Biomarkers of Immunosuppression

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Immunosuppression remains the mainstay of treatment in various liver diseases, particularly in the setting of liver transplantation (LT). The requirement for long-term immunosuppression confers morbidity and mortality caused by toxicity associated with persistent use of these agents. In an era of personalized medical care, there is an ever-growing need to identify more specific markers of the immunosuppression state.

The gold standard to assess the graft status after LT is a liver biopsy, but this is an invasive procedure and is not suitable for continuous monitoring. Currently, immunological monitoring relies mainly on clinical judgment and on immunosuppressive drug levels. However, this provides only a surrogate marker of the actual level of immunosuppression in an individual. Moreover, standard liver biochemical tests have low sensitivity and specificity as markers of graft rejection and show poor correlation with the severity of histopathological findings.

The ideal biomarker should be noninvasive or minimally invasive, rapidly available, sensitive, specific, and cost-effective.¹ Table 1 lists the ideal properties and the common limitations in the identification of a suitable biomarker. Over the last two decades, several promising biomarkers have been identified to help move immunosuppression to a more tailored approach. These can be divided into three types of biomarkers: (1) those reflecting individual response to immunosuppressants, (2) those associated with the risk for rejection (alloreactivity/tolerance), and (3) those associated with graft dysfunction/liver injury. Table 2 summarizes the existing and promising biomarkers of immunosuppression.

BIOMARKERS THAT REFLECT THE INDIVIDUAL RESPONSE TO IMMUNOSUPPRESSANTS

In clinical practice, therapeutic drug monitoring of immunosuppressive drugs is currently based on measuring serum

Abbreviations: ACR, acute cellular rejection; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; ATG, anti-thymocyte globulin; ATP, adenosine triphosphate; CNJ, calcineurin inhibitor; CSC, chemoattractant chemokine; GcfDNA, graft-derived circulating cell-free DNA; HPRT, hypoxanthine guanine phosphoribosyl transferase; IFN- γ , interferon- γ ; IMPDH, inosine-5'-monophosphate dehydrogenase; LT, liver transplantation; 6-MMP, 6-methyl mercaptopurine; 6-MP, 6-mercaptopurine; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; NFAT, nuclear factor of activated T cell; PK, pharmacokinetics; TGN, thioguanine nucleotide; 6-TIMP, 6-thioinosine monophosphate; TPMT, thiopurines methyltransferase; Treg, regulatory T cell; 6-TU, 6-thiouracil; XO, xanthine oxidase.

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TABLE 1. PROPERTIES AND LIMITATIONS OF AN IDEAL BIOMARKER OF IMMUNOSUPPRESSION

Ideal Properties of a Biomarker	Limitations
Noninvasive or minimally invasive	Laborious methods of measurements
Highly sensitive and specific	Poor standardization
Rapidly available	Not cross-validated among laboratories
Not too laborious	Expensive consumables
Cost-effective	Time-consuming
Robust and reproducible	Difficult to automate

drug concentration levels. Table 3 shows target trough levels of tacrolimus and cyclosporine at different time points after LT. However, such pharmacokinetics (PK) monitoring of immunosuppressants may not predict the individual

TABLE 3. TARGET TROUGH LEVELS OF IMMUNOSUPPRESSANTS (RANGE OF TROUGH LEVEL IN ng/mL)

Time Period	Cyclosporine	Tacrolimus
First week	300-350	12-15
First week to 1 month	200-300	10-12
1-3 months	150-250	8-10
3-12 months	100-200	6-8
> 1 year	Can tolerate <100	5-7

effects on immune cells. Therefore, the direct determination of drug targets (e.g., enzyme activity or T cell subsets) as a pharmacodynamics surrogate of the immunosuppressive drug effects may help to better assess the individual response to the immunosuppressant.

