

Management of immunosuppressant agents following liver transplantation: Less is more

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Abstract

Immunosuppression in organ transplantation was revolutionary for its time, but technological and population changes cast new light on its use. First, metabolic syndrome (MS) is increasing as a public health issue, concomitantly increasing as an issue for post-orthotopic liver transplantation patients; yet the medications regularly used for immunosuppression contribute to dysfunctional metabolism. Current mainstay immunosuppression involves the use of calcineurin inhibitors; these are potent, but nonspecifically disrupt intracellular signaling in such a way as to exacerbate the impact of MS on the liver. Second, the impacts of acute cellular rejection and malignancy are reviewed in terms of their severity and possible interactions with immunosuppressive medications. Finally, immunosuppressive agents must be considered in terms of new developments in hepatitis C virus treatment, which undercut what used to be inevitable viral recurrence. Overall, while traditional immunosuppressive agents remain the most used, the specific side-effect profiles of all immunosuppressants must be weighed in light of the individual patient.

Key words: Immunosuppression; Orthotopic liver transplantation; Metabolic syndrome; Acute cellular rejection; Hepatitis C virus

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Core tip: The use of immunosuppressive agents is reviewed in the context of the modern post-orthotopic liver transplantation population. The side effects of mainstay immunosuppressive strategies exacerbate some patient pathologies, and combinations of different immunosuppressants could be more specifically tailored to patient needs. Acute cellular rejection and malignant complications are also discussed with respect to im-

munosuppressive strategies. Finally, hepatitis C virus and its impact on immunosuppression is re-evaluated in light of recent developments in viral clearance.

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INTRODUCTION

The use of immunosuppression in organ transplantation was revolutionary for its time, and its results were quickly embraced for their efficacy at suppressing host rejection of a graft.

However, given the increasing impact of metabolic syndrome (MS) as a public health issue^[1], immunosuppression and its side effects may pose a greater risk to patients than rejection of a newly-transplanted organ. In fact, metabolic complications of immunosuppressive therapy were at one point the leading cause of morbidity and mortality for patients following orthotopic liver transplantation (OLT)^[2]. Reduction of immunosuppression is a widely-recognized strategy to addressing this issue^[3].

Cardiovascular disease (CVD) and renal disease account for 19.3% and 6.8% of nonhepatic causes of death in post-OLT patients, respectively^[4]. In patients who survive at least 3 years, non-hepatic cause of death accounts for 58% of all-cause mortality post-OLT^[5]. In their evaluation of liver transplantation as a cardiovascular risk factor, Madhwal *et al*^[6] (2012) found that up to two-thirds of patients develop MS after OLT. Clearly, the trend towards post-transplant metabolic disturbances must be addressed; the first place to start is optimizing post-transplant immunosuppressive therapies^[7].

Further, with recent advances in the treatment of hepatitis C virus (HCV)^[8,9], long-term metabolic complications posed by immunosuppression must be weighed more heavily against the immediate issue of organ rejection. Just as the introduction of immunosuppression made longer-term complications a new focus in transplantation, eradication of this chronic liver infection leaves room to focus on metabolic issues.

BRIEF OVERVIEW OF LIVER IMMUNOLOGY: THE LIVER IS IMMUNOPRIVILEGED

From a physiological standpoint, the liver is one of the first organs exposed to the absorbed contents of the external environment. Handling of newly-acquired blood content immediately after absorption from the external environment necessitates that the liver maintain its own unique balance of immunity vs tolerance. The

special status of the liver as immunoprivileged is well-recognized; for example, there is a paucity of hepatic B- and T-cell mediated autoimmune disease, and some autoimmune hepatitis liver markers are found in healthy and ill people alike^[10]. Further, transplant tolerance is known to occur at greater frequency for liver transplant recipients, compared to other vascularized organ recipients^[11]. At the same time, there remains much to be learned about liver immunology. For example, the role of humoral alloreactivity in ABO-compatible liver transplantation is still being elucidated^[12-14].

