

stimulation that is reflected in DSA production and then in a reduced long-term transplant survival.

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SYSTEMATIC REVIEW AND REMOVAL OF UNACCEPTABLE ANTIGENS SIGNIFICANTLY IMPROVES THE CHANCE OF A KIDNEY TRANSPLANT FOR HIGHLY SENSITISED PATIENTS. A SINGLE CENTRE APPROACH IN THE UK

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Highly sensitized patients can wait many years for an offer of a transplant due to the many unacceptable antigens listed with UK Transplant. Previous work at our center has shown that cumulative antibodies detected at <5000 MFI using Luminex Single Antigen beads correlated with a negative flow cytometric and CDC crossmatch. We therefore instigated a monthly multi-discipline team meeting to discuss highly sensitized, long waiting patients, deselecting antigens less than 5000 MFI. Whilst the median calculated Reaction Frequency (cRF) only fell from 100% to 95%, the median number of listed unacceptable antigens was halved from 41 to 21. Using the new UK transplant matching tool this resulted in a predicted increase in the median number of potential tier 1-3 donors from just 5 in 10,000 to 112 in 10,000 donors. Over the past two years, 10% of transplants at this hospital have been from this cohort of highly sensitized patients. The number of patients waiting longer than 1000 days for a transplant has reduced from 23 to just seven and some of these transplants have been from living donors who were previously excluded due to unacceptable antigens. The pre-transplant crossmatch was negative in all cases and outcomes are good with a median 12 month creatinine of 143 $\mu\text{mol/L}$.

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IMPACT OF ANTI-HLA-DP AND -DQA ANTIBODIES IN RENAL TRANSPLANTATION: A PRELIMINARY STUDY

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The role of anti-HLA-DP and -DQA antibodies in renal graft has been poorly understood for a long time. However, the introduction of the solid phase assay, allowing their identification and better understanding of their clinical importance in kidney transplantation. This study describes the incidence of anti-DP and -DQA antibodies in a cohort of 126 (80 male, 46 female) patients transplanted between January 2016 and December 2017 at the University Hospital of Parma. The mean age at transplantation was 53 years. Pre- and post-transplant sera were identified using the Luminex Single Antigen (LSA, Luminex Single Antigen class I and II, One Lambda). Anti-DP and -DQA antibodies were detected in 17 of 126 (13%) patient post-transplant sera. Of these, 71% (12/17) of the patients had antibodies against these loci before the transplantation (three of these were re-transplants), whereas 29% (5/17) developed them *de novo* within the follow-up period. Nine of these 17 (53%) patients had previous pregnancies or blood transfusions. Among post-transplant antibodies detected, anti-DP specificities showed a mean fluorescence intensity (MFI) level less than 3000 (positivity cut-off, AIBT Guide Lines 07/2016), while anti-DQA specificities also reached MFI values greater than 3000. In summary, we have shown that anti-DP and -DQA antibodies are common in primary renal transplants but they commonly accompany other antibodies: this makes their impact on graft survival hard to determine. Moreover, typing for HLA-DP and -DQA antigens is still not routinely performed, so it's difficult to classify if an anti-DP antibody as donor-specific, or not. Therefore, the currently available LSA panel for anti-DP and -DQA antibody detection, only covers a small number of the identified alleles. Future studies and extended follow-ups are then warranted to further characterize the role of these antibodies.

