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DONOR-SPECIFIC ANTIBODIES AS A PREDICTOR OF GRAFT REJECTION AFTER LIVER TRANSPLANTATION

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The main reason for graft loss is the rejection of the donor organ, which may occur at different time after transplantation and may be caused by the recipient's organism reaction against donor's human leukocyte antigen (HLA) proteins. Donor-specific antibodies (DSA) are produced in patient's organism as a response to foreign HLA antigens. DSA and the percentage of panel reactive antibodies (PRA) are the main indicators of antibody-mediated rejection in solid organ transplantation. Recipients with pre-formed DSA have demonstrated an increased risk of early graft loss [1, 2]. At the same time, it was shown, that *de novo* DSA production accelerates graft loss [3, 4].

Aim. The purpose of our study was to evaluate the effects of already existed and/or *de novo* generated DSAs in liver transplantation as predictors of graft rejection and to establish an interconnection between blood biochemical parameters (Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin level) with the level of DSA in patients with liver transplant.

Methods. Blood for testing was collected to tubes without anticoagulant, centrifugated at 1500 rpm per 10 minutes [5] and processed immediately (for biochemical parameters measurement) or stored at −70 °C (for antibody detection). xMAP-Luminex next generation flow cytometry technology and LABScreen Single antigen beads reagent (Onelambda, USA) were used for anti-HLA determination. Analysis of results with further calculation of PRA percentage for antibodies to Class I & II separately was performed on HLAFusion 4.6 software. Briefly: patient's serum was centrifugated at 10 000 rcf during 10 min, treated with 100 mM EDTA (Invitrogene, USA) and incubated with LABScreen beads, covered with HLA Class I &II antigens. Positive reaction was detected with second anti-human phycoerythrin (PE)-labelled IgG. The presence of antibodies to specific HLA antigen was determined by the numerical value of the mean fluorescence intensity (MFI). Serum was considered positive when PRA value was higher than 0% (PRA > 0%) and the mean MFI was higher than 600 (MFI ≥ 600).

Total bilirubin level was detected photometrically. The activity of ALT and AST was determined spectrophotometrically on the automatic analyzer COBAS C 111 (Roche, Switzerland) in accordance with the manufacture's instruction.

Results and Discussion. The group of examined participants consisted patients diagnosed with different liver diseases (hepatic cirrhosis and biliary atresia). Patients therapy consisted of plasmapheresis (removal of blood plasma components, in particular anti-HLA antibodies), immunosuppressive drug "Tacrolimus" (decreases T-lymphocytes production), "Solu-Medrol" pulse therapy (synthetic glucocorticoid anti-inflammatory drug). It should be noted, that multiple transfusions before transplantation were associated with an increase in DSA and PRA levels, which increases the probability of the formation of DSA in the recipient and subsequent rejection of the transplanted organ (Figure, *A*). The use of the above drugs in various combinations depending on the patient's medical history led to changes in levels of antibodies to HLA antigens and blood biochemical parameters.

Before graft rejection we observed high values of PRA, DSA and increased level of key biochemical parameters in bloodstream, which indicated start of liver disfunction development. It should be noted, that levels of PRA and DSA grew earlier than ALT and AST activity (Figure, *B*) and total bilirubin concentration (Figure, *C*). We can speculate, that PRA increase as well as *de novo* generated

