their immunosuppression. A potential deadly new virus haunted us 2020: SARS-CoV-2.
Patients and Methods: After the complete lockdown in Spring 2020 our transplant Center in Linz continued the living donor laparoscopic nephrektomy. 5 living kidney transplantations have been carried out from June 4 to September 9, 2020. We matched these data with the numbers in the three previous years.
Results: From June 4, 2020, till September 9, 2020, five kidney living donor transplantations have been performed. All donors and recipients have been screened for COVID 19 infection. They were fully informed about risks of surgery and immunosuppression during the pandemic. All the recipients and donors remained COVID negative during the follow-up. Now all of them are already vaccinated. The number of living transplants has been constant to the same months of 2017, 2018 and 2019.
Conclusion: Living donor kidney transplantation should be continued. Donors and recipients should be carefully selected. They have to be informed about all risks.

KIDNEY TRANSPLANTATION IN COMBINATION WITH VASCULAR REPAIR – POSSIBILITIES AND LIMITATIONS. PRESENTATION OF THREE CASES AND A RETROSPECTIVE ANALYSIS OF A SIMPLIFIED ILIAC CALCIFICATION SCORE

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In the clinical routine of renal transplantation peripheral vascular disease is a frequent comorbidity and severe iliac artery calcification may be a limiting factor. In some cases even surgical vascular reconstruction is necessary in the course of renal transplantation.

We reviewed the records of patients transplanted in our center in the year 2011 "to reach a 10 year follow up period" for concomitant vascular reconstructions. Three cases very much representing the possibilities and limitations of vascular surgery in connection with renal transplantation took place within this one year period and are presented in the following.

The first patient was transplanted for the 3rd time. Both nonfunctioning renal grafts were still in situ. During resection of the old transplant the external iliac artery disintegrated and had to be resected over a length of 4 cm. It was reconstructed with a Dacron tube. The new arterial anastomosis was placed within the Dacron graft. The further course of this patient was uneventful for the next 7 years. However, in the year 2018 the patient died after 6 months of palliative chemotherapy suffering from a metastasized pancreatic carcinoma.

The second Patient was also transplanted for the 3rd time. However on both sides a transplant nephrectomy was performed. Intraoperatively a resection of the common and external iliac vein as well as the external iliac artery was necessary due to calcification and scaring of the transplant remnant. The new kidney graft was anastomosed to the arterial and venous Dacron grafts used for reconstruction. Five days later thromboses of the venous outflow occurred. Surgical revision with cold perfusion of the kidney graft and thrombectomy of the renal vein was completed with transposition of the venous anastomosis to the inferior vena cava as bail out procedure. Unfortunately, on the next day rethrombosis with rupture of the graft and severe bleeding with the need for emergency graft resection appeared.

The third patient had an uneventful primary renal transplantation into the left iliac fossa. Yet 4 month later he presented himself with an enlarging false aneurysm at the site of the arterial anastomosis. During the operative correction, severe bleeding from the common iliac vein occurred caused by the callosity around the aneurysm. After fast disconnection and cold perfusion of the kidney graft, the extern iliac artery and vein were resected and replaced by PTFE tubes. Finally the kidney was reconnected to these PTFE tubes. At 10 years of follow-up the graft is still functioning well. The patient's creatinine level is currently at 1.1 mg/dl.

These cases highlight the importance of iliac calcification as a risk factor in renal transplantation. However, over the last decades the age and comorbidities of the patients assigned for renal transplantation increased and retransplantations became more frequent.

For risk prediction and patient selection several sores have been established to quantify the calcification of the iliac arteries. We used the simplified iliac calcification score to retrospectively compare the patient cohorts of 2004 and 2019, analyzing the development of patient characteristics over a period of 15 years.

Mean age at transplantation was 44.5 years in 2004 and increased to 52.2 years 15 years later, this difference was highly significant $p < 0.027$. The calcification score of the internal iliac artery was higher within the cohort of 2019 compared to 2004 (1.03 vs. 0.75 in 2004, $p = 0.304$). The score of external iliac artery calcification also increased (0.63 vs. 0.33, $p = 0.189$), however these differences reached no statistical significance.

Further analysis of the iliac calcification score concerning complications and graft and patient parameters will be presented.

ABSTRACT

LUNG TRANSPLANTATION FOR ACUTE RESPIRATORY DISTRESS SYNDROME: A MULTICENTER EXPERIENCE

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Acute respiratory distress syndrome (ARDS) is a rapidly progressive lung disease with a high mortality rate. Although lung transplantation (LTx) is a well-established treatment for a variety of chronic pulmonary diseases, LTx for acute lung failure (due to ARDS) remains controversial. We reviewed post-transplant outcome of ARDS patients from three high-volume European transplant centers. Demographics and clinical data were collected and analyzed.

Viral infection was the main reason for ARDS (n = 7/13, 53.8%). All patients were admitted to ICU and required mechanical ventilation, 11/13 were supported with ECMO at the time of listing. They were granted a median LAS of 76 (IQR 50–85) and waited for a median of 3 days (IQR 1.5–14). Postoperatively, median length of mechanical ventilation was 33 days (IQR 17–52.5), median length of ICU and hospital stay were 39 days (IQR 19.5–58.5) and 54 days (IQR 43.5–127). Prolongation of peripheral postoperative ECMO was required in 7/13 (53.8%) patients with a median duration of 2 days (IQR 2–7). 30-day mortality was 7.7%, 1-year and 5-year survival rates were calculated as 71.6% and 54.2%, respectively.

