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Standing at a crossroad: Biomarkers for Rejection or Tolerance

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Standing at a crossroad: Biomarkers for Rejection or Tolerance **Jong Young Choi, M.D., Ph.D.** Liver Transplant Center, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea Liver transplantation (LT) is an eventual treatment for patients with end-stage liver disease or early hepatocellular carcinoma (HCC), improving of hepatic function and treating HCC simultaneously. In the setting of LT, three specific mechanisms that induce tolerance has been outlined as follows: donor passenger leukocytes (PLs), high-dose antigen effect, and the proliferation of Tregs along with the depletion of effector T cells (Tefs). PLs may activate recipient T-cells in lymphoid organs after LT, which induce a defective activation and eventual apoptosis of allo-reactive T-lymphocytes. Moreover, high-antigen dose leads to tolerance by exhausting the finite T cell clone size. Grafting the liver, a large organ, leads to a low density of alloreactive T cells, resulting in exhaustion of T cells and subsequent tolerance. Finally, the proliferation of Tregs and the deletion of Tefs induced by tolerogenic APCs and hepatocytes may induce tolerance in LT patients. With the above unique immunological features in human liver allograft, there have been many studies to withdraw IS (ISW) in LT patients. Classical inclusion criteria for ISW trial are as follows: LT patients with > 3 years post-transplant, normal liver function, no episode of rejection in the past 1 year, and no significant co-morbidities. Approximately, 5% - 20% of selected LT patients could achieve operational tolerance after LT. Tolerance is defined as long-term graft acceptance without IS therapy along with no graft rejection at one year since withdrawal of IS. Clinically, recent study suggested that operational tolerance after LT is associated with time after LT (> 10 years), male, old age, and living donor LT. In early period after LT, as allo-immune response is relatively high, success rate of tolerance is low. Several markers have been suggested as potential biomarkers of tolerance. In animal model, lower level of intrahepatic IL-4 transcripts was observed in tolerated rat liver allografts than in rejected allografts. In another study, upregulated expression of miRNAs, including miRNA-146a, miRNA-15B, and miRNA-451, also suggested as biomarkers for tolerance induction. Recently, a novel set of tolerance biomarker genes, encoding lectin galactose-binding soluble 1, fibrinogen- like protein 2 (FGL2), CD39, phosphodiesterase 3B, killer cell lectin-like receptor G1 (KLRG1), FOXP3 and TGFβ, has also been suggested as potential biomarkers for

tolerance. This novel gene sets showed a maintenance of expression in tolerant patients, which were decreased in rejection. Moreover, in the view of immune cells and mechanisms underlying tolerance, donor-derived dendritic cells (DCs), natural killer T cells, CD4+ regulatory T (Treg) cells play important roles in successfully minimizing IS or tolerance after LT. Indeed, in our previous study, an increase in the Treg cells implies the possibility of depreciation and tolerance during tapering IS, which may be used as biomarker for tolerance. Moreover, in our study, FoxP3+ Treg cells in liver histology were also increased in tolerance patients compared to those in rejection patients. As gut microbiota modulates systemic immune functions along the gut-liver axis, identification of functional microbiome affecting immune homeostasis may provide the possibility of biomarker for assessing immune status and tolerance. Although gut dysbiosis partially recovers within 12 to 24 months post-LT, gut microbial composition of long-term post-LT patients was still different from healthy controls according to our study. In our study, Faecalibacterium was the most decreased in the long-term post-LT patients along with a decrease in Treg with an increase in T helper 17 (Th17) cells, which were recovered by administration of F. prausnitzii and butyric acid in in vitro analysis. Moreover, in tolerant patients, Faecalibacterium was marginally increased, coupled with an increase in Treg cells. These findings provide insight into the potential use of functional microbiomes, especially Faecalibacterium, as a biomarker for assessing immune status and tolerance in long-term post-LT patients. Although several above biomarkers for tolerance have been suggested, further studies for finding biomarkers guiding ISW and tolerance are still needed. Meanwhile, LT patients have a risk of rejection after LT. Although liver is tolerogenic allograft, LT can lead to the activation of effector T cells, effector B cells, and natural killer cells, resulting liver allograft damage. As potential biomarkers for rejection, profile of serum miRNAs showed an association with acute cellular rejection (ACR). Elevated expression of rejection-associated genes, encoding chemokines CXCL10 and CXCL9, IRF1 and STAT1, was also noted in non-tolerant patients compared to tolerant patients, suggesting the possible usage of this novel genes as biomarkers for rejection. Moreover, recent studies revealed the importance of Treg and Th 17 cells in rejection. The population of Th17 in peripheral blood is correlated positively with histologic score of liver tissue in LT patients with rejection; reciprocally, a proportion of Tregs were negatively correlated with rejection severity. An early reduction in the number of Treg cells and an increase in the number of Th17 cells after LT were also associated with acute rejection, which could be a potential biomarker for predicting rejection. There have also been several studies to evaluate microbial changes related to rejection after LT. In the early phase of post-LT, gut dysbiosis could persist and even get worse with a decrease in potentially beneficial genera, including Faecalibacterium, Bifidobacterium, and Lactobacillus. Moreover, in animal model and patients with ACR after LT, there have been an increase in Bacteroides with a decrease in Peptostreptococcus and Faecalibacterium. As this gut microbial imbalance was improved in tolerant patients according to our study, we could assume that functional microbiomes including Faecalibacterium may have potential role as biomarker for rejection and tolerance in LT patients. As cute rejection might increase the risk of graft failure and death in LT recipients, further studies identifying and validating appropriate biomarkers for rejection are urgently needed. In conclusion, the liver is immune tolerogenic organ with the unique immunological feature, which could lead to achieve

tolerance in some selected patients. In contrast, LT patients still have a risk of rejection, particularly in cases when ISs are tapered. Therefore, it is important to find biomarkers for predicting successful ISW in LT patients. Moreover, further research is needed to find and evaluate methods improving immunological balance, which can lead to tolerance, along with reducing the risk of rejection.