

Immune monitoring post liver transplant

Siddharth Sood, Adam G Testro

Siddharth Sood, Adam G Testro, Liver Transplant Unit Victoria, Level 8 HSB, Austin Health, University of Melbourne, Melbourne, Victoria 3084, Australia

Author contributions: Sood S and Testro AG were involved in the writing of this manuscript.

Correspondence to: Adam G Testro, Head of Liver Immunology Research, Liver Transplant Unit Victoria, Level 8 HSB, Austin Health, University of Melbourne, Studley Road, Heidelberg, Melbourne, Victoria 3084,

Australia. adam.testro@austin.org.au

Telephone: +61-3-94965353 Fax: +61-3-94963487

Received: November 27, 2013 Revised: January 14, 2014

Accepted: January 17, 2014

Published online: March 24, 2014

Abstract

Many of the causes of short and late morbidity following liver transplantation are associated with immunosuppression or immunosuppressive medications. Current care often involves close monitoring of liver biochemistry as well as therapeutic drug levels. However, the postoperative course following liver transplantation can often be associated with significant complications including infection and rejection, suggesting an inadequacy in current immune function monitoring. Many assays have been tested in the research setting to identify possible biomarkers that may be used to predict clinical events such as acute cellular rejection, and therefore allow modification of a patient's immunosuppressive regimen prior to a clinical event. However, these generally require significant laboratory processing and have had difficulty becoming established in common clinical use outside the research setting. One assay, Cylex ImmuKnow has been food and drug administration approved but has had variable results. In this review we discuss the assays that have been used to assess monitoring of immune function after liver transplantation and consider possible future directions.

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

Key words: Immune function monitoring; Review; Biomarkers; Liver transplantation

Core tip: Although many research assays have attempted to identify potential biomarkers that may be used to monitor immune function after liver transplantation, most require significant laboratory processing and are not clinically feasible. The rejection cascade is complex and not completely understood, with many likely interactions between innate and adaptive immune processes. Therefore, no single test is likely to provide a fool-proof window to the immune response and a combination of assays may be necessary. However, nothing can replace the clinical judgement of an expert transplant clinician for pooling together data to individualize immunosuppression therapy.

Sood S, Testro AG. Immune monitoring post liver transplant. *World J Transplant* 2014; 4(1): 30-39 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v4/i1/30.htm> DOI: <http://dx.doi.org/10.5500/wjt.v4.i1.30>

INTRODUCTION

Although the use of modern immunosuppression has greatly increased the life expectancy of organ transplant recipients, they are not without problems. Mortality within the first year following liver transplantation (OLTx) usually occurs within the first three months with causes including infection, primary graft failure, rejection and technical complications^[1]. Causes of late mortality include cardiovascular disease (9%-22%), *de novo* malignancy (16%-23%), infections (6%-19%), chronic rejection and graft failure (5%-19%) and chronic renal failure (5%-10%)^[2-5]. Many of the causes of short and late mortality following OLTx are related to immunosuppression, with an estimated 40%-70% of all post-transplant mortality attributable to immunosuppression or immunosuppressants^[5,6].

Table 1 Clinically available immune monitoring after adult liver transplantation

	Sensitivity	Specificity
Currently available		
Liver biochemistry	High	Low
Therapeutic drug levels	Low	Low
ImmuKnow	Low	High
Liver histology	Gold standard	Gold standard
Future possibilities		
PlexImmune	Only Paediatric studies published	
? Combination assays		

To minimize side-effects, clinicians often empirically attempt to minimize dosages. Only very few patients are trialed or able to completely withdraw successfully from all immunosuppression. Tailored therapy for each patient, based on a functional measure of their individual immune response, would clearly be preferable to empiric reduction of therapy in all patients^[7].

The challenge in balancing the risks of over and under immunosuppression is complicated by the lack of reliable means of predicting patients' immunosuppressive needs. OLTx in particular, presents unique challenges compared with other solid organ transplants. The liver is an immunotolerant organ but rejection rates remain at 30%-40%^[8-10]. Despite this, some individuals have the potential for complete withdrawal of immunosuppression. Furthermore, the postoperative course after OLTx is often complicated, with biliary strictures and recurrent diseases shrouding the diagnosis of rejection and confusing the management of a patient's immune function post transplant. Therefore, it has long been suggested that we monitor transplant patients for their functional immunity to optimize therapy^[11,12].

An ideal immune function assay would be based on whole blood, require minimal handling, be reproducible and standardized across laboratories, relatively cheap, and offer a rapid turn around that would allow interpretation of results and corresponding adjustments in immunosuppression early enough to prevent complications or drug related side-effects.

Currently available standard of care in most centres to monitor immune function involves liver biochemistry, drug levels and clinical events (Table 1). Several other potential bio-markers and diagnostic parameters have been suggested in order to confront the immune monitoring challenge and are summarized in Table 2. In this review, we examine the current available options for monitoring the immune system after liver transplantation.

LIVER BIOCHEMISTRY

Clinicians have traditionally relied on liver biochemistry (LFT) in making non-invasive assessments regarding graft function after OLTx. An increase in LFTs is seen during rejection but is non-specific and many other important aetiologies need to be considered. These include but are not limited to biliary strictures, hepatic artery

thrombosis, cholangitis, recurrent viral hepatitis and drug induced injuries. There is often a delay between the first LFT abnormality being noted, and patients undergoing a liver biopsy for diagnosis of rejection. It is an imprecise and late marker of graft injury.

THERAPEUTIC DRUG MONITORING

Calcineurin inhibitors

Cyclosporine and tacrolimus are the two commonest drugs used in maintenance following OLTx and inhibit the phosphatase activity of calcineurin through binding of cyclosporine-cyclophilin and tacrolimus-FKBP12 complexes. This inhibits T-cell activation, but because calcineurin and the nuclear factor activated T-cell pathway are not T-cell specific, calcineurin inhibitors (CNIs) are often associated with significant toxicity^[13]. In particular, tacrolimus has high rates of diabetes, while cyclosporin is associated with increased hypertension and dyslipidemia^[14]. Furthermore, both drugs are associated with end-stage renal failure that can complicate up to 20% of patients following OLTx^[15].

Tacrolimus (> 90%) and cyclosporine (> 50%) are concentrated in erythrocytes, and therefore whole blood is used to measure the therapeutic drug levels^[16]. Most centres use an ELISA to measure trough levels of tacrolimus, while large clinical trials of OLTx patients treated with cyclosporine show lower rates of rejection and nephrotoxicity complications with monitoring based on either AUC₀₋₄ or the concentration 2 h following administration^[17-19]. Therefore many units (including our own) perform a level 2 h (C2) following the patient's morning dose.

Setting a therapeutic target for the CNIs has been difficult with standard protocols generalized to managing large number of recipients, but not specific to each patient's individual clinical situation^[20]. CNIs also have a poor dose-level correlation, an unpredictable level-effect association, individual pharmacokinetic differences, and an unclear level-toxicity relationship^[21,22]. Side-effects are seen even with CNI levels below the "therapeutic range"^[23]. Further problems arise as the monoclonal antibodies used to detect certain metabolites may not capture all biologically active forms of the CNIs^[24,25].

Given the level of drug determined by immunoassay is not correlated with immunosuppressive drug efficacy or the level of immunosuppression^[22,26,27] the United States Food and Drug Administration (FDA) has gone so far as to reclassify assays for measuring tacrolimus and cyclosporin blood levels indicating that no suitable therapeutic ranges exist and these tests should not be used alone to adjust drug dosing^[28].