Given the lack of alternative treatment options the herein presented results support the concept of offering live-saving LTx to carefully selected ARDS patients.

THE EFFECT OF PRONE POSITIONING AFTER LUNG TRANSPLANTATION - A SINGLE CENTER EXPERIENCE

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Background: Prone positioning (chest/face down and back up) became a standard treatment for acute respiratory distress syndrome (ARDS) patients to improve impaired gas exchange. The aim of this study was to evaluate the potential benefit of prone positioning after lung transplantation (LTx) in patients with impaired primary gas exchange.
Methods: We retrospectively analyzed LTx recipients transplanted between 01/2014 and 12/2019 (n = 553). Of whom, 165 patients (29.8 %) were placed in prone position. Demographics and clinical data of these patients were collected. A subgroup analysis was performed for patients who were placed in prone position on prolonged extracorporeal membrane oxygenation (ECMO).
Results: During the study period 155 (28%) patients were placed in prone position immediately after LTx for a median of 19 (15–26) hours. Patients requiring prone positioning were mainly suffering from idiopathic pulmonary fibrosis (IPF) with a mean age of 44.3 (A $\pm$ 1.4) years. Before prone position, median PO2/FiO2 (P/F ratio) was 179 (120–280) mmHg and median dynamic lung compliance (Cdyn) was 24.1 (18.3–30.6) ml/cmH2O. Both parameters significantly increased after proning - median P/F ratio increased to 353 (255–414; $P < 0.0001$) mmHg and median Cdyn to 28.3 (21.3–35.2; $P = 0.008$) ml/cmH2O. Forty one patients were placed in prone position while being supported by postoperatively prolonged femoro-femoral veno-arterial (VA) ECMO). No complications related to prone positioning (such as kinking of ECMO lines, dislocation of ECMO cannulas) were reported. Further, in this subgroup, P/F ratio (148 (81.3–219.3) mmHg to 317 (153.9–403.3) mmHg; $P = 0.0002$) and Cdyn (16.6 (12.2–26.4) ml/cmH2O to 21.8 (14.6–29.8) ml/cmH2O; $P = 0.05$) improved significantly by proning. Nevertheless, length of mechanical ventilation, length of intensive care unit (ICU) and hospital stay were significantly longer with a median of 2 (1.8–9.3), 12 (7–29) and 35 (21–53) days in the proning group compared to 1.4 (0.9–2.8), 7 (4–13) and 25 (18–37) days in the non-proning group (all $P < 0001$).
Conclusion: To conclude, we could demonstrate in this study, that prone positioning after LTx in patients with a complex postoperative course, significantly improves oxygenation. Our results demonstrate that placing patients in prone positioning after LTx with prolonged ECMO support is feasible and can be safely performed. Further studies are needed to confirm our findings.

COMPARISON OF MRNA-1273 AND BNT16B2 SARS-COV-2 MRNA VACCINE IMMUNOGENICITY IN KIDNEY TRANSPLANT PATIENTS

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Background: Kidney transplant patients are at high risk for severe COVID-19 disease or death associated with COVID-19. However, rates of seroconversion after SARS-CoV-2 vaccination are low in this population. Here, we analyzed whether rates of seroconversions differ between the two currently available mRNA-based SARS-CoV-2 vaccines in kidney transplant recipients.
Methods: In this retrospective cohort study involving 300 prevalent kidney transplant patients, anti-Spike (S) protein IgG antibody titers were measured (Abbott Architect assay) 3 to 6 weeks after administration of the second dose of either mRNA-1273 or BNT162b2 SARS-CoV-2 vaccine.
Results: Median time for antibody measurement was 28 days (IQR 26–33 days). In the overall cohort, anti-S-antibody positivity was detected in 50% of patients. After correction for age, diabetes status, sex, serum albumin and serum creatinine, the odds ratio for anti-S-antibody seroconversion was significantly higher ($P = 0.02$) for mRNA-1273 vaccinated patients compared to BNT162b2 (odds ratio: 2.07, 95% confidence interval: 1.11–3.86). Results were similar after exclusion of patients with a history of SARS-CoV-2-infection ($N = 20$; OR: 2.08, 95% CI: 1.09–3.94).
Conclusion: In conclusion, vaccination with mRNA-1273 confers 2.07-fold higher odds of anti-S-antibody seroconversion than vaccination with BNT162b2 in prevalent kidney transplant patients.

HEALTH RELATED QUALITY OF LIFE, WORKABILITY AND RETURN TO WORK OF PATIENTS AFTER LIVER TRANSPLANTATION

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Background: Health-related quality of life (HrQoL) and workability are related parameters to measure success of therapy. Both are insufficiently explored in patients after liver transplantation (LT). Particularly little is known about the attitude to return to work, employment status before LT, and how frequently there is any employment at any time after LT. Thus, this study was designed to evaluate these parameters.
Methods: This is a single-center retrospective cohort study including 150 adult outpatients after LT. Liver transplantations had been performed between 1993 and 2018. The study was carried out from February to July 2018. Exclusion criteria were combined transplantations, positive screening for current alcohol abuse, and anxiety or depression. To evaluate HrQoL and fitness to work the patients were tested with the Short-form-36, the Chronic Liver Disease Questionnaire and the Workability Index.
Key-results: Return rate of sufficiently filled in questionnaires was 46.8% (66 patients). The mean age of patients was 59.9 years (SD = 10.8), ranging from 25 to 78 years. HrQoL was partly comparable to the normal population. Workability sum scores with a mean value of 31.61 (SD 9.79) suggested moderate workability at present. While 28.8% of respondents were ever employed after LT, 45.5% currently wished to work or would have wished to work.
Conclusions: This study is the first to assess subjective workability, attitude to return to work, and if any employment after LT was reported. Access to employment for those who wish to work could contribute to further improve HrQoL following transplantation.