The liver is rich with parenchymal hepatocytes, but also contains non-parenchymal immune cells that serve as a first barrier to antigens arriving from portal circulation. Hepatic nonparenchymal cells include the largest population of fixed resident macrophages in the body, Kupffer cells, as well as other reticuloendothelial cells^[15]. The parenchymal hepatocytes further contribute to immunity by secreting 80%-90% of complement components and pathogen-recognition receptors (PRR), as well as synthesizing membrane-bound PRRs to catch portal antigens^[16].

Along with antigens arriving from portal circulation, about 10⁸ peripheral blood lymphocytes pass through the liver every 24 h^[17]. These cells are squeezed through fenestrated capillaries that may open a window to T-cell priming^[18]. The status of the liver as a major reservoir of immune cells has major implications, then, both as far as catching portal circulation antigens as well as immunomodulation.

For example, the privileged status of the liver might be used in the future to induce complete graft tolerance. As a promising example, animal models have shown a lifetime tolerance to liver grafts: In 20% of outbred pig liver recipients, lifetime tolerance can be achieved without the use of immunosuppressants^[19]. The possibility of lifetime tolerance in humans, too, remains optimistic. Ramos *et al*^[20] (1995) review their experience withdrawing immunosuppressive therapy after witnessing patient noncompliance with immunosuppression. They were able to accomplish immunosuppression-free tolerance in 16 patients for 3 to 19 mo, continuing efforts to completely wean 28 patients, but failed in 15 patients without any graft loss or demonstrable loss of function due to rejection.

Not only is the liver self-protective, but there is evidence of immunocompetence conferred to other organs: Simpson *et al*^[21] (2006) showed that patients who receive combined same-donor liver and kidney transplants are immunoprotected compared to patients who receive kidney transplant after liver transplant. The authors speculate that the identical genotypes of the transplanted organs facilitates immunoprotective effects. There is, however, conflicting data: Katznelson *et al*^[22] (1996), comparing incidence of acute rejection between 248 combined liver and kidney transplantations to a control group of 206 kidney-alone transplantations, found that 3-year survival rates are not significantly different.

Despite a key role in immunoregulation generally, human leukocyte antigen (HLA) histocompatibility has little clinical significance to liver allograft outcome^[23,24]. On the other hand, HLA compatibility may play a more subtle role in OLT than we see clinically, where immuno-suppressive regimens may paint broad enough strokes to obfuscate nuances. Neumann *et al.*^[25] (2003) report that HLA compatibility is associated with significantly less acute rejection, but no difference in graft survival. This peculiar behavior of the liver compared to other organs may further reflect the possibility that acute rejection is not as harmful to OLT as previously thought. A better understanding of the mechanisms of liver immunology is necessary to identify how to maximize the utility of histocompatibility^[26].

IMMUNOSUPPRESSANT AGENT OVERVIEW

There are three signal pathways targeted by immuno-suppressive agents. The first is calcineurin-mediated nuclear factor of activated T-cells activation *via* the T-cell receptor (TCR) and CD3 meeting an antigen presented on an major histocompatibility complex (MHC) protein; the second is a B7/CD28 costimulatory signal required for TCR-MHC complex synapsing; and the third signal is mediated by interleukin 2 (IL-2) as a ligand to CD25, through adaptor proteins JAK3 and PI-3K to mechanistic target of rapamycin (mTOR) regulation of cyclin-dependent kinases and cyclins to control the cell cycle^[27].

Figure 1 depicts the process of an antigen-presenting cell (APC) travelling to a lymphoid organ, where it meets T cells in the paracortex. A native T-cell interacts with the APC, and if a set of several interactions and conditions are met, then the native T-cell replicates many times. This is called T-cell activation, and T-cell clonal expansion, and creates numerous effector T-cells that are specific to the antigen originally presented by the APC. These effector T-cells proceed to leave the lymph nodes and head back to the liver, where they can detect antigen and effect an immune response.