Optimising CNI drug dosing

CNI dosing is impacted by the variable metabolism of the drugs. Tacrolimus is metabolised by CYP3A enzymes in the small intestine and the enzymatic activity can vary by a factor of 5 between patients^[29]. Genetic polymorphisms of CYP3A have shown higher tacrolimus clearance and lower levels in some kidney transplant re-

Table 2 Summary of assays for immune function monitoring

		Advantages	Disadvantages
Antigen-specific assays:	Limiting dilution assays, mixed lymphocyte reactions, ELISPOT	Measure individual antigen specific response	Need donor cells, Laboratory intensive
Antigen non-specific:	ImmuKnow Cytokine levels/polymorphisms Immune competence scores Regulatory T cells (Tregs)	Available, FDA approved Readily available Associated with rejection	Inconsistent results Inconsistent results Lack of published validation studies Laboratory intensive. Lack of published validation studies
Identifying operational tolerant recipients:	Soluble CD30 Tregs, Gene expression, dendritic cell types, delayed type hypersensitivity	Able to identify recipients in whom immunosuppression could be withdrawn	Lack of association with clinical outcomes in OLTx Laboratory intensive. Only few recipients suitable

FDA: Food and drug administration; ELISPOT: Enzyme-linked immunosorbent spots; OLTx: Liver transplantation.

recipients^[30] while attempts to evaluate pharmacodynamics directly through monitoring of CNI biological activity have demonstrated correlation between peak levels of CNIs and residual gene expression (by nuclear factor of activated T-cells), but not clinical events^[31].

High-performance liquid chromatography was developed for evaluating four cyclosporine degradation products and two related compounds (CyB and CyG)^[32]. Initially developed to test quality control of generic formulations, future studies may consider evaluating whether these could have a closer association with outcomes than the cyclosporin blood level^[20].

Other drugs

The CNIs are often used in combination with other immunosuppressants. Steroids and induction agents such as basiliximab (anti-IL2) have no specific monitoring mechanisms apart from side-effects, while the optimal dosing and levels of the mTOR inhibitors remain uncertain.

Even if the biological activity of each individual drug could be accurately determined, this would not provide an objective net biomarker of immune function as the cross-reactive effects of the drugs would remain uncertain. As such, therapeutic drug monitoring may continue to assist clinicians in managing patients, but is unlikely to be the dominant method of future immune system monitoring following OLTx.

Clinical events

One of the major influences on drug dosing and immunosuppression following liver transplantation is the presence of complications. In particular, patients who develop sepsis or malignancy following transplantation often have their immunosuppression empirically reduced. Correspondingly, patients undergoing rejection are treated with increased medication. Clearly this is a crude method of monitoring immunosuppression and the purpose of immune monitoring is to optimise immunosuppression prior to the occurrence of clinical events.

Biopsies

Acute cellular rejection is diagnosed on histology based on the commonly accepted Banff criteria^[33]. Sampling

graft tissue has the further advantage that it can reveal the local ongoing antidonor immune responses^[34] and protocol biopsies provide a more accurate marker of graft function compared to liver biochemistry^[13]. Surveillance biopsies of the transplanted organ may represent the gold standard for directly assessing the extent of immune activity within the allograft. However, serial biopsies are invasive and almost impractical outside of a research setting^[35].

Immune monitoring assays

Although commonly used, the aforementioned tests have significant disadvantages and do not provide an accurate marker of a patient's immune system following OLTx. As a consequence, clinical events and side-effects remain common causes of morbidity and mortality. Many assays have been developed and evaluated with varying results but are yet to achieve use outside of research settings. In general, these assays can be broadly classified as antigen-specific or non-antigen specific and will be discussed below.

ANTIGEN-SPECIFIC ASSAYS

Donor specific assays

Functional donor specific assays may allow detection of immunological states favouring alloimmune quiescence over reactivity^[36]. Functional or cytokine kinetics assays may then be applied to determine preemptively whether immunosuppression dosing should be altered.

Limiting dilution assays (LDA) are an example which can provide more precise quantification of immunity to a given stimulus and allow estimation of frequencies of antigen-specific cells participating in an immune response^[37]. It requires recipient peripheral blood mononuclear cells (PBMCs) interacting with donor stimulator cells. This can then be used to determine production of different cytokines in the presence of supernatant cultures such as interferon-gamma, interleukin (IL)-5, IL-4, IL-10, IL-13 or TNF- α present in the well^[37]. LDA has been employed to show a highly significant correlation between the donor-specific and third-party stimulated IL-4 and IL-10 produced from recipient PBMCs with

stable liver graft function compared with rejectors, independent to level of immunosuppression^[38].

The main limiting step is availability of donor cells that can be difficult to obtain from cadaveric transplants unless cells are harvested at time of surgery from the spleen or lymph nodes and cryopreserved for future donor-specific assays^[20]. Furthermore, the assays often require substantial laboratory work and may need significant amounts of blood and cells for repeated stimulations/experiments.

Mixed lymphocyte reaction

Mixed lymphocyte reaction (MLR) assays provide an estimate of the primary *in vitro* response to the direct recognition of allogenic molecules^[37]. Their main value is in assessing tolerance - that is MLR responsiveness in the face of clinically evident donor-specific tolerance.

Studies with ³H-thymidine mixed leukocyte responses (MLR) show that enhanced donor-specific alloreactivity persists longer among children with early rejection and is associated with early and late liver rejection^[39,40]. To account for the significant variation that is often seen in donor-specific alloresponses, values are often expressed as a ratio to a third-party response known as the immunoreactivity index. A ratio under 1 suggests low rejection risk^[40]. However, this assay is non-antigen specific, requires prolonged stimulation and larger amounts of blood than would be routinely feasible in transplant populations^[41].

Further enhancements to MLR include combination of results with carboxyfluorescein diacetate succinimidyl ester (CFSE) labelling by flow cytometry^[42]. CFSE is an intracellular fluorescent label that divides equally amongst daughter cells and can be used to study cell division^[37]. It measures the proliferative response of recipient lymphocytes after culture or stimulation with donor cells. Unlike many other immune monitoring studies, this has been investigated in an interventional study of 51 adult OLTx recipients. Immunosuppression was increased, decreased or maintained depending on results from the MLR compared with 64 OLTx recipients who had standard of care with empirical based management. This showed trends towards improved rates of rejection and survival, but not sufficient to reach significance ($P < 0.05$)^[42]. A MLR-CFSE assay has also been used to distinguish between rejection on suspicious biopsies^[43].

To overcome the issues of prolonged stimulations and blood sample requirements common in MLR assays, Ashokkumar *et al.*^[41] evaluated a CD154⁺ (CD40L) T-helper and T-cytotoxic cells MLR as measures of rejection risk^[41]. This requires < 24 h of stimulation and only 3 mL of blood. These authors identified pre OLTx CD154⁺ cytotoxic T memory cell responses were associated with significantly increased risk (HR = 7.355, $P = 0.02$) for rejection. This assay can be ordered as PlexImmune™ (Plexison, Pittsburgh, United States) with results in the United States available 2 d after obtaining blood samples. Only small studies have been published to date with PlexImmune in paediatric liver and small intestinal transplant recipients. The assay requires extraction of PBMCs not

only from the recipient but also the donor. In some cases when donor cells have been insufficient or unavailable, “surrogate PBMCs” have been used^[41] but their validity is uncertain in a clinical population.

Enzyme-linked immunosorbent spots

Enzyme-linked immunosorbent spots (ELISPOT) quantifies the frequency of previously activated (memory) T cells that respond to donor antigens by producing a selected cytokine *in vitro*. Recipient T cells are cultured with donor cells on tissue culture plates coated with a cytokine-specific antibody that is detected using labeled secondary antibodies. Each detected spot represents an effector or memory T cell which has been primed to the stimulating antigens^[37].