ABSTRACT

FRAMEWORK FOR SOLID ORGAN TRANSPLANTATION IN EUROPE DURING COVID-19 PANDEMIC

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Introduction: Since the effect of COVID-19 pandemic on solid organ transplantation (SOT) is unclear, an online survey on the specific framework of leading European transplant centers ($n = 155$) in 31 European countries was conducted between April 24–May 15, 2020.
Methods: A questionnaire was designed to collect information on (i) restrictions in SOT, (ii) protective measures, (iii) (non-) governmental information policies, and (iv) individual opinions on how to deal with SOT during COVID-19 pandemic.
Results: The response rate was 37.4% (58/155). (i) Overall, in 84.5% an effect of COVID-19 pandemic on SOT in Europe was reported. In 49% of these a limited capacity was mentioned, in 51% reasons for restricted resources were strategic preparedness. As a result, SOT was totally or partially suspended for several weeks. (ii) 93.1% of centers implemented protective measures against COVID-19. (iii) (non-) governmental information policies were felt to be adequate in 90%. (iv) A continuation of transplant activities was desired by 97% of centers.
Conclusion: Results of this survey suggested the need of more ICU-capacities during COVID-19 pandemic in order to guarantee adequate and timely treatment of other patient cohorts in surveyed countries.

IMPLEMENTATION OF A MOBILE APPLICATION FOR OUTPATIENT CARE AFTER LIVER TRANSPLANTATION

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Background: In the face of the Covid-19 pandemic and the need for social distancing new therapeutic tools like mobile health applications might gain in importance for outpatient care. Objective of the present study was to assess if and to what extent the implementation of a free available transplant application in a cohort of liver transplant recipients was feasible.
Methods: Patients of the aftercare program at the Department of Transplant Surgery Graz in June 2016, were first asked to complete a survey concerning knowledge about mobile health and their management of everyday life. After using the application for 2 months a second survey evaluated whether the implementation of the application in the daily routine was feasible.
Results: Among 135 patients, 124 (91.9%) agreed to participate. Seventy-one (57.3%) owned a mobile device with which they could use the application, 42 patients (33.8%) decided to try it out for 2 months. The majority stated that the application supported them for therapy management and surveillance of vital parameters. Successful implementation of the application was feasible in 57.1% of patients after 2 months testing period.
Conclusion: The technical prerequisites are only partially met and should be improved. Older patients need extensive support and motivation.

EFFECT OF WAITING TIME ON MORTALITY AFTER SECOND KIDNEY TRANSPLANTATION

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Background: The median half-live of kidney transplants is about 10–15 years. As the availability of donor organs is limited it is of valuable knowledge, when a second transplantation shows an increase of life span. Therefore we compared patients on waiting list with patients who received a second transplant.
Methods: As such a comparison done with conventional methods inherits some bias like the lead time bias, we used a recently developed method called target trial emulation for the statistical analysis. Data used in our study originated from the Austrian Dialysis and Transplant registry as well as from Eurotransplant. We included 2346 patients who received a second transplantation in the years 1980–2019 and were older than 18 years. The analysis of patient survival was adjusted for sex, dialysis vintage, age at transplantation, year and duration of first transplantation as well as time to wait listing for the second transplantation.
Results: After second transplantation, the restricted mean survival time was increased at 10 years compared to patients remaining on waiting list (5.8 months, 95 % confidence interval 0.9–11.1). However, this time span decreased, when the patients were longer on waiting list and were virtually not present at all after more than 8 years on waiting list (0.1 month, 95 % confidence interval -14.3–15.2).
Conclusion: These data let us conclude that a second kidney transplantation leads to prolonged patient survival when compared to treatment by dialysis on the waiting list. But the difference between the two groups diminishes with longer waiting time. Though the quality of life usually increases which might be an argument for retransplantation.

2021 REPORT OF THE GUARDIAN REGISTRY: AN INTERNATIONAL CONSORTIUM EXAMINING THE EFFECT OF CONTROLLED HYPOTHERMIC PRESERVATION IN HEART TRANSPLANTATION

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Methods and Results: The Global Utilization And Registry Database for Improved heArt preservatioN (GUARDIAN) study is an international multi-center retrospective-prospective registry assessing the impact of organ preservation technology on clinical outcomes after heart transplantation. An analysis of 164 patients from 8 global centers, including 151 receiving donor hearts preserved with the Paragonix SherpaPak Cardiac Transport System (PGNX) and 113 patients receiving donor hearts transported by conventional preservation methods (ICE). Baseline characteristics were similar in the two groups with the exception of travel distance to donor organ (ICE = 418 km vs PGNX = 704 km, $P < 0.001$) and total ischemic time (ICE = 184 min vs PGNX = 213 min, $P < 0.001$). Peri-operatively, the PGNX group saw a reduction in cardioversion (ICE = 25% vs PGNX = 14%, $P = 0.02$) and post-operatively, a reduction in severe PGD (ICE = 14% vs PGNX = 6%, $P = 0.02$).
Conclusions: Despite longer ischemic times, the Paragonix cohort experienced favorable peri- and post-operative outcomes. The Paragonix SherpaPak Cardiac Transport System appears to be a safe and effective preservation method and further study is warranted.