Many current immunosuppressive drugs target either those extracellular interactions or the intracellular signals that are highlighted in Figure 1. Calcineurin inhibitors (CNIs) prevent T-cell activation *via* an intracellular pathway, for example; on the other hand, the anti-IL-2 receptor antibodies basiliximab and daclizumab prevent IL-2 receptor activation^[28].

Immunosuppressant drugs can function as depleting agents, or as non-depleting agents. Depleting immuno-suppressive therapies cause destruction of T cells and/or B cells^[27], whereas non-depleting agents affect the immune system by preventing immune cell proliferation.

CNIs have been the mainstay of immunosuppression since they were discovered, and increased 1-year patient and graft survival to greater than 80%^[29,30]. CNIs serve to prevent transcription of the autocrine factor IL-2, preventing cell proliferation. These drugs halt the phosphatase activity of calcineurin, which is an

intracellular signal transduction protein that mediates response to antigenic peptides. CNIs include cyclosporin A (CsA) and tacrolimus (TAC). There is no direct reduction in T or B cell count, so these agents are considered non-depleting.

Second, there is the non-depleting immunosuppressant, mycophenolate mofetil (MMF). MMF is a prodrug of mycophenolic acid (MPA), which inhibits inosine monophosphate dehydrogenase (IMPDH). Because IMPDH catalyzes the rate-limiting step of *de novo* guanosine synthesis, both genetic replication and transcription are inhibited. Further, MPA is five times more effective at inhibiting the type II isoform of IMPDH - the isoform expressed in activated lymphocytes^[31]. Because it is specific to the lymphocyte isoform of IMPDH, MPA prevents lymphocyte proliferation and transcription of activation-associated genes. As far as small molecules go, MMF is one of the more specific immunosuppressant agents. Side effects of MMF include nonimmune issues such as diarrhea and anemia^[27], but, more importantly, exacerbation of cytomegalovirus infection^[32].

Finally, the third major class of immunosuppressive drugs are called mTOR inhibitors. Found in soil from an Easter Island bacteria called *Streptomyces Hygroscopicus*, this class blocks IL-2-mediated autocrine leukocyte proliferation *via* inhibition of an intracellular signal transduction mechanism - thus, mTOR inhibitors are considered non-depleting agents^[33,34]. Everolimus (EVR) and sirolimus (SIR) are the two best-known mTOR inhibitors.

Besides their use as immunosuppressants, another interesting property of mTOR inhibitors is their effect on longevity and age-related diseases. It is well-established that one way to increase longevity is through dietary restriction, and this effect is partially mediated by the mTOR pathway. Inhibition of mTORC1 has been associated with protection against neurodegenerative disease, heart disease, metabolic diseases, and a host of other age-related diseases^[35]. Rapamycin administration, specifically, has repeatedly been shown to increase both mean and median lifespan in genetically heterogenous mice^[36].

Immunosuppressive steroids such as methylprednisone are considered essential to graft tolerance induction, yet their use is highly associated with metabolic complications^[37]. Several studies have examined outcomes of steroid-free or reduced-steroid immunosuppressive maintenance regimens^[38,39]. In a prospective, randomized, placebo-controlled, double-blinded study, Lerut *et al.*^[40] (2014) report that in a cohort of 156 patients, patients treated with minimal or steroid-free immunosuppression displayed excellent outcomes over a period of 5 years. They report 5-year biopsy results, finding that histology presents similarly across both groups. In support of steroid withdrawal, other prospective studies of prednisone withdrawal post-OLT have demonstrated no difference in 2-year and 1-year survival of patients treated with or without