TABLE 2. SUMMARY OF EXISTING AND PROMISING BIOMARKERS OF IMMUNOSUPPRESSION

Biomarkers	Clinical Utility	Availability
Drug levels	Drug concentration levels as a marker of immunosuppression (e.g., tacrolimus, cyclosporine)	Commercially available
TPMT genotype/phenotype	Useful to identify TPMT-deficient patients at risk for myelosuppression	Commercially available
Target enzyme: IMPDH	May predict risk for rejection or MPA-associated toxicity	Commercially available
Target enzyme: mTOR	May predict risk for rejection or mTOR inhibitor-associated toxicity	Research tool
NFAT	May assist to identify transplant recipients at higher risk for adverse effects and may be used to better guide CNI therapy	Research tool
CYP3A5 genotype	May assist in determining optimal tacrolimus starting dose	Commercially available
Total lymphocyte/CD3 ⁺ counts	Useful in ATG dose titration	Commercially available
IFN- γ	Predictive of the risk for ACR	Research tool
IL-2	Use for risk stratification and immunosuppression selection	Research tool
CD154	Predictive of the risk for ACR after LT and assists immunosuppression minimization	Research tool
CD26 and CD28 T cell surface antigens	Associated with ACR and/or malignancy after LT	Research tool
Tregs	Predictive of the risk for ACR, can be used to decide immunosuppression conversion	Research tool
Immune cell function assay	Measures intracellular ATP production	Commercially available
Liver function tests (AST, ALT, ALP, bilirubin)	Marker of cell-mediated immune function	Commercially available
Chemokines	Commonly used as a marker of liver injury and hence ACR; however, low sensitivity and specificity	Research tool
GcfDNA	May predict early allograft dysfunction	Research tool
	Early detection of transplant injury ("liquid biopsy"); guide changes in immunosuppression and minimization	Research tool

Abbreviation: mTOR, mammalian target of rapamycin.

FIG 1 Thiopurine metabolic pathway. Abbreviations: HPRT, hypoxanthine guanine phosphoribosyl transferase; 6-MP, 6-mercaptopurine; 6-MMP, 6-methyl mercaptopurine; 6-TIMP, 6-thiouridine monophosphate; 6-TU, 6-thiouracil; XO, xanthine oxidase.

Azathioprine, commonly used in autoimmune hepatitis, is converted to mercaptopurine, which is further metabolized into active thioguanine nucleotides (TGNs). The TGN metabolites act as purine antagonists and induce cytotoxicity and immunosuppression by inhibition of RNA, DNA, and protein synthesis. Genetic polymorphisms in the thiopurines methyltransferase (TPMT) gene are associated with decreased TPMT activity and the development of myelotoxicity because of high TGN metabolite concentrations (Fig. 1). Although pretreatment TPMT determination may not be predictive for toxicity when azathioprine is used in autoimmune hepatitis, such testing is still recommended to identify patients with a TPMT deficiency.²

Determination of inosine-5'-monophosphate dehydrogenase (IMPDH) activity before LT might be useful to identify transplant recipients at higher risk for acute cellular rejection (ACR) or mycophenolic acid (MPA)-associated side effects.³ In the context of calcineurin inhibitors (CNIs), determination of residual nuclear factor of activated T cell (NFAT)-regulated gene expression may help to identify transplant recipients at higher risk for opportunistic infections, malignancy, ACR, and cardiovascular complications, and complement CNI PK to better guide CNI therapy.⁴ In addition, serum creatinine is commonly used to monitor renal function and as a marker of CNI-associated kidney injury.

Pharmacogenetics (PG) is based on the identification of constitutive genetic markers located in the genes influencing drug response. The UGT1A9 gene, which encodes the enzyme uridine diphosphoglucuronate-glucuronosyltransferase 1-9, may serve as a biomarker

to predict initial dosing of MPA in patients cotreated with tacrolimus. IMPDH1 and IMPDH2 genotype may explain some of the variability in the response to and toxicity of MPA. The CYP3A5*3 allele may influence sirolimus PK and may be useful for the initial dose adjustment of sirolimus provided that CNIs are not coadministered.