ABSTRACT

BASELINE LUNG ALLOGRAFT DYSFUNCTION AFTER LUNG TRANSPLANTATION IS NOT ASSOCIATED WITH DONOR FACTORS

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Background: After lung transplantation (LTx), lung function usually increases over the first 12 months. The peak forced expiratory volume (FEV1) reached within this first year serves as the baseline value for the further clinical course and for CLAD diagnosis. However, in a number of patients FEV1 fails to increase to adequate levels and remains below 65% of the predicted value, a condition termed baseline lung allograft dysfunction (BLAD). Risk factors of BLAD and how it impacts survival and CLAD diagnosis are still poorly understood.
Methods: We analysed patients who received primary double lung transplantation between January 2010 and December 2018 at our center. Lobar transplantations, pediatric patients and patients lost within the first 12 months were excluded. Post-transplant lung function trajectories were analyzed. Patients who failed to reach normal lung function (defined as FEV1 > 65% of the predicted value) formed a BLAD group. A control group consisted of patients who achieved adequate FEV1-values. Demographics and outcome factors including length of mechanical ventilation, PGD and graft survival were compared. A binary logistic regression analysis of donor parameters was performed to determine risk factors of BLAD.
Results: Of 598 patients included in the study, 77 (12.9%) did not reach an adequate lung function. Patients in group I reached their best FEV1 sooner (median 61 days; IQR: 15-282) compared to the control group (median 296 days; IQR: 113-613) ($P < 0.001$). Intraoperative factors of both groups were comparable. Patients in the BLAD group required ECLS bridging ($P = 0.040$) and postoperative prolonged ECMO ($P < 0.001$) more often. Rates of PGD 3 at T72 were comparable at all time points (2.7% vs 1.8%; $P = 0.643$). Median length of mechanical ventilation (48 hours vs 34 hours; $P = 0.003$) and median ICU stay (8 days vs 6 days; $P = 0.002$) were significantly longer in BLAD patients. In contrast, long-term graft survival was similar (5 years: 87.1% vs 84.4%, Log Rank: $P = 0.554$). None of the donor parameters included in the regression model (donor age, BMI, paO2, paCO2, Oto score, smoking >20 pyrs and organ ischemic time) reached statistical significance.
Conclusion: Although BLAD may complicate the diagnosis of CLAD, it was not associated with impaired long-term outcomes. Donor quality was not associated with BLAD but affected patients had a complicated perioperative course more often than control patients.

RETROSPECTIVE MONOCENTRIC STUDY OF RUXOLITINIB COMPASSIONATE USE FOR ACUTE, CHRONIC AND OVERLAP GRAFT-VERSUS-HOST DISEASE (GVHD), INCLUDING GVHD AFTER INTERVENTIONAL DONOR LYMPHOCYTE INFUSION

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Introduction: Ruxolitinib (RUX) has demonstrated safety and efficacy in acute and chronic GVHD. Approvals for both indications by FDA and EMA are anticipated. However, overlap GVHD and donor lymphocyte infusion (DLI) -induced GVHD were excluded from the pivotal phase 3 trials.
Methods: We report a retrospective monocentric study of RUX compassionate use in 68 patients (median age, 51.9 [19.4-73.6 years] after hematopoietic stem cell transplantation for hematological malignancies for treatment-refractory acute ($n = 28$), chronic ($n = 33$) and overlap ($n = 7$) GVHD, of which 21.4%, 33.3% and 14.3% were DLI-induced, respectively. Primary endpoint was best response after initiation of RUX. Secondary endpoints included current response/response at last follow-up feasibility of steroid taper, duration of RUX treatment, overall survival after initiation of RUX, cytomegalovirus (CMV) reactivation during RUX. Treatment lines prior to RUX and beyond RUX were assessed.
Results: Acute GVHD (aGVHD) as indication for RUX was grade II (17.9%), grade III (71.4%) or grade IV (10.7%); and in overlap GVHD it was grade I (57.1%), grade II (28.6%) or grade III (14.3%). Chronic GVHD (cGVHD) as RUX indication was mild (9%), moderate (45.5%) or severe (45.5%); in patients with overlap GVHD it was mild (14.3%), moderate (71.4%), or severe (14.3%). Patients with aGVHD received 1 (60.7%), 2 (21.4%), 3 (10.7%), or >3 (7.2%) lines of GVHD-treatment before starting RUX. Patients with cGVHD received 1 (21.2%), 2 (33.3%), 3 (27.3%), or >3 (18.2%) prior treatment lines. In overlap GVHD, 1 (42.9%), 2 (42.9%), or 3 (14.3%) treatment lines were given prior to RUX. Efficacy population was defined by RUX treatment for more than 14 days, or until death. Sixty-six patients were evaluable for efficacy. Best response following initiation of RUX in aGVHD was complete remission (CR) (63%), partial remission (PR) (7.4%) and less than PR (29.6%). Best response in cGVHD was CR (21.9%), PR (50%), stable disease (SD) (15.6%), mixed response (MR) (9.4%) and progressive disease (PD) (3.1%). In patients with overlap GVHD, RUX initiation resulted in CR (57.1%), PR (28.6%), or PD (14,3%). Responses in DLI-associated GVHD (80% CR and 20% PR in response-evaluable patients with aGVHD post DLI, $n = 5$; and 9% CR and 36% PR in cGVHD post DLI, $n = 11$); were similar to those in the non-DLI associated setting. The median duration of RUX intake was 5.1 months in aGVHD, 20.2 months in cGVHD, and 20.6 months in overlap GVHD. Steroid taper during RUX treatment was feasible, with a median methylprednisolone equivalent decreasing from 1.5mg/kg to 0.0mg/kg in aGVHD, 0.1mg/kg to 0.0mg/kg in cGVHD, and 0.3mg/kg to 0.0mg/kg in overlap GVHD. Overall survival probabilities 2 years after initiation of RUX were 66% for aGVHD, 86% for cGVHD, and 86% for overlap-GVHD.
Conclusion: Our study confirms in a real-world setting that RUX is an effective treatment for steroid-resistant/dependent acute and chronic GVHD. Adding to previous evidence from the phase 3 studies, RUX can also be expected to be effective in situations such as advanced GVHD therapy line (≥ 3rd line), DLI-induced GVHD, and overlap GVHD.