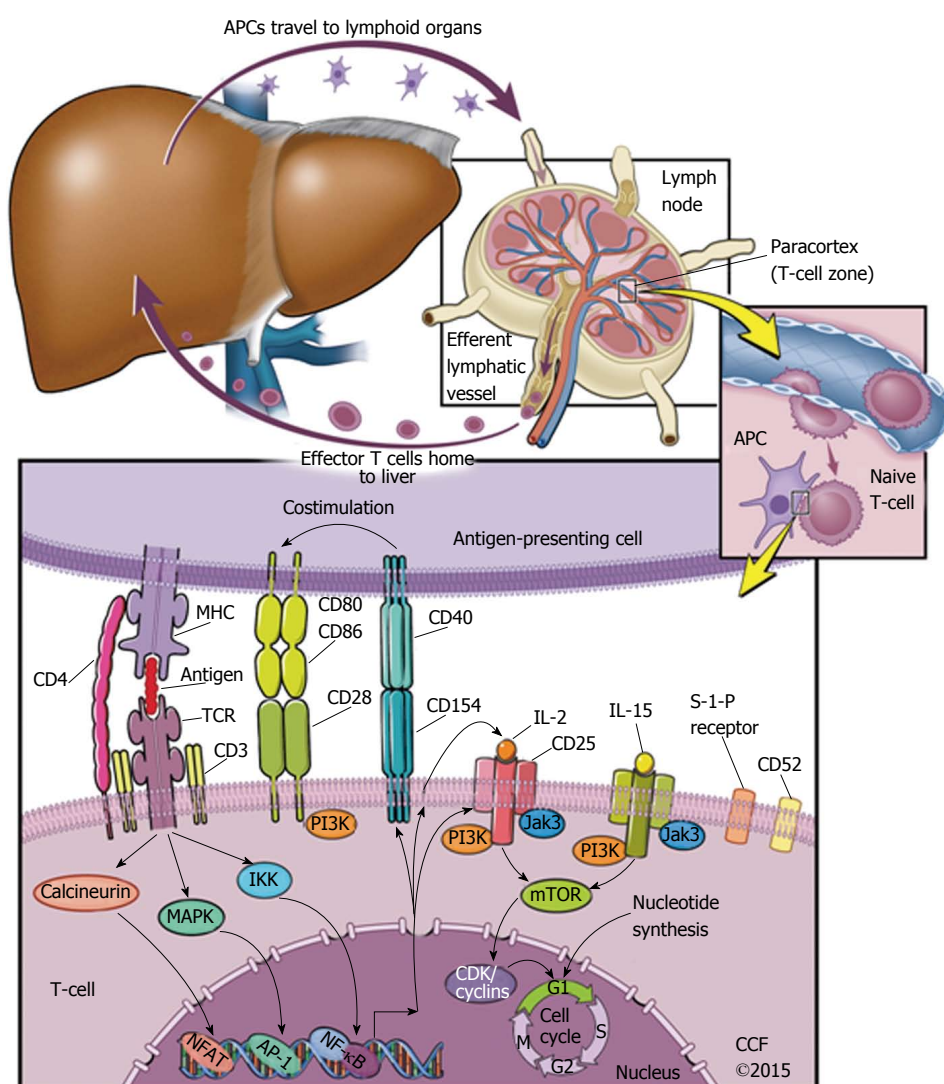


Figure 1 Signaling pathways targeted by modern immunosuppressive agents. Starting from the top left, an antigen-presenting cell (APC) migrates from local tissue to lymphoid organs. In the paracortex of the lymphoid organ, the APC meets a naive T-cell. If the naive T-cell has a T-cell receptor (TCR) that binds the antigen as it is presented by a Major Histocompatibility Complex on the APC, a set of other T-cell-APC interactions are likely to follow. This includes T-cell CD4 binding to the MHC on an APC, as well as costimulatory signals via extracellular receptors CD28 or CD40. After this set of T-cell-APC interactions begins, a set of intracellular signals follow towards the nucleus of the T-cell. The naive T-cell is then activated, and begins to replicate. This replication is called clonal expansion, and produces a population of T-cells that eventually migrate back to the tissue that contains antigen. AP-1: Activator protein 1; CD: Cluster of differentiation; CDK: Cyclin-dependent kinase; IKK: Inhibitor of kappa-B kinase; IL: Interleukin; Jak3: Janus-associated kinase 3; PI3K: Phosphatidylinositol 3-kinase; MAPK: Mitogen-activated protein kinase; mTOR: Mechanistic target of rapamycin; NFAT: Nuclear factor of activated T-cells; NF- κ B: Nuclear factor kappa-light-chain-enhancer of activated B cells; TCR: T-cell receptor; MHC: Major histocompatibility complex.

prednisone^[41,42]. Today, the use of steroids is generally limited to tolerance induction and treatment of acute cellular rejection (ACR); many studies have further investigated the possibility of steroid-free transplantation^[43-45].

Depleting immunosuppressant agents are mostly antibody-based. The first monoclonal antibody to be approved for use in humans was OKT3, an anti-CD3 antibody that modulates T-cell activation^[46,47]. There are currently a host of biologics directed against T-cell proliferation, these are specific enough that their metabolic impact is significantly less than that of CNIs^[48,49].

Issues associated with biological immunosuppression are unlike those of smaller molecules because they act extracellularly and are specific to their antigen^[50], in

contrast to small molecules such as tacrolimus that affect intracellular processes across a wider variety of cells. For example, one OKT3 side effect is called cytokine release syndrome, where widespread T-cell activation outweighs the antibody-mediated T-cell destruction that follows it^[51]. This complication can become more severe if it leads to a positive-feedback loop, potentially causing a "cytokine storm"^[52].

