

HLA

Immune Response Genetics

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**Abstracts for the 31st European Immunogenetics and Histocompatibility
Conference and the 25th Annual Meeting of the German Society
for Immunogenetics (Joint Meeting)
Mannheim/Heidelberg, Germany, May 30 – June 2, 2017**

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ABSTRACT BOOK

31st European Immunogenetics and Histocompatibility Conference (EFI)

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A RAPID METHOD TO ISOLATE HIGHLY PURIFIED T OR B CELLS FROM BLOOD, LYMPH NODE OR SPLEEN SAMPLES FOR USE IN DONOR-RECIPIENT CROSSMATCH ANALYSIS

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The crossmatch assay is used as part of a pre-transplant immunologic risk assessment to determine the compatibility between donor-recipient pairs. Isolated T or B cells from the donor are mixed with recipient serum and the presence of donor-specific antibodies is detected through a complement-dependent killing assay (CDC-crossmatch) or by flow cytometry (flow crossmatch). Isolation of specific cell types can be time consuming, and multiple methods must often be validated in laboratories that receive a variety of sample types. We have developed methods (EasySep) to isolate T or B cells directly from whole blood (WB) in 25 minutes, or lymph node (LN) and spleen samples in 11 minutes, without RBC lysis, sedimentation or density gradient centrifugation. Unwanted cells, platelets and RBCs were immunomagnetically labelled and then placed into a magnet. Labelled unwanted cells were retained, while untouched T or B cells were poured or pipetted off. Isolation of T or B cells using this method was tested on WB, peripheral blood mononuclear cells (PBMC) (model system for LN, which typically have few RBC) as well as on a suspension of PBMC/WB and a B cell line (model system for spleen, which has a high B cell content). Purities following T cell isolation were 97% \pm 4 (n = 10) from WB, 94% \pm 6 (n = 11) from PBMC (mock LN), and 94% \pm 6 (n = 12) from mock spleen (mean \pm SD). B cell purities were 97% \pm 4 (n = 10) from WB, 94% \pm 7 (n = 18) from PBMC, and 95% \pm 9 (n = 6) from mock spleen. On average, 650,000 T cells and 70,000 B cells were recovered per mL of WB. Starting with 5 x10⁷ cells, 10 million T cells and 2.8 million B cells were recovered from PBMCs, while 10 million T cells and 1.8 million B cells were recovered from mock spleen samples. Isolations can be automated using RoboSep. This new method enables the isolation of highly purified T or B cells from multiple sample sources using the same reagents, thus simplifying validation for a busy HLA laboratory.

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LONG-TERM OUTCOMES AND DISCARD RATE OF KIDNEYS BY DECADE OF EXTENDED CRITERIA DONOR AGEMaria Messina¹, Luigi Biancone², Fabrizio Fop¹, Paola Magistroni³, Silvia B. Vanzino³, Claudia M. Rosso³, Cristiana Caorsi³, Antonio Amoroso²¹Nefrologia, Dialisi e Trapianto, Torino, Italy, ²University of Torino, Torino, Italy, ³Città della Salute e della Scienza Hospital, Torino, Italy**Correspondence:** pmagistroni@cittadellasalute.to.it

Extended criteria donors represent nowadays a main resource for kidney transplantation, and recovery criteria are becoming increasingly inclusive. However, the limits of this approach are not clear as the effects of extreme donor ages on long-term kidney transplantation outcomes is not known. To address these issues, we performed a retrospective study on extended criteria donor kidney transplantation. In total, 647 consecutive extended criteria donor kidney transplantations performed over 11 years (2003–2013) were included. Donor, recipient, and procedural variables were classified according to donor age decades (group A, 50–59 years old [n = 91]; group B, 60–69 years old [n = 264]; group C, 70–79 years old [n = 265]; and group D, 80 years old [n = 27]). Organs were allocated in single- or dual-kidney transplantation after a multi-step evaluation including clinical and histologic criteria. Long-term outcomes and main adverse events were analyzed among age groups and in either single- or dual-kidney transplantation. Kidney discard rate incidence and causes were evaluated. Median follow-up was 4.9 years (25th; 75th percentiles: 2.7; 7.6 years); patient and graft survival were comparable among age groups (5-year patient survival: group A, 87.8%; group B, 88.1%; group C, 88.0%; and group D, 90.1%; P = 0.77; graft survival: group A, 74.0%; group B, 74.2%; group C, 75.2%; and group D, 65.9%; p = 0.62) and between dual-kidney transplantation and single-kidney transplantation except for group D, with a better survival for dual-kidney transplantation (P = 0.04). No difference was found analyzing complication incidence or graft function over time. Kidney discard rate was similar in groups A, B, and C (15.4%, 17.7%, and 20.1%, respectively) and increased in group D (48.2%; odds ratio, 5.1 with A as the reference group; 95% confidence interval, 2.96 to 8.79). Discard rate and long-term outcomes are similar among extended criteria donor kidney transplantation from donors ages 50–79 years old. Conversely, discard rate was strikingly higher among kidneys from octogenarian donors, but appropriate selection provides comparable long-term outcomes, with better graft survival for dual-kidney transplantation.