MELATONIN AND GLYCINE PROTECT UTERUS AGAINST ISCHEMIA/ REPERFUSION INJURY IN A RAT MODEL

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Background: Uterus transplantation (UTx) is the first and only available treatment for women with absolute uterine factor infertility. Up to date, there is a limited number of successful UTx with livebirth and the majority was achieved with live donors. Ischemia/reperfusion injury (IRI) remains a significant problem to be solved in UTx. Melatonin and glycine have been shown to possess direct cytoprotective activities mainly due to antioxidative and anti-inflammatory properties. The aim of this study was to investigate the protective effects of melatonin and glycine and their combination on IRI in a rat model of UTx.
Methods: Eighty rats were assigned to eight groups, including sham and IRI (n = 10/group). Prior to 1 h of uterus warm ischemia followed by 1 h of reperfusion rats were treated with melatonin (50 mg/kg, via gavage 2 h before intervention) and glycine (as enriched food diet for 5 days prior intervention) alone or their combination. Uterus IRI was evaluated by histology, including immunohistochemistry, and biochemical tissue analyses.
Results: Histologically uterus IRI was significantly attenuated by pretreatment with melatonin (P = 0.019) and glycine (P = 0.044) alone as well as their combination (P = 0.003). Increase of myeloperoxidase expression after IRI was significantly reduced by melatonin (P = 0.004), glycine (P < 0.001) or their combination (P < 0.001). The decline in superoxide dismutase activity was significantly diminished in the melatonin (P = 0.027), glycine (P = 0.038) and combined treatment groups (P = 0.015) when compared to the IRI control group.
Conclusions: Melatonin, glycine and their combination significantly reduce oxidative stress induced cell damage after IRI in a small animal UTx model. Further clinical studies are mandatory to evaluate the protective effects of these well characterized substances in uterus IRI.

MANAGEMENT OF PREGNANCY-INDUCED ALLOSENSITIZATION IN HEART TRANSPLANT PATIENTS

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Background: Allosensitization represents a growing problem in heart transplantation. The leading causes for sensitization are previous blood transfusions, pregnancy, transplantation and cardiac surgery. Presence of antibodies can lead to severe early antibody-mediated rejection and worse outcome after heart transplantation. In this analysis, we evaluate allosensitization due to pregnancy in heart transplant patients.
Methods: We reviewed our internal database for all cases between September 2013 and July 2021, where additional human leukocyte antigen (HLA) analysis of children was used to assess allosensitization in adult female patients prior to heart transplantation. Records were reviewed for panel-reactive-antibody test results (PRA), presence of pregnancy-induced HLA alloantibodies before and after transplantation, unacceptable antigens and episodes of antibody-mediated rejection 2 (pAMR 2).
Results: Between Sep. 2013 and July 2021, 73 adult female patients received orthotopic heart transplantation at our institution. In 19 cases we performed additional HLA typing of the recipient´s children or the child´s father. Even though only six patients had positive PRA testing with a median PRA of 27%, a total of 15 patients showed presence of pregnancy-induced alloantibodies. In 9 cases we defined unacceptable antigens prior to transplantation. In 5 cases, HLA typing of children was only available on the day of transplantation or afterwards. Eight patients in this group of allosensitized recipients suffered from an episode of pAMR 2 after a median time of 19.5 days. Analyzing the course of pregnancy-induced HLA antibodies showed a sharp increase of alloantibodies at the time of rejection in 4 patients. The other 4 patients had a constant high level of HLA alloantibodies before and after transplantation with no detectable trend at time of rejection. Treatment for pAMR 2 was initiated in addition to our standard immunosuppressant regimen and involved immunoadsorption plasmapheresis followed by repeated cycles of photopheresis in 3 cases, only immunoadsorption plasmapheresis in 2 cases, intravenous IVIG in 1 case and treatment with the monoclonal CD38 antibody daratumumab in 2 cases. Survival and freedom of repeated pAMR 2 was 100% after a median follow-up time of 1161 days.
Conclusion: Women after pregnancy are often highly sensitized and require precise immunological evaluation and attentive postoperative management. Even in cases where repeated HLA mismatch is avoided, transplantation seems to cause an immunogenic response with a strong growth in preexisting HLA alloantibodies, putting women with pregnancy-induced HLA alloantibodies at risk for antibody-mediated rejection. Analyzing the HLA phenotype of female patients and their children prior to heart transplantation in combination with bead-based immunoassay analysis of antibodies could be used as an additional tool to prevent antibody-mediated rejection caused by resynthesis of HLA alloantibodies after transplantation.