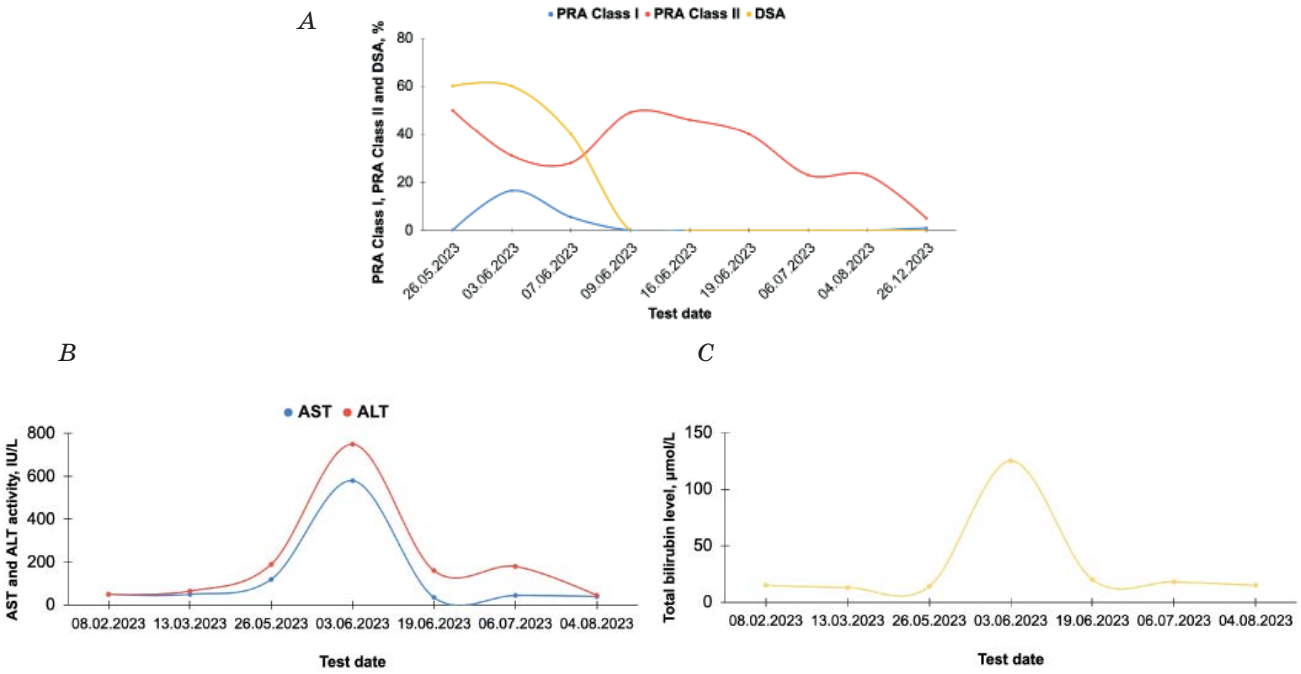

Fig. **Monitoring results of PRA, DSA (*A*), activity of ALT and AST (*B*) and total bilirubin (*C*) in liver transplanted patient**
For this case: 20.07.2022 - liver allotransplantation from a living family donor (brother). 03.06.2023 - liver graft rejection crisis. 05.06.2023 –«Solu-Medrol» pulse therapy.

DSA can be the reason of graft rejection, which was confirmed by the changes of key biochemical markers of liver functioning (Figure 1, *B, C*). We observed directly proportional dependence between biochemical blood parameters and PRA and DSA. They have the same trend of growth and decline depending on the effectiveness of the therapy.

After immunosuppression therapy, DSA were not detected, blood biochemical parameters returned to normal reference values (total bilirubin = 15 µmol/L; AST = 40 IU/L; ALT = 45 IU/L), however, general anti-HLA antibodies level was still present therefore antibody monitoring should be continued to prevent the possibility of graft rejection and to adjust immunosuppression protocols.

Conclusions. The DSA and PRA levels as well as total bilirubin and ALT and AST activity corresponded to each other and could be used for comprehensive both pre- and post-transplantation screening of patients requiring liver transplantation or re-transplantation. Detection of DSA and PRA was important at the same level as measurement of classical biochemical parameters of liver function (ALT, AST etc.) for monitoring of graft status and prevention of acute or chronical rejection and choosing correct immunosuppression protocol.

Key words: HLA antibodies, donor-specific antibodies, transplantation screening.

Authors' contribution. AVK carrying out serological tests (ant-HLA antibody detection), data analysis, writing original draft, NAT determination of blood biochemical parameters (total bilirubin, ALT, AST), AOM conceptualization, data curation, writing original draft, HYA conceptualization, supervision.

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