Besides OKT3, biologic agents have shown varying degrees of success and outcomes. For example, basiliximab is an IL-2 receptor antagonist that reduces rates of ACR at the expense of increased disease progression in HCV liver transplant patients^[53]. Basiliximab, though, has been found to successfully treat graft-vs-host disease^[54]. For those patients with

metabolic dysfunction, avoidance of steroid immunosuppression may be enough of a concern to warrant use of basiliximab.

Another biologic used to treat multiple sclerosis and Crohn's disease, natalizumab, is associated with significant liver injury as a side effect^[55]. While the medical field is ripe for biologic development, implementation of biologics requires close evaluation.

On the other hand, some biologics open doors that might otherwise stay shut. Rituximab is an anti-CD20 monoclonal antibody that has been successfully used as immunological prophylaxis for ABO-incompatible (ABOi) liver transplantation^[56]. Further, Yoshizawa *et al.*^[57] (2005) report that ABOi living-donor liver transplantation is possible without humoral rejection. Their protocol involves hepatic artery infusion and prophylactic use of rituximab, but, unlike previous attempts, does not involve splenectomy. Tanabe *et al.*^[58] (2010) later explain that ABOi has progressed to the point that it is as effective as ABO compatible transplantation, in part due to rituximab prophylaxis. Perfection of ABOi OLT will hopefully lead to other organ allocation advancements, such as humanized livers grown in non-human primates^[59]. Immunosuppression in this context may become a hot topic in coming years.

ACR: DIAGNOSIS AND TREATMENT

ACR is most likely to occur within the first 6 wk of transplant, and is a very common event; in a study of 762 patients, the incidence of ACR post-transplantation is 64%. Several factors are associated with the occurrence of ACR, such as cold ischemic time of the organ, lower age of recipient, presence of edema, and HLA-DR mismatch^[2].

There are three distinguishing features of ACR, each visible on histological examination. The first feature is portal triad inflammation, indicated by mixed inflammatory infiltrate; the second feature is damage to the bile ducts, specifically nonsuppurative cholangitis involving interlobular ductal epithelia; third, venous endotheliitis^[60]. Venous endotheliitis is the most reliable diagnostic sign of ACR, but it is worth noting that phasic increase and decrease in lymphocyte aggregation may affect biopsy results^[2].

