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„Rola oceny swoistości przeciwciał anty-HLA w analizie ryzyka immunologicznego u potencjalnych biorców alloprzeszczepu nerki”

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki medyczne

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Streszczenie w języku angielskim

Virtual crossmatch (VXM) is a new powerful tool in pre-transplant risk assessment. However, the ability of VXM to predict physical crossmatch (PXM) results remains controversial. It is also questionable whether all donor-specific antibodies (DSA) detected by the solid-phase single antigen bead (SAB) assay negatively affect kidney transplantation outcomes. The following dissertation deals with both of these issues in the context of the experience of one transplantation center (Infant Jesus Clinical Hospital in Warsaw) and the current rules for the allocation of kidney recipients in Poland.

The aim of the first part of our study was to evaluate the predictive potential of VXM results, measured by SAB, for prediction of CDC-XM (complement-dependent cytotoxicity crossmatch) and FLXM (flow cytometry crossmatch) results of DSA in sensitized patients.

As part of the first objective, 261 CDC-XM and FLXM measurements were performed for 180 potential kidney transplant candidates, each with a single HLA–A, –B, or –DR DSA against a potential deceased donor. Analysis was conducted with two SAB datasets of four-month distant and collected prior to and after PXM results. Optimal MFI (mean fluorescence intensity) thresholds and likelihood ratios were assigned based on low (<2000 MFI), medium (2001–5000 MFI) and high risk (>5000 MFI). The impact of VXM predictability was determined by the ROC curves comparison. In addition, inter-assay changes of MFI were evaluated.

The accuracy of VXM to predict CDC-XM was inferior to that of FLXM with the AUC (area under ROC curve) of 0.644 vs. 0.849 values, respectively. In contrast, the initial ROC analysis showed that the VXM prediction was good for both T-FLXM with ROC value of 0.849 and by B-FLXM with ROC value of 0.706 for a single antigen of HLA–A, –B, or –DR DSA. In fact, the best VXM prediction was for FLXM with good sensitivity for B-FLXM against HLA-DR-specific DSA (0.851). Similar results of VXM predictability were observed for pre- and post-crossmatch ROC curves.

VXM predictability is better for positive/negative FLXM than for positive/negative CDC-XM results to evaluate a single HLA–A, –B, –DR DSA disparity. This may be related to the fact that VXM and FLXM rely on binding of antibodies to beads or cells, respectively. In contrast, VXM is less predictive for CDC-XM because the latter measures complement-dependent cytotoxic function. In order to improve the VXM's ability to predict PXM results, the following modifications may be considered: increasing the sensitivity of CDC-XM, incorporation of a

systematic update of anti-HLA SAB results based on data from the immunization event monitoring system, extending the range of HLA antigen typing in potential donors, and introducing procedures eliminating interferences in the anti-HLA SAB assay.

The subject of the second part of the dissertation was the evaluation of the possible clinical significance of low pre-transplant DSA in living donor kidney recipients. We analyzed a group of patients with HLA–A, –B, and –DR DSA reactivities below a virtual crossmatch (VXM) value of 5000 MFI but with all VXM DSA reactivities at HLA–DQ, –DP, and –Cw, which were not typed routinely for donors prior to transplantation. We also investigated the incidence of persistent and *de novo* DSAs in available posttransplant SAB assays.

From the historical cohort of living donor recipients transplanted between 2014 and 2018 at our center (n = 82), 55 patients met the inclusion criteria, namely: these patients were > 18 years old with non-HLA identical sibling donors, who were not desensitized, who had available pre-transplant SAB results, and who had negative both complement-dependent cytotoxicity crossmatch (CDC-XM) and flow cytometry crossmatch (FLXM) results. An additional donor HLA typing, performed for all 55 recipients, identified donor additional HLA-DQ, -DP, and -Cw DSA reactivities. These patients were then divided by SAB reactivity into three groups: 1) those with DSA-positive reactivities; 2) those with non-donor-specific anti-HLA reactivities (NDSA); and, 3) those who were anti-HLA-negative. All these recipients were followed for three years and checked for their *de novo* or persistent DSA.

In the studied cohort, DSA-positive, NDSA reactive, and anti-HLA negative recipients constituted 33%, 36%, and 31% of 55 patients, respectively. Non-routinely considered pre-transplant HLA-DQ, -DP, and -Cw DSA-positive reactivities were shown in as many as 78% of DSA-positive cases (group 1) with the lowest MFI value of 319 to DP4 and the highest MFI of 5767 to DQ2. Of the pre-transplant HLA–A, –B, and –DR DSA reactivities, only –DR52 DSA reactivity reached the highest MFI value of 2191. These detected DSAs did not reduce the mean estimated glomerular filtration rate (eGFR) values and did not increase the incidence of proteinuria in recipients. While the 3-year graft survival tended to be lower in the DSA-positive group (94.4%) with one recipient who lost kidney transplant, the difference was not significantly different (p = 0.7) from the NDSA (100%) and negative (100%) groups. In terms of the incidence of *de novo* acute antibody-mediated rejection (AMR) at three years after transplantation, no case has been reported in the cohort. This may suggest that low DSA-positive recipients do not

experience higher rejection rate. However, DSA-positive recipients had a tendency for a higher frequency of C4d deposits in peritubular capillaries (PTC) and *de novo* DSA.

Our 3-year follow-up of patients with low pre-transplant DSA found no association with a deterioration in graft function and worse graft survival. Furthermore, we did not observe an increase in AMR in our patients with low DSA. A larger cohort and a longer follow-up period may be needed to evaluate the tendency of low DSA-positive recipients towards the higher incidence of C4d deposits in PTC and/or *de novo* DSA.