Anti-thymocyte globulin (ATG) is used for steroid resistant-ACR in LT. It is thought to exert its effects via lymphocyte depletion. Monitoring of total lymphocyte counts, or more specifically CD3⁺ T cells, have been shown to be useful to determine ATG doses.⁵

BIOMARKERS ASSOCIATED WITH THE RISK FOR REJECTION

Cytokines

Multiple cytokines have been implicated in mediating the effector and regulatory effects of the immune response. The impact of immunosuppressive drugs on the levels of cytokines, such as interleukin-2 (IL-2) and interferon- γ (IFN- γ), has shown wide interindividual variability, which suggests that monitoring cytokines may be useful both for predicting the risk for rejection and reflecting personal susceptibility to immunosuppressive drugs. Their levels before and after transplantation can help to identify liver transplant recipients at high risk for ACR.^{6,7}

Other cytokines that have shown promise include IL-4, IL-6, IL-15, IL-18, IL-23, and TNF- α . However, many of these cytokines cannot differentiate between ACR and infections, making their utility limited in clinical practice.

T Cells

T lymphocytes play a central role in the cellular-mediated process of acute rejection after LT. Donor-specific CD154 expression in T cytotoxic memory cells, as well as CD26- and CD28-expressing T cells, have shown correlation with risk for ACR in transplant recipients.⁸

Regulatory T cells (Tregs) are defined by their capacity to suppress effector immune responses, and have been considered as potential biomarkers for LT to both monitor immunosuppression and predict clinical events. Low numbers of circulating Tregs, best characterized phenotypically at present as CD4⁺D25^{high}FoxP3⁺ T cells, before

transplantation may help identify recipients at high risk for ACR.⁹

The immune cell function assay (or ImmuKnow) measures intracellular adenosine triphosphate (ATP) production that occurs in T lymphocytes, and ATP levels have been shown to correlate with T lymphocyte activity and, consequently, cell-mediated immune function. However, a meta-analysis of five studies was not conclusive of the use of this assay to identify LT patients at risk for AR.¹⁰

Robust biomarkers are required in the setting of both spontaneous and induced operational tolerance to identify patients suitable for withdrawal or minimization of immunosuppressive drugs. Using gene expression analysis and cell immunophenotyping, researchers have shown natural killer cells and $\gamma\delta$ T cells to be associated with liver allograft tolerance.¹¹

BIOMARKERS ASSOCIATED WITH GRAFT DYSFUNCTION OR INJURY

Traditionally, the suspicion of ACR is usually driven by the rise of serum liver function tests [bilirubin, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP)] after LT. They are cheap, rapidly processed, and widely available. However, several reports have clearly shown that elevated liver enzymes have a low sensitivity and specificity for ACR and show a weak correlation with the severity of histopathological findings.

Chemoattractant chemokines (CXC), such as CCL-2, CXCL-9, and CXCL-10, are small molecular weight proteins (8-14 kDa) that direct leukocyte navigation and are associated with inflammatory and immune response after transplantation. In LT, high serum concentrations were associated with early allograft dysfunction.¹² Graft-derived circulating cell-free DNA (GcfDNA), a marker of cell death released from necrotic or apoptotic cells in the transplanted organ, is another promising new biomarker of allograft dysfunction.¹³

CONCLUSION

Currently, immunological monitoring in the treatment of liver disease relies mainly on clinical judgment and on immunosuppressive drug levels, without a proper assessment of the real suppression of the immunological

system. In LT, a relatively high proportion of recipients experience under or over immunosuppression, resulting in opportunistic infections, malignancies and toxicity, and rejections, respectively. Therefore, it is crucial to identify potential biomarkers of immune activity, to minimize and tailor immunosuppression.

It is becoming evident that a single biomarker is not able to reflect all of the alterations of the immune system. Hence a panel of different biomarkers will likely be needed to properly evaluate the immunological suppression and to modify treatment according to patient needs. Effectively, truly personalized immunosuppression has the potential to shift emphasis from reaction to prevention and to reduce cost of health care.

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