ELISPOT has been proposed as a surrogate marker of allogenic responsiveness in renal transplantation^[44-46]. Pretransplant IFN- γ ELISPOT has been associated with rejection risk following renal transplant^[44,45,47] which suggests that IFN- γ -producing cells represent cells that have been sensitized to the graft antigens. Thus providing an *ex vivo* reflection of the evolving *in vivo*, donor-reactive immune response which may allow patients without a positive response to reduce or withdraw their immunosuppression^[7]. Apart from IFN- γ , granzyme B (GrB) has been studied in a small number of paediatric OLTx recipients but failed to predict the occurrence of rejection^[48].

The labor-intensiveness and time-consuming nature of these assays, the need for donor cells, the questionable reliability for stored cells along with some inconsistent correlations with clinical outcomes have prevented their broad acceptance as reliable immune monitoring tools^[7,49].

Chimerism

After OLTx, haematopoietic donor cells are transferred with the graft from donor to recipient. These chimeric cells may persist in the recipient and be detectable even years post-transplant^[50]. It has been hypothesised that developing chimerism may be desirable after OLTx and potentially associated with tolerance^[51]. This could allow immunosuppression to be reduced in patients who have detectable chimerism. However, a meta-analysis has failed to demonstrate a significant association between microchimerism and rejection, but techniques of varying sensitivity were used to measure the degree of chimerism^[52]. The value and role of chimerism after liver transplantation remains uncertain, and may also differ depending on the time post-transplant^[53].

ANTIGEN NON-SPECIFIC

ImmuKnow

As immunosuppressive drugs ultimately target T-cell function, it would seem logical that assessing T-cell function would provide a potential biomarker for monitoring immune function after transplantation^[54]. ImmuKnow (Cylex Ltd, United States) was developed as a biomarker

to guide immunosuppressant dosing following solid organ transplantation and was approved by the United States FDA in 2002. ImmuKnow measures adenosine triphosphate produced after stimulation of T-cells with plant lectin phytohemagglutinin (PHA) mitogen^[54]. Whole blood is used to ensure that CNIs are maintained during incubation. After overnight incubation, CD4 cells are selected using paramagnetic particles coated with a monoclonal antibody to CD4^[54]. ImmuKnow does not correlate with CD4 cell numbers, and the assay is theorized to provide an independent variable^[54].

Studies in OLTx recipients have reported contradictory results for ImmuKnow in predicting acute rejection and infection^[55-62]. Most of these studies are retrospective, have limited follow-up, heterogenous in study design, and often include multiple solid organ transplants in the analysis despite immunosuppression protocols and clinical event risks differing substantially amongst different transplant populations.

Further, many of these studies only employ single time point measurements and risk potential bias and the effect of confounders. For example, one study assessing ImmuKnow and infection risk declared lower values in patients who suffer an infection following transplant. However, one of the triggers to run the assay in this study was an event such as fever or raised liver biochemistry^[63]. Furthermore, a single result cannot be expected to predict the long-term immune function of the patient. Ideally serial measures, correlated with changes in immunosuppressant dosing, would be needed to adequately assess the immune response post OLTx.

To coincide with the multiple studies demonstrating conflicting results, there have been two opposing meta-analyses published^[64,65]. One recent meta-analysis by Ling *et al.*^[64] suggests a sensitivity of 0.43 (95%CI: 0.34-0.52) and specificity of 0.75 (95%CI: 0.72-0.78) of ImmuKnow for predicting rejection with a diagnostic odds ratio 1.19 (95%CI: 0.65-2.20). This study incorporated multiple organ transplants and when a sub-analysis of liver transplant patients was conducted, results suggested poor sensitivity but improved specificity (sensitivity 0.11 95%CI: 0.01-0.33, specificity 0.94 95%CI: 0.91-0.95).

A separate meta-analysis in liver transplant recipients identified 4 studies which assessed ImmuKnow for both infection risk and rejection, one further study assessing infection specifically, and a further study examining rejection risk alone. All but one study were retrospective, and in general had small patient numbers with short or undeclared periods of follow-up. In this meta-analysis, the ImmuKnow assay was identified as having a diagnostic odds ratio of 14.7 with sensitivity 83.8% and specificity 75.3% for diagnosing infection. When evaluating rejection, a diagnostic odds ratio of 8.8 (sensitivity 65.6%, specificity 80.4%) was noted alongside significant variation amongst studies included in analysis. In particular, the sensitivity ranged from 9.1%-85.7%^[65].

A possible explanation for the perceived poor sensitivity of ImmuKnow in detecting rejection may be that it relies on T cell stimulation with PHA mitogen, which is a

non-specific antigen that stimulates the adaptive immune system. With the renewed interest in Toll-like receptors, current evidence suggest that the innate immune system also plays a central role in rejection and allorecognition^[66-69]. By only stimulating the adaptive immune system, we postulate that the poor sensitivity may reflect ImmuKnow failing to recognize and therefore measure the contribution made by innate immune mediators to rejection processes.

Clearly there have been issues with several studies that incorporate ImmuKnow. However, the assay is FDA approved and with few other options, the assay is employed in several centres. However, there are often no clear protocols and use varies even amongst individual clinicians in the same centre^[55]. A large, formal, multi-centre randomized controlled trial would resolve many questions regarding ImmuKnow in regards to its ability to be an objective biomarker of immune function in OLTx patients.

Cytokine genetic polymorphisms

Productions of cytokines vary amongst individuals, and detecting possible polymorphisms in the responsible genes could help in stratifying patients for risk of clinical outcomes. However, in a meta-analysis studying the impact of cytokine gene polymorphism on graft acceptance in clinical transplantation, the only genetic risk factor associated with acute liver rejection was IL-10 polymorphism at position 1082^[70] which is associated with low *in vitro* production of IL-10^[71].

Circulating cytokine levels

Circulating cytokine levels have the benefit of being reasonably easy to determine. However, analysis of published clinical studies correlating circulating levels with immunological status after liver transplantation are confusing and often contradictory^[49]. This probably reflects the multitude of confounding factors that impact this patient population, including surgical stress, the associated ischaemia-reperfusion injury, blood transfusions, hepatic regeneration and infectious complications^[72].

Immune competence scores

Some have evaluated multiple factors such as complement and immunoglobulin levels in an attempt to determine an immune competence score to assist in determining risk of infection^[73]. This scoring system assigned two points for each of the following: increased levels of baseline IgG, increased levels of baseline IgA, and decreased levels of pre-OLTx C3. This score was found to have a relative risk of infection of 1.99 ($P < 0.001$) and would be both relatively cheap and employs pathology tests already available in many labs^[73]. However, to our knowledge it has not been validated in larger cohorts and would not take into account the multitude of other factors involved in a patient's immune function after the transplant operation.

Regulatory T Cells (Treg)

In adult allograft recipients there is evidence that Tregs

are involved in transplantation tolerance by directly inhibiting the proliferation of effector T cells. A substantial number of donor Tregs detach from the liver graft during perfusion and continue to migrate into the recipient after OLTx. These suppress the direct pathway alloresponses and are theorized to contribute to chimerism-associated tolerance *in vivo* in the early stage after transplantation^[74].

Lower levels of these regulatory cells have been identified in patients undergoing acute rejection^[75,76] while patients completely weaned off immunosuppression demonstrate higher numbers in their grafts and peripheral circulation^[77-81]. Despite this, Treg analysis still requires significant laboratory work to isolate PBMCs and perform laboratory analysis and are not currently marketed or used in clinical settings that we are aware of.

Soluble CD30

Both CD4 and CD8 cells express CD30 after primary alloantigenic stimulation. Although there is some suggestion that soluble CD30 may be a useful marker in kidney transplantation^[82,83], studies in adult^[84] and paediatric^[85] liver transplantation have failed to reveal a role in predicting rejection outcomes.

Operational tolerance

The liver allograft can often be maintained after transplantation with low levels of immunosuppression and in some cases be withdrawn completely without histological damage from rejection - defined as operational tolerance (OT)^[86]. It is estimated that OT rates after OLTx are as high as 20%-25%^[87,88]. It appears that OT recipients have different cellular immunophenotypic or peripheral blood transcriptional profiles compared with healthy volunteers, recipients on immunosuppression or those experiencing rejection^[80,86]. Several studies have sought to identify which patients are likely to achieve OT which could then facilitate drug withdrawal in this select group.