ABSTRACT

TREATMENT OF JC-VIRUS-ASSOCIATED PROGRESSIVE MULTIFOCAL LEUKENCEPHALOPATHY WITH ALLOGENIC BK-VIRUS-SPECIFIC T CELLS: FIRST APPLICATION IN AUSTRIA

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Introduction: In patients with HI-Virus (HIV) acquired immune deficiency syndrome (AIDS), polyoma JC-virus (JCV)-associated progressive multifocal leukencephalopathy (JCV-PML) is a life-threatening complication with a high mortality rate of approximately 50% at six months after diagnosis. There is no proven treatment except to optimize the conditions for immune-reconstitution by rapid suppression of HIV replication using combination antiretroviral therapy (cART). In cases with rapid neurologic deterioration the rate of immune reconstitution of CD4 cells may not prevent disability or death. The harvest and application of allogenic virus-specific T cells (VSTs) has been advocated as a treatment strategy for JCV-PML by allowing for viral clearance until the host immune system has reconstituted sufficiently to overcome JCV-PML. In the setting of JCV-PML, VSTs of the closely to JCV-related Polyoma BK-virus (BKV) were shown to efficiently clear JCV from cerebrospinal fluid (CSF) and blood, as both BKV and JCV share relevant immunogenic epitopes presented by host major histocompatibility complex (MHC). Here we report to our knowledge the first case of allogenic BKV-specific T cell therapy in Austria. The patient is a 26-year-old male suffering from severe JCV-PML caused by AIDS who presented with profound CD4 cell suppression (7/μL) before reinstallation of cART.
Material and Methods: Allogenic haploidentical cells from a blood-related donor were obtained by leukapheresis. Following a GMP-certified 12-day expansion protocol using overlapping peptides of BKV/JCV Large-T antigen and VP-1, and stimulation with IL-15 (5 ng/mL) on day 9 we were able to achieve six doses of 2×10^5 VSTs per kg bodyweight (patient weight: 80 kg). The T cells within the VST product were composed of 75% CD4 cells and 25% CD8 cells. For administration, cells were thawed and immediately injected intravenously via 1mL syringes. We performed monthly lumbar punctures and obtained brain MRI scans in order to assess JCV load in CSF and imaging progression of PML.
Results: Overall, we administered five doses of 2×10^5 VSTs /kgBW in 4-5 weekly periods. JC-virus load was assessed at about one week before or after VST infusion. On the day before the first infusion JCV load in CSF was $8{,}6x10^4$ copies, which was reduced to 5.7×10^3 copies 50 days after the first infusion. In parallel, we noticed neurological improvement. At baseline, the patient was intubated and tetraplegic. Over the course of each VST dose, we observed the patient slowly regaining consciousness and improvement in motor skills and respiratory function. On brain MRI with T2-weighted sequences, the expansion of PML lesions in the brainstem and cerebellum continuously slowed down. Subsequently, at six months, we detect a tendency for regression in several lesions and no sign of immune-reconstitution inflammation syndrome (IRIS). At this point, there is no evidence of adverse effects of this treatment.
Discussion: Allogenic BKV-VST therapy is an innovative and causal therapy for JCV-PML. In our patient we observed clinical, serological and radiological improvement. Recovery was slow, which we believe is attributed to extreme disease severity. In our case, this experimental treatment was safe. We believe BKV-VST should be considered as a promising treatment option in JCV-PML in the future, particularly in severe cases.

EDGE-TO-EDGE MITRAL VALVE REPAIR AS A BRIDGE-TO-ELIGIBILITY FOR HEART TRANSPLANTATION

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Objective: Heart transplantation (HTx) is well established in end-stage heart failure (HF). Successful transplantation depends largely on disease stability during the time on the waiting list and on pulmonary arterial pressure prior to transplantation. Percutaneous edge-to-edge mitral valve repair using the MitraClip system has been shown to improve mitral regurgitation (MR) and secondary pulmonary hypertension. We here present our initial experience with MitraClip implantation as bridge-to-transplant in a series of patients with end-stage HF and severe functional MR.
Material and Methods: We retrospectively analysed 10 patients with end-stage HF (mean age 51±14, eight males) who underwent MitraClip implantation for severe functional MR at our center between May 2015 and January 2021 immediately before or while on the waiting list for HTx. Laboratory, echocardiographic, and hemodynamic findings were evaluated before MitraClip implantation and at follow-up.
Results: MitraClip implantation was uncomplicated in all patients. Substantial improvement in MR was achieved in eight patients (MR 3.0 to 1.8±0.8, $P = 0.002$). In the follow up visits, 3–12 months following clip implantation, eight patients improved in NYHA functional class (NYHA 2.75 to 2.0, $P = 0.005$). NT-proBNP decreased from 6572±4912 to 3072±1469 ng/l ($P = 0.048$), systolic pulmonary artery pressure decreased from 58±8 to 45±10 mmHg ($P = 0.002$) and mean pulmonary artery pressure from 41.5±11 to 28.8±8 mmHg ($P = 0.025$). So far, eight patients were successfully transplanted, one patient died from sepsis before HTx and one patients is awaiting HTX- listing 90 days after MitraClip implantation.
Discussion: MitraClip implantation is feasible, safe and is associated with functional and hemodynamic improvement in high-risk end-stage HF patients with functional MR while on the waiting list for HTx. This strategy appears effective as bridge-to-transplant in selected patients.

