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The aim of this study was the estimation of the effect of long-term kidney transplantation on T and B regulatory lymphocytes. Patients who had received a transplant 17+ years ago (group A) and 1 year ago (group B) took part in the study. The subpopulations of CD4 + CD25 + FOXP3 (Tregs), CD19 + CD24++CD38++ (Bregs1), CD19 + CD27 + CD24++ (Bregs2) and naïve, switched, non-switched memory and double negative (DN) B-lymphocytes were measured by flow cytometry. Patients of group A (N = 20, M/F = 11/9) did not differ with patients of group B (N = 40, M/F = 23/17) regarding age (57 vs 56 years, $p = 0.065$) and eGFR (63.5 ± 19.2 vs 63.6 ± 17, 1 mL/min/1,73 m2, $p = 0.132$). Significant differences between groups were observed in respect of the percentage of Tregs (2.55 vs 4.7%, $p < 0.001$), the percentage and number of Bregs1 (1.6 vs 0.0%, $p < 0.001$ and 0.53 vs 0.0/μL, $p = 0.001$) and Bregs2 (0.0 vs 2.55%, $p < 0.001$ and 0.0 vs 2.0/μL, $p = 0.001$). Group A also exhibited a significant reduction of B-lymphocytes (55 vs 99/μL, $p = 0.034$), which mainly referred to naïve (18 vs 41/μL, $p = 0.005$), switched (7.26 vs 14/μL, $p = 0.005$), and non-switched memory (1.22 vs 13/μL, $p < 0.001$) B-cells, with a simultaneous increase of DN cells (22 vs 11/μL, $p = 0.028$). Long-term kidney transplant recipients show a reduction of the percentage of Tregs, a rise of Bregs1 and a decline of Bregs2 as well as significant alterations of B-lymphocyte subpopulations.

## P100 | Pre-transplant flow cytometric crossmatch in patients undergoing Rituximab treatment: employment of Pronase and anti-CD20

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A limitation in the application of cytometric cross-match (FCXM) pertains to patients highly immunized against HLA antigens, undergoing desensitization protocols with Rituximab, an α-CD20 chimeric monoclonal antibody. It has been demonstrated that in the context of pre-transplant cross-match, the presence of Rituximab in patients' serum can lead to false-positive results for B lymphocytes, making data interpretation challenging. Specifically, the drug in the recipient's serum binds itself to the CD20 on the donor's B lymphocytes and is detected by the secondary α-Human IgG Ab. In this case, a positive result for B lymphocytes could occur even in the absence of donor-specific Ab. The development of a protocol involving the treatment of donor cells with the enzyme Pronase and subsequent incubation with α-CD20 Ab (not detectable by α-human IgG Ab) has aimed to saturate CD20 receptors and prevent binding with Rituximab. In this context, sera from 24 patients undergoing Rituximab treatment were collected to be cross-matched with cells from 15 healthy donors with known HLA typing. At first, the drug bioavailability has been assessed excluding 5 sera. The remaining 19 sera underwent a search for anti-HLA Ab using SAB. Subsequently, the titration of α-CD20 Ab was performed and the standard deviation was calculated to determine the cut-off. During the study, 42 FCXM tests were conducted: out of the 13 expected positive cross-matches due to the presence of donor-specific Ab, 9 (69%) remained positive with B lymphocytes even after treatment. The remaining 4 yielded negative results: the likely cause is attributed to the low expression on the cell surface of the involved antigens and the low Ab's MFI values. Out of the remaining 29 cross-matches with SAB negative sera, 28 (97%) yielded negative results as expected. In conclusion, the collected data demonstrate the effectiveness of the studied protocol without compromising the sensitivity and specificity of the test.

## P101 | Deciphering HLA antibody reactivity patterns: A cluster-based analysis of SAB assay data

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This study provides a nuanced exploration of anti-HLA antibody reactivity profiles using Single Antigen Bead (SAB) assay data. Employing k-means clustering, we

segmented a comprehensive dataset into 10 distinct clusters, each characterizing unique immunological patterns. Our analysis, visualized through a detailed heatmap, revealed significant variations in mean fluorescence intensities (MFIs) across these clusters, indicative of diverse antibody sensitization profiles. Notably, cluster 2 emerged as a focal point due to its unexpected reactivity pattern. While generally exhibiting low MFIs (average MFI < 1500), a pronounced spike was observed against specific alleles like A*29:01 (MFI: 24,000) and B*27:05 (MFI: 22,500), suggesting a targeted immune response. In contrast, cluster 7 displayed consistently high reactivities across a range of HLA alleles, with MFIs often surpassing 10,000, indicative of a broad or polyclonal sensitization. Intriguingly, cluster 5, with moderate overall reactivity (average MFI: 3000–4000), showed selective heightened responses to alleles such as B*15:10 (MFI: 17,000), hinting at specific immunological events or exposures. Conversely, cluster 9 presented minimal reactivity across all HLA alleles, with most MFIs below 500, possibly reflecting a low level of sensitization or antibody presence. This comprehensive analysis sheds light on the intricate landscape of anti-HLA antibody responses patterns, revealing how specific clusters may correspond to different immunological histories or sensitization patterns. The distinct reactivity profiles underscore the potential for using such data in understanding patient-specific immune responses, which could be pivotal in fields like transplant immunology and personalized medicine.

## P102 | Can Molecular HLA mismatch scores predict antibody-mediated rejection in desensitized kidney transplant recipients?

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We investigated in desensitized kidney transplant recipients whether high HLA mismatch epitope scores and high donor-specific HLA antibody (DSA) levels pre- or post-desensitization are associated with development of antibody-mediated rejection (AMR). Thirty-three adult patients who received a living-donor kidney transplant at our center between December 2018, and March 2023, after desensitization therapy due to DSA ≥1000 MFI were analyzed. Patients with and without biopsy-proven AMR during the first three post-transplant months were compared for the four different molecular HLA mismatch scores (Predicted Indirectly ReCognizable HLA Epitopes (PIRCHE), HLA-Matchmaker, Amino Acid Mismatch Score and Electrostatic Mismatch Score 3D), demographics, and immunological risk factors. Twelve patients (36.3%) experienced AMR at a median of 11 days post-transplantation (IQR: 6–43). AMR attributable to existing DSA before desensitization occurred in 11 patients (91.7%); specifically, 6 cases (50%) could be linked to pregnancy-related antibodies against paternal HLA mismatches and 5 cases (41.7%) to DSA originating from prior blood transfusions. While no differences were noted between groups regarding the number of HLA mismatches, pregnancies, pre-transplant blood transfusions, and previous transplants, the strength of dominant class I DSA before desensitization was higher in patients with AMR than in patients without AMR ($P = 0.034$) (Table 2). PIRCHE and HLA Matchmaker scores were slightly higher in AMR patients without reaching clinical significance. Kidney transplant recipients who are pre-sensitized with DSA continue to face a higher risk of AMR, even after the disappearance of antibodies through desensitization. The risk of AMR is pronounced in patients with high levels of class I DSA. Larger-scale studies are necessary to demonstrate whether the PIRCHE and HLA Matchmaker scores are valuable in assessing the AMR risk in desensitized patients.

## P103 | Clinical utility of 1:16 serum dilution as a predictor of response to therapeutic plasma exchange for HLA antibody-mediated rejection treatment and overall survival in lung transplant recipients: A two center study

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