Gene expression

Martínez-Llordella *et al.*^[89] identified and validated a “tolerant genetic fingerprint” using transcriptional profiling from transplant PBMCs. This identified a modest number of genes capable of identifying tolerant liver recipients with good accuracy. In particular, NK and $\gamma\delta$ TCR⁺ T cells were the main PBMC subsets associated with tolerance-associated transcriptional patterns.

Although transcriptional profiling of peripheral blood may allow identification of some patients capable of completely weaning off immunosuppression, data directly supporting these assays and their ability to monitor the net immunosuppressive state are yet to be published and not available in clinical settings^[20].

Dendritic cells

In humans, 2 major types of blood dendritic cells have been described^[90]. Monocytoid DC (CD11c⁺) can be derived from circulating monocytes in response to granulocyte-macrophage colony-stimulating factor and IL-4 and induce Th1 cell differentiation *in vitro* and may

be specialized for induction of immunity. Plasmacytoid DC (CD123⁺) develop after stimulation with IL-3 and CD125⁺ (CD40L) and promote Th2 responses which can be for induction of tolerance^[91]. The ratio of these cells may be important, with flow cytometry demonstrating operationally tolerant patients exhibiting higher incidence of plasmacytoid dendritic cells (theorised to induce tolerance) compared with myeloid dendritic cells^[92,93].

Delayed-type hypersensitivity

In OLTx patients, the trans vivo delayed-type hypersensitivity (DTH) assay has been shown to be valuable in identifying OT recipients^[94]. This technique involves transfer of PBMCs plus donor antigen in the footpads of naive, severe combined immunodeficiency mice and measuring for response^[94]. This has the advantage of evaluating *in vivo* cell-mediated allogenic immunity without direct exposure of patients^[95]. The logical limitation is the need to have immunodeficient mice available and this makes the assay unfeasible outside research.

Identifying patients who can achieve OT would prove valuable in reducing immunosuppression and related side-effects in these recipients. It would also reduce the ad hoc nature that is sometimes employed to withdraw immunosuppressants following OLTx. However, only a small proportion of patients are likely to have the potential to achieve full operational tolerance and other methods of immune monitoring are therefore needed for the majority of patients.

CONCLUSION

Immune function monitoring following OLTx remains a difficult area, but an area in which even small advances would likely result in significant improvements to morbidity and long-term mortality for patients following liver transplantation today. Many options for immune monitoring have been considered, and vary in methodology from predicting risk of clinical complications, varying dosing of immunosuppressants, and identifying those who may be able to develop operational tolerance.

No single method or assay has been able to meet the diagnostic requirements while answering the basic technical requirements: an assay that is standardized, reproducible, cost-effective, easy and intuitive to perform^[35]. Most vary in degree of promise based on ease of execution, precision, specificity, reproducibility and cost, as well as the type of information they provide^[96]. It is possible that multiple assays or a combination assay may be needed in the same patient at different times to distinguish an accurate immunological profile in the future^[37]. In particular, combining assays from both arms of the immune system (innate and adaptive) may provide clinicians a more comprehensive net immune response of a patient.

Many antigen specific assays also suffer from being based on PBMC which excludes the red cells from. This can pose several issues. Firstly, both the CNIs and mTOR inhibitors are found in whole blood rather than extracted PBMCs, and whole blood has been considered the best

matrix for monitoring immune function^[20,97]. Secondly, extraction of PBMCs is often a process that requires significant laboratory effort and its applicability outside research settings in commercial laboratories would likely be personnel and cost-prohibitive.

Without available objective markers of immune function, drug levels, liver biochemistry and clinical events are often used to guide immunotherapy. This approach is crude and drug side-effects and clinical complications remain common^[63]. Although the ImmuKnow assay offered early promise and is FDA approved, some conflicting results have limited its widespread acceptance. A formal randomised controlled trial would help in answering many questions regarding the assay given the issues in many of the trials previously undertaken.

The rejection cascade is complex and not firmly understood, with many likely interactions between innate and adaptive immune processes. Therefore no single test is likely to provide a foolproof window to the immune response. As such, nothing can replace the clinical judgement of an expert transplant clinician for pooling together data to individualize immunosuppression therapy^[20] but an unmet need exists to measure immune function and assess the risk of clinical complications objectively in OLTx patients^[41].