PREVENTION STRATEGIES AGAINST CMV-INFECTION AFTER LIVER TRANSPLANTATION

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Background: There is a variety of prevention strategies against cytomegalovirus (CMV) viremia / disease after liver transplantation (LT). Thus, this retrospective cohort study performed in a prospective manner with a 1-year follow-up has been performed comparing anti-CMV immunoglobulins (IGs) with Valganciclovir.
Patients and Methods: A total of 257 patients underwent LT between January 2008 and May 2020. While 134 patients received anti-CMV-IGs (7500 IU for 4 days) between 2008 and October 2016, 111 patients were treated with Valganciclovir (up to 900 mg/d for 3-6 months dependent on renal function and side effects) since November 2016. Patients with viremia in both groups were treated at least for 14 days with Ganciclovir or Valganciclovir. Primary endpoint of the study was CMV viremia within 1 year after LT. Secondary endpoints were leukopenia, renal function, patient and graft survival.
Results: Demographics were comparable in both groups (i.e. age (56.6 +/- 11.2 years), gender (78.8% male)). Viremia was 36.7% ($n = 90$) within 1 year after LT with 43.3% ($n = 58$) after anti-CMV IGs vs. 28.8% ($n = 32$) after Valganciclovir ($p>0.05$). The incidence of viremia was dependent of the donor-recipient match with 39.6% ($n = 21$) and 60,6% ($n = 20$) in D-/R+ and D+/R+ after anti-CMV IGs, and 12.0% ($n = 3$) and 25,7% ($n = 9$) after Valganciclovir ($P = 0.018$; $P = 0.007$), respectively. The overall 1-year mortality rate was comparable in both groups with 13.1% ($n = 32$). Kidney function based on serum creatinine was significantly improved with anti-CMV IGs at 1 year after LT.
Conclusion: While Valgancyclovir protected nicely CMV positive recipients from CMV viremia the effect of anti-CMV IGs was inferior in this constellation without any impact on CMV disease; however, anti-CMV IGs are in favor to a better side effect profile especially concerning renal function and thus it should be the preferred prevention strategy.

COMBINED HEART AND KIDNEY TRANSPLANTATION: HOW HYPOTHERMIC MACHINE PERFUSION ENABLES DELAYED KIDNEY IMPLANTATION

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Introduction: Combined heart and kidney transplantation (HKTx) is a well-established procedure in a highly demanding patient population. Due to the growing complexity of heart recipients, the early postoperative period is crucial for the timing of the kidney. In the era of dynamic perfusion strategies, hypothermic machine perfusion (HMP) became a standard clinical procedure for kidney transplantation with favourable outcomes compared to SCS (static cold storage). We introduce a new strategy for delayed kidney implantation applying HMP in patients undergoing HKTx.
Methods: Within the last twenty years, 26 patients (median age 59, IQR 48–63 years) underwent HKTx at our center. In the last twelve months, six patients were transplanted using HMP for delayed kidney implantation. Hence, we are retrospectively describing this case series.
Results: Five male patients (median age 58 years) and one female patient (59 years) with end-stage heart failure (2 ischemic cardiomyopathy (CMP), 3 dilative CMP, 1 restrictive CMP) and cardiorenal syndrome received the transplants. Five patients had at least one previous cardial surgical procedure. Two were bridged with mechanical circulatory support (Impella for 6 days, veno-arterial extracorporeal life support for 4 days) and underwent high urgency (HU) transplantation. Overall, four patients received high urgency status. Three patients were upgraded for HKTx due to progressive kidney failure during the waiting period (median 21 months). Median SCS time for the heart was 197 minutes. All patients were transferred to the ICU for initial stabilization after HTx with adequate graft function but moderate catecholamine and inotropic support. Once patients started clearing lactate and entered the weaning phase, they were transferred to the OR for kidney implantation. Mean overall cold ischemic time (SCS and HMP) for the kidney was 24 hours. On average, kidney HMP was performed for 18.2±10 hours.

All patients had a normal cardiac function with none or minimal medical support within a week after HNTx. One patient, transplanted under HU conditions, died after 20 days due to severe sepsis. In one patient primary non function of the kidney occurred following a critical early postoperative period with major volume shifts.
Conclusion: Implementation of HMP for delayed kidney transplantation in increasingly complex HNTx recipients offers the possibility to stabilize the patients hemodynamically after HTx.

ABSTRACT

RELAXIN AND ERYTHROPOIETIN REDUCES ISCHEMIA-REPERFUSION INJURY IN RAT UTERUS

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Uterus transplantation is a new potential alternative to gestational surrogacy for treatment of absolute uterine factor infertility. Ischemia-reperfusion injury (IRI) is associated with early allograft dysfunction in organ recipients. Therefore, various substances are under investigation for reducing IRI. Relaxin (RLX) is a peptide hormone that has antifibrotic, antioxidant, anti-inflammatory and cytoprotective properties. It was used in several in vivo studies showing its effect in reducing IRI and improving organ quality. Erythropoietin (EPO) is an endogenous hormone, which has similar reported properties as RLX, although acting via different mechanisms. The aim of this study is to investigate the protective effect of RLX and EPO alone and their combination in an experimental rat uterus IRI model.