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PRE-TRANSPLANT HLA ANTIBODY SCREENING BY SOLID PHASE ASSAYS: INCIDENCE OF ANTI-HLA IGM ANTIBODIES IN KIDNEY TRANSPLANT CANDIDATESElvira Poggi^{1,2}, Giuseppina Ozzella^{1,2}, Antonio Giuseppe Bianculli², Annarita Manfreda², Lucia Spano², Damiano Colasante², Maria Rosaria Fazio², Silvia Sinopoli², Antonina Piazza^{1,2}¹National Council of Researches, IFT Unit of Rome S. Camillo Hospital, Rome, Italy, ²Regional Transplant Center of Lazio, Rome, Italy**Correspondence:** elvira.poggi@cnr.it

A positive lymphocytotoxic crossmatch represents an absolute contraindication to kidney transplantation. This paradigm is widely accepted for IgG antibodies but few data on the clinical relevance of IgM antibodies are available and so their role is still controversial. Some studies report that they can be protective for transplant, while other suggest that IgM antibodies are harmful for the graft. Pre-transplant antibody screening by CDC technique does not allow us to discriminate IgG and IgM isotypes. Instead, serum treatment with DTT can indirectly highlight this because it is able to inactivate the IgM pentamer. Moreover, in both cases it is not possible to know if these antibodies are specific for HLA molecules. The new solid-phase techniques permit us to investigate anti-HLA antibodies and to discriminate their isotype. Since September 2016, 189 kidney transplant candidates on the waiting list at the Lazio Regional Transplant Center, were analyzed to evaluate the incidence of IgM antibodies. The antibody characterization was performed using FlowPRA Screening Test to detected either anti-HLA IgM alone, otherwise unknown, or in combination with IgG antibodies and by Luminex Single Antigen Beads to identify antibody specificity. The incidence of anti-HLA IgM antibodies was 10% (19/189). Only IgM antibodies were detected in ten (53%) patients, both IgM/IgG antibodies were presented in nine (47%) patients. IgM positive-group showed in 6 cases only anti-HLA class I antibodies ($2000 \geq \text{MFI} \leq 7000$) and 4 cases only class II antibodies ($4000 \geq \text{MFI} \leq 12000$). The antibody characterization of IgM/IgG positive-group evidenced in 3 patients the same IgM and IgG specificity, while 6 patients showed additional IgM specificity respect to evidenced IgG antibody specificity. In conclusion, our study suggests widening the antibody screening to anti-HLA IgM antibodies in transplant candidates. The strength and specificity of detected IgM antibodies highlighted the importance of their accurate characterization to understand the clinical significance and to improve graft survival.

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FEASIBILITY OF EPLET-BASED MATCHING FOR ALLOCATION OF DECEASED DONOR RENAL TRANSPLANTS

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Retrospective studies have shown a poor correlation between the number of broad and eplet mismatches at the same HLA locus. If eplet mismatches are to be incorporated in the deceased donor allocation pathway, using high resolution HLA typing to calculate the number of eplet mismatches is not practical in the short time-frame available. We aimed to determine whether low / intermediate resolution HLA typing could be

used to accurately calculate eplet mismatches. 264 patients who underwent renal transplant between 2003 and 2007 were included. Prospective serological HLA typing and retrospective 4-digit Sanger sequencing was performed for donor / recipient pairs. Two-digit molecular typing was derived from the 4-digit sequencing results. The number of eplet mismatches was calculated using HLAMatchmaker for 2-digit typing and compared with the 4-digit typing derived eplet mismatches. Correlation and agreement of HLA-A, -B, -C, -DR and -DQ mismatches between the 2- and 4-digit results were analyzed. There was close correlation between the number of eplet mismatches calculated using serological and four-digit molecular typing methods at HLA-A, -B and -DR loci with coefficients above 0.95 (Spearman correlation). In contrast, there was less correlation at HLA-C and -DQ loci with coefficients of 0.87 and 0.80, respectively. The correlation coefficients between the number of eplet mismatches calculated using 2-digit and 4-digit molecular typing at all loci were above 0.98 ($p < 0.001$). Consistency and absolute agreement in the number of eplet mismatches was similar using 2-digit and 4-digit molecular typing across class I and II loci. In contrast, consistency and absolute agreement was generally lower for serological typing, particularly at HLA-C (consistency: 0.875 and absolute agreement: 0.875) and HLA-DQ loci (consistency: 0.801 and absolute agreement: 0.792). There is good correlation and agreement between 2- and 4-digit typing for total eplet mismatches at all loci. These results suggest that 2-digit molecular HLA typing may be sufficient for the allocation of donor kidneys by eplet-based matching in deceased donor allocation pathways. Further studies evaluating the correlation and agreement in a broader ethnically diverse population group are required.

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COMPARISON OF ANTI-HLA ANTIBODY DETECTION METHODS IN CADAVERIC TRANSPLANT CANDIDATES IN TURKEY: A SINGLE CENTER EXPERIENCE

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Chronic kidney failure can result from diabetes, obesity and hypertension which are the most abundant diseases worldwide. The definitive treatment of chronic kidney failure is transplantation. However, organ rejections can occur due to the presence of anti-HLA antibodies after transplantation. Therefore detection of anti-HLA antibodies is important to prevent hyperacute or acute rejections. In this study crossmatch tests were performed before transplantation to 416 cadaveric transplant candidates who were admitted to Tepecik Training and Education Hospital Tissue Typing Laboratory between 2014 and 2016.

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