REFERENCES

- 1 **Abbasoglu O**, Levy MF, Brkic BB, Testa G, Jeyarajah DR, Goldstein RM, Husberg BS, Gonwa TA, Klintmalm GB. Ten years of liver transplantation: an evolving understanding of late graft loss. *Transplantation* 1997; **64**: 1801-1807 [PMID: 9422423]
- 2 **Neuberger J**. Liver transplantation. *J Hepatol* 2000; **32**: 198-207 [PMID: 10728805]
- 3 **Sudan DL**, Venkataramani A, Lynch J, Lynch JC, Fox IJ, Jr, Shaw BW, Langnas AN. Causes of late mortality in survivors of liver transplantation [abstract]. *Transplantation* 1999; **67**: S564
- 4 **Rabkin JM**, de La Melena V, Orloff SL, Corless CL, Rosen HR, Olyaei AJ. Late mortality after orthotopic liver transplantation. *Am J Surg* 2001; **181**: 475-479 [PMID: 11448447]
- 5 **Gelson W**, Hoare M, Dawwas MF, Vowler S, Gibbs P, Alexander G. The pattern of late mortality in liver transplant recipients in the United Kingdom. *Transplantation* 2011; **91**: 1240-1244 [PMID: 21516069 DOI: 10.1097/TP.0b013e31821841ba]
- 6 **Asfar S**, Metrakos P, Fryer J, Verran D, Ghent C, Grant D, Bloch M, Burns P, Wall W. An analysis of late deaths after liver transplantation. *Transplantation* 1996; **61**: 1377-1381 [PMID: 8629300]
- 7 **Gebauer BS**, Hricik DE, Atallah A, Bryan K, Riley J, Tary-Lehmann M, Greenspan NS, Dejeo C, Boehm BO, Hering BJ, Heeger PS. Evolution of the enzyme-linked immunosorbent spot assay for post-transplant alloreactivity as a potentially useful immune monitoring tool. *Am J Transplant* 2002; **2**: 857-866 [PMID: 12392292]
- 8 **Wallia A**, Parikh ND, Molitch ME, Mahler E, Tian L, Huang JJ, Levitsky J. Posttransplant hyperglycemia is associated with increased risk of liver allograft rejection. *Transplantation* 2010; **89**: 222-226 [PMID: 20098286 DOI: 10.1097/TP.0b013e3181c3c2ff]
- 9 **Gómez-Manero N**, Herrero JI, Quiroga J, Sangro B, Pardo F, Cienfuegos JA, Prieto J. Prognostic model for early acute rejection after liver transplantation. *Liver Transpl* 2001; **7**: 246-254 [PMID: 11244167 DOI: 10.1053/jlts.2001.22460]
- 10 **Ziolkowski J**, Paczek L, Niewczas M, Senatorski G, Olszakowska-Jedynak U, Wyzgal J, Foroniewicz B, Mucha K, Zegarska J, Nyczkowski P, Zieniewicz K, Patkowski W, Krawczyk M, Ziarkiewicz-Wroblewska B, Gornicka B. Acute liver transplant rejection: incidence and the role of high-doses steroids. *Transplant Proc* 2003; **35**: 2289-2291 [PMID: 14529918]
- 11 **Schulick RD**, Weir MB, Miller MW, Cohen DJ, Bermas BL, Shearer GM. Longitudinal study of in vitro CD4+ T helper cell function in recently transplanted renal allograft patients undergoing tapering of their immunosuppressive drugs. *Transplantation* 1993; **56**: 590-596 [PMID: 8105569]
- 12 **Ahmed M**, Venkataraman R, Logar AJ, Rao AS, Bartley GP, Robert K, Dodson FS, Shapiro R, Fung JJ, Zeevi A. Quantitation of immunosuppression by tacrolimus using flow cytometric analysis of interleukin-2 and interferon-gamma inhibition in CD8(-) and CD8(+) peripheral blood T cells. *Ther Drug Monit* 2001; **23**: 354-362 [PMID: 11477316]
- 13 **Naesens M**, Sarwal MM. Monitoring calcineurin inhibitor therapy: localizing the moving target. *Transplantation* 2010; **89**: 1308-1309 [PMID: 20458272 DOI: 10.1097/TP.0b013e3181dc13fa]
- 14 **Post DJ**, Douglas DD, Mulligan DC. Immunosuppression in liver transplantation. *Liver Transpl* 2005; **11**: 1307-1314 [PMID: 16237688 DOI: 10.1002/lt.20614]
- 15 **Gonwa TA**, Mai ML, Melton LB, Hays SR, Goldstein RM, Levy MF, Klintmalm GB. End-stage renal disease (ESRD) after orthotopic liver transplantation (OLTx) using calcineurin-based immunotherapy: risk of development and treatment. *Transplantation* 2001; **72**: 1934-1939 [PMID: 11773892]
- 16 **Nagase K**, Iwasaki K, Nozaki K, Noda K. Distribution and protein binding of FK506, a potent immunosuppressive macrolide lactone, in human blood and its uptake by erythrocytes. *J Pharm Pharmacol* 1994; **46**: 113-117 [PMID: 7517447]
- 17 **Thervet E**, Pfeffer P, Scolari MP, Toselli L, Pallardó LM, Chadban S, Pilmore H, Connolly J, Buchler M, Schena FP, Carreño CA, Dandavino R, Cole E. Clinical outcomes during the first three months posttransplant in renal allograft recipients managed by C2 monitoring of cyclosporine microemulsion. *Transplantation* 2003; **76**: 903-908 [PMID: 14508352 DOI: 10.1097/01.TP.0000089006.00653.64]
- 18 **Tanaka K**, Lake J, Villamil F, Levy G, Marotta P, Mies S, de Hemptinne B, Moench C. Comparison of cyclosporine microemulsion and tacrolimus in 39 recipients of living donor liver transplantation. *Liver Transpl* 2005; **11**: 1395-1402 [PMID: 16237691 DOI: 10.1002/lt.20508]
- 19 **Levy G**, Grazi GL, Sanjuan F, Wu Y, Mühlbacher F, Samuel D, Friman S, Jones R, Cantisani G, Villamil F, Cillo U, Clavien PA, Klintmalm G, Otto G, Pollard S, McCormick PA. 12-month follow-up analysis of a multicenter, randomized, prospective trial in de novo liver transplant recipients (LIS2T) comparing cyclosporine microemulsion (C2 monitoring) and tacrolimus. *Liver Transpl* 2006; **12**: 1464-1472 [PMID: 17004259 DOI: 10.1002/lt.20802]
- 20 **Levitsky J**. Next level of immunosuppression: drug/immune monitoring. *Liver Transpl* 2011; **17** Suppl 3: S60-S65 [PMID: 21761551 DOI: 10.1002/lt.22385]
- 21 **Naesens M**, Kuypers DR, Sarwal M. Calcineurin inhibitor nephrotoxicity. *Clin J Am Soc Nephrol* 2009; **4**: 481-508 [PMID: 19218475 DOI: 10.2215/CJN.04800908]
- 22 **Venkataraman R**, Shaw LM, Sarkozi L, Mullins R, Pirsch J, MacFarlane G, Scheller D, Ersfeld D, Frick M, Fitzsimmons WE, Virji M, Jain A, Brayman KL, Shaked A. Clinical utility of monitoring tacrolimus blood concentrations in liver transplant patients. *J Clin Pharmacol* 2001; **41**: 542-551 [PMID: 11361051]
- 23 **Ekberg H**, Tedesco-Silva H, Demirbas A, Vitko S, Nashan B, Gürkan A, Margreiter R, Hugo C, Grinyó JM, Frei U, Vanrenterghem Y, Daloze P, Halloran PF. Reduced exposure to

- calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562-2575 [PMID: 18094377 DOI: 10.1056/NEJ-Moa067411]
- 24 **Jusko WJ**, Thomson AW, Fung J, McMaster P, Wong SH, Zylber-Katz E, Christians U, Winkler M, Fitzsimmons WE, Lieberman R. Consensus document: therapeutic monitoring of tacrolimus (FK-506). *Ther Drug Monit* 1995; **17**: 606-614 [PMID: 8588229]
- 25 **Yatscoff RW**, Copeland KR, Faraci CJ. Abbott TDx monoclonal antibody assay evaluated for measuring cyclosporine in whole blood. *Clin Chem* 1990; **36**: 1969-1973 [PMID: 2242580]
- 26 **Rovira P**, Mascarell L, Truffa-Bachi P. The impact of immunosuppressive drugs on the analysis of T cell activation. *Curr Med Chem* 2000; **7**: 673-692 [PMID: 10702633]
- 27 **Wieland E**, Olbricht CJ, Süsal C, Gurragecha P, Böhler T, Israeli M, Sommerer C, Budde K, Hartmann B, Shipkova M, Oellerich M. Biomarkers as a tool for management of immunosuppression in transplant patients. *Ther Drug Monit* 2010; **32**: 560-572 [PMID: 20814352 DOI: 10.1097/FTD.0b013e3181efb3d2]
- 28 **US FDA Centre for Devices and Radiological Health**. Class II Special Controls Guidance Document: Cyclosporine and Tacrolimus Assays; Draft Guidance for Industry and FDA. In: Devices DoCL. Rockville, USA, 2002
- 29 **Lampen A**, Christians U, Guengerich FP, Watkins PB, Kolars JC, Bader A, Gonschior AK, Dralle H, Hackbarth I, Sewing KF. Metabolism of the immunosuppressant tacrolimus in the small intestine: cytochrome P450, drug interactions, and interindividual variability. *Drug Metab Dispos* 1995; **23**: 1315-1324 [PMID: 8689938]
- 30 **MacPhee IA**, Fredericks S, Tai T, Syrris P, Carter ND, Johnston A, Goldberg L, Holt DW. The influence of pharmacogenetics on the time to achieve target tacrolimus concentrations after kidney transplantation. *Am J Transplant* 2004; **4**: 914-919 [PMID: 15147425 DOI: 10.1111/j.1600-6143.2004.00435.x]
- 31 **Zahn A**, Schott N, Hinz U, Stremmel W, Schmidt J, Ganten T, Gotthardt D, Meuer S, Zeier M, Giese T, Sommerer C. Immunomonitoring of nuclear factor of activated T cells-regulated gene expression: the first clinical trial in liver allograft recipients. *Liver Transpl* 2011; **17**: 466-473 [PMID: 21445930 DOI: 10.1002/lt.22254]
- 32 **Bonifacio FN**, Giocanti M, Reynier JP, Lacarelle B, Nicolay A. Development and validation of HPLC method for the determination of Cyclosporin A and its impurities in Neoral capsules and its generic versions. *J Pharm Biomed Anal* 2009; **49**: 540-546 [PMID: 19124213 DOI: 10.1016/j.jpba.2008.11.027]
- 33 **Demetris A**, Adams D, Bellamy C, Blakolmer K, Clouston A, Dhillon AP, Fung J, Gouw A, Gustafsson B, Haga H, Harrison D, Hart J, Hubscher S, Jaffe R, Khettry U, Lassman C, Lewin K, Martinez O, Nakazawa Y, Neil D, Pappo O, Parizhskaya M, Randhawa P, Rasoul-Rockenschaub S, Reinhold F, Reynes M, Robert M, Tsamandas A, Wanless I, Wiesner R, Wernerson A, Wrba F, Wyatt J, Yamabe H. Update of the International Banff Schema for Liver Allograft Rejection: working recommendations for the histopathologic staging and reporting of chronic rejection. An International Panel. *Hepatology* 2000; **31**: 792-799 [PMID: 10706577 DOI: 10.1002/hep.510310337]
- 34 **Sawitzki B**, Schlickeiser S, Reinke P, Volk HD. Monitoring tolerance and rejection in organ transplant recipients. *Biomarkers* 2011; **16** Suppl 1: S42-S50 [PMID: 21707443 DOI: 10.3109/1354750X.2011.578754]
- 35 **Israeli M**, Klein T, Brandhorst G, Oellerich M. Confronting the challenge: individualized immune monitoring after organ transplantation using the cellular immune function assay. *Clin Chim Acta* 2012; **413**: 1374-1378 [PMID: 22333671 DOI: 10.1016/j.cca.2012.01.033]
- 36 **Levitsky J**. Operational tolerance: past lessons and future prospects. *Liver Transpl* 2011; **17**: 222-232 [PMID: 21384504 DOI: 10.1002/lt.22265]
- 37 **Truong DQ**, Bourdeaux C, Wieërs G, Saussoy P, Latinne D, Reding R. The immunological monitoring of kidney and liver transplants in adult and pediatric recipients. *Transpl Immunol* 2009; **22**: 18-27 [PMID: 19800003 DOI: 10.1016/j.trim.2009.09.008]
- 38 **Chung SW**, Yoshida EM, Cattal MS, Hu Y, Gorczynski RM. Donor-specific stimulation of peripheral blood mononuclear cells from recipients of orthotopic liver transplants is associated, in the absence of rejection, with type-2 cytokine production. *Immunol Lett* 1998; **63**: 91-96 [PMID: 9761370]
- 39 **Sindhi R**, Magill A, Bentejewski C, Abdullah A, Tresgaskes M, Seward J, Janosky J, Zeevi A. Enhanced donor-specific alloreactivity occurs independently of immunosuppression in children with early liver rejection. *Am J Transplant* 2005; **5**: 96-102 [PMID: 15636616 DOI: 10.1111/j.1600-6143.2004.00639.x]
- 40 **Khera N**, Janosky J, Zeevi A, Mazariegos G, Marcos A, Sindhi R. Persistent donor-specific alloreactivity may portend delayed liver rejection during drug minimization in children. *Front Biosci* 2007; **12**: 660-663 [PMID: 17127326]
- 41 **Ashokkumar C**, Talukdar A, Sun Q, Higgs BW, Janosky J, Wilson P, Mazariegos G, Jaffe R, Demetris A, Dobberstein J, Soltys K, Bond G, Thomson AW, Zeevi A, Sindhi R. Allo-specific CD154+ T cells associate with rejection risk after pediatric liver transplantation. *Am J Transplant* 2009; **9**: 179-191 [PMID: 18976293 DOI: 10.1111/j.1600-6143.2008.02459.x]
- 42 **Tanaka Y**, Tashiro H, Onoe T, Ide K, Ishiyama K, Ohdan H. Optimization of immunosuppressive therapy based on a multiparametric mixed lymphocyte reaction assay reduces infectious complications and mortality in living donor liver transplant recipients. *Transplant Proc* 2012; **44**: 555-559 [PMID: 22410068 DOI: 10.1016/j.transproceed.2012.01.038]
- 43 **Tanaka Y**, Ohdan H, Onoe T, Mitsuta H, Tashiro H, Itamoto T, Asahara T. Low incidence of acute rejection after living-donor liver transplantation: immunologic analyses by mixed lymphocyte reaction using a carboxyfluorescein diacetate succinimidyl ester labeling technique. *Transplantation* 2005; **79**: 1262-1267 [PMID: 15880082]
- 44 **Augustine JJ**, Siu DS, Clemente MJ, Schulak JA, Heeger PS, Hricik DE. Pre-transplant IFN-gamma ELISPOTs are associated with post-transplant renal function in African American renal transplant recipients. *Am J Transplant* 2005; **5**: 1971-1975 [PMID: 15996247 DOI: 10.1111/j.1600-6143.2005.00958.x]
- 45 **Nickel P**, Presber F, Bold G, Biti D, Schönemann C, Tullius SG, Volk HD, Reinke P. Enzyme-linked immunosorbent spot assay for donor-reactive interferon-gamma-producing cells identifies T-cell presensitization and correlates with graft function at 6 and 12 months in renal-transplant recipients. *Transplantation* 2004; **78**: 1640-1646 [PMID: 15591953]
- 46 **Hricik DE**, Rodriguez V, Riley J, Bryan K, Tary-Lehmann M, Greenspan N, DeJelo C, Schulak JA, Heeger PS. Enzyme linked immunosorbent spot (ELISPOT) assay for interferon-gamma independently predicts renal function in kidney transplant recipients. *Am J Transplant* 2003; **3**: 878-884 [PMID: 12814480]
- 47 **Näther BJ**, Nickel P, Bold G, Presber F, Schönemann C, Pratschke J, Volk HD, Reinke P. Modified ELISPOT technique—highly significant inverse correlation of post-Tx donor-reactive IFN-gamma-producing cell frequencies with 6 and 12 months graft function in kidney transplant recipients. *Transpl Immunol* 2006; **16**: 232-237 [PMID: 17138059 DOI: 10.1016/j.trim.2006.09.026]
- 48 **Truong DQ**, Cornet A, Wieërs G, Robert A, Reding R, Latinne D. Pre- and post-transplant monitoring of granzyme B enzyme-linked immunosorbent spot assay in pediatric liver recipients. *Transpl Immunol* 2008; **19**: 215-219 [PMID: 18602007 DOI: 10.1016/j.trim.2008.06.001]
- 49 **Reding R**, Gras J, Truong DQ, Wieërs G, Latinne D. The immunological monitoring of alloreactive responses in liver transplant recipients: a review. *Liver Transpl* 2006; **12**: 373-383