Materials and methods: A total of 80 Sprague Dawley rats were randomly assigned into 8 groups (n = 10/group). Rats were injected intravenously with a single bolus of EPO (4000 IU/kg), RLX (5μg/kg), EPO+RLX or saline 30 minutes before ischemia. Ischemia was induced for 60 minutes by clamping aorta 0.5-1 cm above the bifurcation and ligating ovarian arteries, followed by 120 minutes of reperfusion. Sham-operated rats underwent the same dissection procedure except for artery occlusion. Tissue samples were taken after 120 minutes of reperfusion. Uterine tissue malondialdehyde (MDA) and superoxide dismutase (SOD) levels were measured. Immunostaining with anti-myeloperoxidase (MPO) antibodies was performed. Also, uterine tissue samples were stained with eosin and hematoxylin for morphology evaluation. Tissue infiltration with inflammatory cells, vasoconstriction, hemorrhage, necrosis, edema, thrombosis, endometrial loss of cells were graded by a blinded pathologist using a predefined score.

Results: There were no differences in MDA and SOD levels between all sham-operated groups. Uterine IRI led to a higher tissue MDA level, which was significantly attenuated in groups pretreated with RLX and EPO/RLX compared to saline pretreated group ($P < 0.001$, $P < 0.001$ respectively). Moreover, RLX alone or in combination with EPO preserved tissue SOD activity compared to control group. EPO alone had no significant effect on MDA levels or in increasing SOD activity. Tissue MPO expression was lower in all sham-operated groups. The highest MPO expression was observed in experimental group, pretreated with saline. All other IRI groups presented with significantly lower MPO expression indicating lower inflammation in uterine tissue.

Sham-operated rats showed normal uterine tissue morphology—normally organized endometrial glands, regular morphology of epithelium, no tissue edema. Tissue morphology was significantly distorted in intervention group, pretreated with saline. Epithelial cells showed cytoplasm loss, increased height of surface epithelium and increased number of cell layers. Uterine tissue in RLX group showed less irregularity of uterine glands, less edema and polymorphonuclear cells compared to intervention group, pretreated with saline.

Conclusions: These preliminary results suggest that EPO and RLX may have protective anti-inflammatory, antioxidative effects on uterus in experimental ischemia-reperfusion injury model. Furthermore, RLX also preserved a protective effect on uterine tissue morphology. Although these results look promising, more readouts should be taken into account when drawing robust conclusions.

CORONARY ANGIOGRAPHY IS ASSOCIATED WITH INCREASE OF CARDIAC TRANSPLANT RATES IN DONORS ≥ 40 YEARS. AN ANALYSIS OF >12500 EUROTRANSPLANT ORGAN DONORS

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Purpose: Donor age has increased dramatically in Europe. There exist concerns about transmission of coronary artery disease (CAD) in older donors, with associated complications like primary graft failure and graftvasculopathy. Donor coronary angiography (DCA) can detect CAD but is not regularly used in all donors. The aim of this study was to analyse if donor angiography has an impact on heart transplant rates.

Methods: Between 2004 and 2016 all donor heart offers within Eurotransplant were analysed for DCA use and transplantation rates. DCA rate was calculated and compared for different donor risk variables: age, sex, diabetes (DM), hypertension (HT), smoking history (SH) and body mass index >30 (BMI). Influence of donor risk factors on DCA results were analysed. The likelihood of transplantation of donors with and without DCA was compared in the overall donor population as well as within risk groups. Multiple logistic regression models were created to analyse the influence donor risk factors on DCA- rates, -outcome and transplantation rates. P-value of <0.05 was defined as statistical significant.

Results: A total of 12565 donor hearts were offered during the study period. In 2319 (18.5%) DCA's were performed. 107 DCA's were excluded due to unclear results. Median donor age was 45 (31–53) years, 54.4% were male. 3.4% of donors had DM, 20.5% HT, 44,4% SH and 10,0% had a BMI >30. Donor risk variables had a significant impact on DCA use: Age (10 year increments (10a): $P < 0.0001$, OR:2.797), DM ($P < 0.0001$,OR:1.811), HT ($P < 0.0001$,OR:1.349), SH ($P < 0.0001$,OR:1.859), BMI ($P = 0.01$,OR:1.240), male ($P = 0.0002$, OR:1.235). CAD was associated with donor age (10a: $P < 0.0001$,OR:1.545), HT ($P = 0.0006$,OR:1.425), SH ($P < 0.0001$,OR: 1.903), male ($P < 0.0001$, OR:2.247). Transplant rates were significantly higher in donors with normal DCA result (compared to no DCA performed: $P < 0.0001$,OR:2.612), whereas other independent risk factors for transplantation were: Age (10a: $P < 0.0001$, OR 0.775), DM ($P = 0.0019$,OR:0.689), HT ($P = 0.002$,OR:0.84), male ($P < 0.0001$, OR:0.738) and CAD detected by DCA ($P < 0.0001$,OR: 0.832).

Conclusion: DCA is used more often, in donors with potential CAD risk factors. Donor CAD is associated with known risk factors. However, normal DCA results are associated with higher transplant rates independent of CAD risk factors. Higher use of DCA might increase transplant rates, especially in donors with CAD risk.