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FLOW CYTOMETRY CROSSMATCH: FCXM VS. FLOWDSA-XM

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Clinical impact of preformed HLA donor specific antibodies (DSA) ranges from an acceptable risk to an absolute contraindication for kidney transplantation, according to the

technique used to detect them (CDC vs. flow cytometry/solid phase). The new FlowDSA-XM technique combines sensitivity of the classical FCXM with specificity of the single antigen bead assay, allowing a better correlation between crossmatch result and patients' HLA class I and II sensitization detected by solid phase assay. We performed a comparative analysis between FCXM and FlowDSA-XM results by testing 46 sera (9 PRA=0%, 14 HLA class I positive, seven HLA class II positive and 16 HLA class I&II positive) with five different lymphocytes population (typed for all HLA class I&II loci by high resolution PCR-SSP/SSO techniques). The standard FCXM technique allowed detection of IgG-antibodies specific for T and B lymphocytes. The FlowDSA-XM consisted of an incubation of lymphocytes and serum was performed. Then, three different Capture Beads (HLA I A, B, C beads, HLA IIa DQ beads and HLA IIb DR, DQ, DP beads) were added. Lastly, a Lysis/Stain buffer was used to solubilize the HLA molecules and to label the antibodies bound to them. FlowDSA-XM and FCXM showed strong concordance in the nine PRA negative sera and in the 14 anti-HLA class I positive sera; FlowDSA-XM was partially discordant in one of seven anti-HLA class II positive sera because of FCXM B cells showed a very weak positivity while FlowDSA-XM HLA IIb showed a result near cut-off. Moreover, the FlowDSA-XM was more sensitive than the FCXM showing higher values of positivity, especially in the presence of anti-HLA class I DSA and gave "specific" results discriminating between HLA class I and/or II DSA. On the contrary the FCXM gave B positive results for the presence of both HLA class I and class II DSA. In conclusion the FlowDSA-XM, allowing a better interpretation of the crossmatch result, could represent the crossmatch of the new millennium.

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CORRELATION OF THE DE NOVO DONOR SPECIFIC ANTI-HLA ANTIBODY (DNDSA) EMERGENCE WITH IMMUNOSUPPRESSION AND 2-YEAR LIVER TRANSPLANTATION OUTCOME: A PROSPECTIVE STUDY

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The clinical impact of anti-HLA antibodies in the liver transplantation (LT) setting has been unclear, although recent studies have described an increase in the diagnosis of antibody-mediated damage in this population. The aim of our study is to correlate the development of *de novo* donor-specific anti-HLA antibodies (dnDSA) after LT with the immunosuppression, as well as graft outcome. A total of 65 liver transplanted patients (50 males) of the 2010-2015 period (out of 102; 37 patients were excluded due to early graft loss <3 months) were included for analysis. All patients were screened by Luminex for anti-HLA antibodies before and 6 months, 1 year and 2 years after LT. HLA typing was performed for all donor and recipient pairs. *De novo* antibodies emerged in 26 patients (40%) who had 38.5% liver dysfunction or graft loss two years post-transplantation, versus 28.2% in *de novo* negative patients. Fifteen out of 26 positive patients (57.7%) had dnDSA (four class I and 13 class II) with 33.3% graft dysfunction (fibrosis), versus 28.2% in negative patients. Concerning the immunosuppression regimen, patients who received mTORi as maintenance therapy had more dnDSA (31%) and dnDSA class II (24.1%) against 18.8% in patients who did not receive mTORi. Moreover, less HLA compatible patients (0-3 out of 6 matches) had more dnDSA (27.7%) than 18.2% in more compatible patients (4-6 out of 6 matches). Less compatible patients with dnDSA had 35% graft dysfunction, while more compatible patients negative for dnDSA had 14.3% graft dysfunction. In conclusion, the preliminary results of this ongoing study show that *de novo* anti-HLA antibodies seem to affect the 2-year liver graft outcome. HLA compatibility as well as immunosuppression, seem to have an effect on dnDSA emergence. However, the differences are not yet statistically significant.

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DISCOVERY OF A NOVEL HLA-B*08 NULL ALLELE IN A DANISH KIDNEY TRANSPLANT RECIPIENT

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The human leukocyte antigen (HLA) region on chromosome 6 is the most polymorphic region of the genome. According to the IPD-IMGT/HLA Database there were 17,874 HLA allele sequences including 4,950 HLA-B