- [PMID: 16498661 DOI: 10.1002/lt.20704]
- 50 **Monaco AP.** Chimerism in organ transplantation: conflicting experiments and clinical observations. *Transplantation* 2003; **75**: 13S-16S [PMID: 12819484 DOI: 10.1097/01.TP.0000067945.90241.F4]
- 51 **Mineo D, Ricordi C.** Chimerism and liver transplant tolerance. *J Hepatol* 2008; **49**: 478-480 [PMID: 18644653 DOI: 10.1016/j.jhep.2008.06.021]
- 52 **Sahota A, Gao S, Hayes J, Jindal RM.** Microchimerism and rejection: a meta-analysis. *Clin Transplant* 2000; **14**: 345-350 [PMID: 10945206]
- 53 **Wood KJ.** Passenger leukocytes and microchimerism: what role in tolerance induction? *Transplantation* 2003; **75**: 17S-20S [PMID: 12819485 DOI: 10.1097/01.TP.0000067946.90241.2F]
- 54 **Kowalski R, Post D, Schneider MC, Britz J, Thomas J, Deierhoi M, Lobashevsky A, Redfield R, Schweitzer E, Heredia A, Reardon E, Davis C, Bentlejewski C, Fung J, Shapiro R, Zeevi A.** Immune cell function testing: an adjunct to therapeutic drug monitoring in transplant patient management. *Clin Transplant* 2003; **17**: 77-88 [PMID: 12709071]
- 55 **Cabrera R, Ararat M, Soldevila-Pico C, Dixon L, Pan JJ, Firpi R, Machicao V, Levy C, Nelson D, Morelli G.** Using an immune functional assay to differentiate acute cellular rejection from recurrent hepatitis C in liver transplant patients. *Liver Transpl* 2009; **15**: 216-222 [PMID: 19177434 DOI: 10.1002/lt.21666]
- 56 **Xue F, Dong H, Wu J, Wu Z, Hu W, Sun A, Troxell B, Yang XF, Yan J.** Transcriptional responses of *Leptospira interrogans* to host innate immunity: significant changes in metabolism, oxygen tolerance, and outer membrane. *PLoS Negl Trop Dis* 2010; **4**: e857 [PMID: 21049008 DOI: 10.1371/journal.pntd.0000857]
- 57 **Hashimoto K, Miller C, Hirose K, Diago T, Aucejo F, Quintini C, Eghtesad B, Corey R, Yerian L, Lopez R, Zein N, Fung J.** Measurement of CD4+ T-cell function in predicting allograft rejection and recurrent hepatitis C after liver transplantation. *Clin Transplant* 2010; **24**: 701-708 [PMID: 20047619 DOI: 10.1111/j.1399-0012.2009.01169.x]
- 58 **Mizuno S, Hamada T, Nakatani K, Kishiwada M, Usui M, Sakurai H, Tabata M, Sakamoto Y, Nishioka J, Muraki Y, Okuda M, Nobori T, Isaji S.** Monitoring peripheral blood CD4+ adenosine triphosphate activity after living donor liver transplantation: impact of combination assays of immune function and CYP3A5 genotype. *J Hepatobiliary Pancreat Sci* 2011; **18**: 226-232; discussion 232-234 [PMID: 21165655 DOI: 10.1007/s00534-010-0335-8]
- 59 **Cheng JW, Shi YH, Fan J, Huang XW, Qiu SJ, Xiao YS, Wang Z, Dai Z, Tang ZY, Zhou J.** An immune function assay predicts post-transplant recurrence in patients with hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2011; **137**: 1445-1453 [PMID: 21809031 DOI: 10.1007/s00432-011-1014-0]
- 60 **Hwang S, Kim KH, Song GW, Yu YD, Park GC, Kim KW, Choi NK, Park PJ, Choi YI, Jung DH, Ahn CS, Moon DB, Ha TY, Lee SG.** Peritransplant monitoring of immune cell function in adult living donor liver transplantation. *Transplant Proc* 2010; **42**: 2567-2571 [PMID: 20832545 DOI: 10.1016/j.transproceed.2010.04.040]
- 61 **Zhou T, Xue F, Han LZ, Xi ZF, Li QG, Xu N, Zhang JJ, Xia Q.** Invasive fungal infection after liver transplantation: risk factors and significance of immune cell function monitoring. *J Dig Dis* 2011; **12**: 467-475 [PMID: 22118697 DOI: 10.1111/j.1751-2980.2011.00542.x]
- 62 **Mendler M, Kwok H, Franco E, Baron P, Weissman J, Ojogho O.** Monitoring peripheral blood CD4+ adenosine triphosphate activity in a liver transplant cohort: insight into the interplay between hepatitis C virus infection and cellular immunity. *Liver Transpl* 2008; **14**: 1313-1322 [PMID: 18756485 DOI: 10.1002/lt.21529]
- 63 **Xue F, Zhang J, Han L, Li Q, Xu N, Zhou T, Xi Z, Wu Y, Xia Q.** Immune cell functional assay in monitoring of adult liver transplantation recipients with infection. *Transplantation* 2010; **89**: 620-626 [PMID: 20010326 DOI: 10.1097/TP.0b013e3181c690fa]
- 64 **Ling X, Xiong J, Liang W, Schroder PM, Wu L, Ju W, Kong Y, Shang Y, Guo Z, He X.** Can immune cell function assay identify patients at risk of infection or rejection? A meta-analysis. *Transplantation* 2012; **93**: 737-743 [PMID: 22357178 DOI: 10.1097/TP.0b013e3182466248]
- 65 **Rodrigo E, López-Hoyos M, Corral M, Fábrega E, Fernández-Fresnedo G, San Segundo D, Piñera C, Arias M.** ImmuKnow as a diagnostic tool for predicting infection and acute rejection in adult liver transplant recipients: a systematic review and meta-analysis. *Liver Transpl* 2012; **18**: 1245-1253 [PMID: 22740321 DOI: 10.1002/lt.23497]
- 66 **Testro AG, Visvanathan K, Skinner N, Markovska V, Crowley P, Angus PW, Gow PJ.** Acute allograft rejection in human liver transplant recipients is associated with signaling through toll-like receptor 4. *J Gastroenterol Hepatol* 2011; **26**: 155-163 [PMID: 21175809 DOI: 10.1111/j.1440-1746.2010.06324.x]
- 67 **Land WG.** The role of postischemic reperfusion injury and other nonantigen-dependent inflammatory pathways in transplantation. *Transplantation* 2005; **79**: 505-514 [PMID: 15753838]
- 68 **Kreisel D, Goldstein DR.** Innate immunity and organ transplantation: focus on lung transplantation. *Transpl Int* 2013; **26**: 2-10 [PMID: 22909350 DOI: 10.1111/j.1432-2277.2012.01549.x]
- 69 **Leventhal JS, Schröppel B.** Toll-like receptors in transplantation: sensing and reacting to injury. *Kidney Int* 2012; **81**: 826-832 [PMID: 22297675 DOI: 10.1038/ki.2011.498]
- 70 **Warlé MC, Metselaar HJ, Hop WC, Tilanus HW.** Cytokine gene polymorphisms and acute liver graft rejection: a meta-analysis. *Liver Transpl* 2005; **11**: 19-26 [PMID: 15690532 DOI: 10.1002/lt.20316]
- 71 **Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV.** An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet* 1997; **24**: 1-8 [PMID: 9043871]
- 72 **Asakura T, Ohkohchi N, Satomi S.** Changes of serum cytokines associated with hepatic regeneration after living-related liver transplantation. *Transplant Proc* 2000; **32**: 2199-2203 [PMID: 11120132]
- 73 **Carbone J, Micheloud D, Salcedo M, Rincon D, Bañares R, Clemente G, Jensen J, Sarmiento E, Rodriguez-Molina J, Fernandez-Cruz E.** Humoral and cellular immune monitoring might be useful to identify liver transplant recipients at risk for development of infection. *Transpl Infect Dis* 2008; **10**: 396-402 [PMID: 18657086 DOI: 10.1111/j.1399-3062.2008.00329.x]
- 74 **Demirkiran A, Bosma BM, Kok A, Baan CC, Metselaar HJ, Ijzermans JN, Tilanus HW, Kwekkeboom J, van der Laan LJ.** Allosuppressive donor CD4+CD25+ regulatory T cells detach from the graft and circulate in recipients after liver transplantation. *J Immunol* 2007; **178**: 6066-6072 [PMID: 17475831]
- 75 **Stenard F, Nguyen C, Cox K, Kambham N, Umetsu DT, Krams SM, Esquivel CO, Martinez OM.** Decreases in circulating CD4+CD25hiFOXP3+ cells and increases in intra-graft FOXP3+ cells accompany allograft rejection in pediatric liver allograft recipients. *Pediatr Transplant* 2009; **13**: 70-80 [PMID: 18331536 DOI: 10.1111/j.1399-3046.2008.00917.x]
- 76 **He Q, Fan H, Li JQ, Qi HZ.** Decreased circulating CD4+CD25highFoxp3+ T cells during acute rejection in liver transplant patients. *Transplant Proc* 2011; **43**: 1696-1700 [PMID: 21693260 DOI: 10.1016/j.transproceed.2011.03.084]
- 77 **Koshiba T, Li Y, Takemura M, Wu Y, Sakaguchi S, Minato N, Wood KJ, Haga H, Ueda M, Uemoto S.** Clinical, immunological, and pathological aspects of operational tolerance after pediatric living-donor liver transplantation. *Transpl Immunol* 2007; **17**: 94-97 [PMID: 17306739 DOI: 10.1016/

- j.trim.2006.10.004]
- 78 **Martínez-Llordella M**, Puig-Pey I, Orlando G, Ramoni M, Tisone G, Rimola A, Lerut J, Latinne D, Margarit C, Bilbao I, Brouard S, Hernández-Fuentes M, Soullou JP, Sánchez-Fueyo A. Multiparameter immune profiling of operational tolerance in liver transplantation. *Am J Transplant* 2007; **7**: 309-319 [PMID: 17241111 DOI: 10.1111/j.1600-6143.2006.01621.x]
- 79 **Li Y**, Koshiba T, Yoshizawa A, Yonekawa Y, Masuda K, Ito A, Ueda M, Mori T, Kawamoto H, Tanaka Y, Sakaguchi S, Minato N, Wood KJ, Tanaka K. Analyses of peripheral blood mononuclear cells in operational tolerance after pediatric living donor liver transplantation. *Am J Transplant* 2004; **4**: 2118-2125 [PMID: 15575917 DOI: 10.1111/j.1600-6143.2004.00611.x]
- 80 **Pons JA**, Revilla-Nuin B, Baroja-Mazo A, Ramírez P, Martínez-Alarcón L, Sánchez-Bueno F, Robles R, Rios A, Aparicio P, Parrilla P. FoxP3 in peripheral blood is associated with operational tolerance in liver transplant patients during immunosuppression withdrawal. *Transplantation* 2008; **86**: 1370-1378 [PMID: 19034005 DOI: 10.1097/TP.0b013e318188d3e6]
- 81 **Li Y**, Zhao X, Cheng D, Haga H, Tsuruyama T, Wood K, Sakaguchi S, Tanaka K, Uemoto S, Koshiba T. The presence of Foxp3 expressing T cells within grafts of tolerant human liver transplant recipients. *Transplantation* 2008; **86**: 1837-1843 [PMID: 19104431 DOI: 10.1097/TP.0b013e3181818feb4]
- 82 **Cinti P**, Pretagostini R, Arpino A, Tamburro ML, Mengasini S, Lattanzi R, De Simone P, Berloco P, Molajoni ER. Evaluation of pretransplant immunologic status in kidney-transplant recipients by panel reactive antibody and soluble CD30 determinations. *Transplantation* 2005; **79**: 1154-1156 [PMID: 15880060]
- 83 **Süsal C**, Pelz S, Döhler B, Opelz G. Identification of highly responsive kidney transplant recipients using pretransplant soluble CD30. *J Am Soc Nephrol* 2002; **13**: 1650-1656 [PMID: 12039995]
- 84 **Matinlauri I**, Höckerstedt K, Isoniemi H. High serum soluble CD30 does not predict acute rejection in liver transplant patients. *Transplant Proc* 2006; **38**: 3601-3604 [PMID: 17175343 DOI: 10.1016/j.transproceed.2006.10.063]
- 85 **Truong DQ**, Darwish AA, Gras J, Wieërs G, Cornet A, Robert A, Mourad M, Malaise J, de Ville de Goyet J, Reding R, Latinne D. Immunological monitoring after organ transplantation: potential role of soluble CD30 blood level measurement. *Transpl Immunol* 2007; **17**: 283-287 [PMID: 17493532 DOI: 10.1016/j.trim.2007.01.007]
- 86 **Sánchez-Fueyo A**. Hot-topic debate on tolerance: immunosuppression withdrawal. *Liver Transpl* 2011; **17** Suppl 3: S69-S73 [PMID: 21850680 DOI: 10.1002/lt.22421]
- 87 **Lerut J**, Sanchez-Fueyo A. An appraisal of tolerance in liver transplantation. *Am J Transplant* 2006; **6**: 1774-1780 [PMID: 16889539 DOI: 10.1111/j.1600-6143.2006.01396.x]
- 88 **Orlando G**, Soker S, Wood K. Operational tolerance after liver transplantation. *J Hepatol* 2009; **50**: 1247-1257 [PMID: 19394103 DOI: 10.1016/j.jhep.2009.03.006]
- 89 **Martínez-Llordella M**, Lozano JJ, Puig-Pey I, Orlando G, Tisone G, Lerut J, Benítez C, Pons JA, Parrilla P, Ramírez P, Bruguera M, Rimola A, Sánchez-Fueyo A. Using transcriptional profiling to develop a diagnostic test of operational tolerance in liver transplant recipients. *J Clin Invest* 2008; **118**: 2845-2857 [PMID: 18654667 DOI: 10.1172/JCI35342]
- 90 **Robinson SP**, Patterson S, English N, Davies D, Knight SC, Reid CD. Human peripheral blood contains two distinct lineages of dendritic cells. *Eur J Immunol* 1999; **29**: 2769-2778 [PMID: 10508251 DOI: 10.1002/(SICI)1521-4141(199909)29:]
- 91 **Rissoan MC**, Soumelis V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R, Liu YJ. Reciprocal control of T helper cell and dendritic cell differentiation. *Science* 1999; **283**: 1183-1186 [PMID: 10024247]
- 92 **Mazariegos GV**, Zahorchak AF, Reyes J, Chapman H, Zeevi A, Thomson AW. Dendritic cell subset ratio in tolerant, weaning and non-tolerant liver recipients is not affected by extent of immunosuppression. *Am J Transplant* 2005; **5**: 314-322 [PMID: 15643991 DOI: 10.1111/j.1600-6143.2004.00672.x]
- 93 **Tokita D**, Mazariegos GV, Zahorchak AF, Chien N, Abe M, Raimondi G, Thomson AW. High PD-L1/CD86 ratio on plasmacytoid dendritic cells correlates with elevated T-regulatory cells in liver transplant tolerance. *Transplantation* 2008; **85**: 369-377 [PMID: 18301333 DOI: 10.1097/TP.0b013e3181612ded]
- 94 **VanBuskirk AM**, Burlingham WJ, Jankowska-Gan E, Chin T, Kusaka S, Geissler F, Pelletier RP, Orosz CG. Human allograft acceptance is associated with immune regulation. *J Clin Invest* 2000; **106**: 145-155 [PMID: 10880058 DOI: 10.1172/JCI9171]
- 95 **Carrodeguas L**, Orosz CG, Waldman WJ, Sedmak DD, Adams PW, VanBuskirk AM. Trans vivo analysis of human delayed-type hypersensitivity reactivity. *Hum Immunol* 1999; **60**: 640-651 [PMID: 10439310]
- 96 **Castellaneta A**, Thomson AW, Nayyar N, de Vera M, Mazariegos GV. Monitoring the operationally tolerant liver allograft recipient. *Curr Opin Organ Transplant* 2010; **15**: 28-34 [PMID: 19890211 DOI: 10.1097/MOT.0b013e328334269a]
- 97 **Stenton SB**, Partovi N, Ensom MH. Sirolimus: the evidence for clinical pharmacokinetic monitoring. *Clin Pharmacokinet* 2005; **44**: 769-786 [PMID: 16029064 DOI: 10.2165/00003088-200544080-00001]

P- Reviewers: Abbasoglu O, Avolio AW, Liu F, Pande G, Sackak I, Zocco MA **S- Editor:** Wen LL
L- Editor: A **E- Editor:** Wu HL





百世登

Baishideng®

Published by **Baishideng Publishing Group Co., Limited**

Flat C, 23/F., Lucky Plaza, 315-321 Lockhart Road,

Wan Chai, Hong Kong, China

Fax: +852-65557188

Telephone: +852-31779906

E-mail: bpgoffice@wjgnet.com

<http://www.wjgnet.com>