In the clinic, ACR is suspected upon elevated liver function tests preceding jaundice and fever. Unfortunately, blood tests are neither sensitive nor specific for ACR diagnosis^[61,62]. It follows, then, that liver biopsy is the gold standard of liver tissue evaluation^[63]. To provide a level of standardization to biopsy evaluation, the Banff rejection activity index (RAI) assigns a score between zero and three to each characteristic of ACR^[64].

Treatment for ACR includes high-dose steroids, optionally tapering off steroids and/or using biological immunosuppression^[65]. Response to steroids is favorable; however, incidence of steroid-resistant rejection has been found to reach up to 14%^[66].

ACR MORBIDITY AND MORTALITY

Timing of rejection is of major clinical significance in evaluating the potential impact of ACR^[67,68]. While different studies have used different definitions, one commonly accepted cutoff defines early and late ACR as occurring within and after 90 d of transplantation, respectively. Several studies have found that early ACR is common and of lesser significance than late ACR^[69]. For example, in a retrospective review of 231 histologically-confirmed cases of early ACR, Höroldt *et al.*^[64] (2006) report that neither total RAI score nor any of its components were correlated to steroid treatment response or graft survival. Indeed, Thurairajah *et al.*^[70] (2013), in a retrospective review of 970 patients, confirms that early acute rejection cases yield the best 10-year graft survival rates, at 85%.

In contrast to the minimal impact of early ACR, it appears that late ACR is associated with decreased graft survival. Uemura *et al.*^[69] (2008) found that of 1604 patients, 19.0% developed ACR later than 6 mo after the transplant; the only predictor of late ACR here was post-transplant lymphoproliferative disease. Thurairajah *et al.*^[70] (2013) found that 11% of patients developed late ACR, and that the highest rates of late ACR were found in patients with seronegative hepatitis, primary biliary sclerosis, and primary sclerosing cholangitis. Other studies have found that post-transplant lymphoproliferative disorder, decreased age, and increased medication level variability index are associated with and can predict late ACR^[71].

Epidemiological evaluation of ACR is masked by its frequently subclinical character. Bartlett *et al.*^[72] (2002) explain that in a retrospective review examining 15 studies with a total of 1566 patients, 32% of standard protocol post-OLT biopsy samples showed evidence of ACR without any biochemical dysfunction. Since ACR is defined according to biopsy but not serum biomarkers, incidence of ACR may be higher than previously accepted. Another study, Tisone *et al.*^[42] (1999), explains that 80% of acute rejection episodes in their 45-patient cohort resolved spontaneously. The chances of clinically significant acute rejection, then, must be balanced against the risks of liver biopsy. In the future, metabolomic and other noninvasive studies could shed significant light on incidence of ACR^[73-75].

Reduction of immunosuppression in light of ACR is not a new subject: Volpin *et al.*^[76] (2002), in a controlled study, evaluated two methylprednisolone regimens in the treatment of acute cellular rejection. They find that a 6-d taper regimen is safer than the higher-dose standard because ACR impact on graft rejection is minor, and the toxic effects of methylprednisolone outweigh the potential benefit of ACR suppression. Goddard *et al.*^[77] (2002) reviewed the Volpin study, concluding that immunosuppression therapies should be tailored to the individual patient after careful consideration of the interaction between past medical history and

immunosuppression side effects.

More recently, Rodríguez-Perálvarez *et al.*^[78] (2013) report that standard TAC trough concentrations are set too high (at 10-15 ng/mL), and that target TAC levels between 7 and 10 ng/mL are associated with longer graft survival while maintaining safety against rejection.

In contrast to the safety of simply reducing TAC, a randomized prospective trial of SIR monotherapy conversion regimen efficacy^[79] found that liver transplant patients have no demonstrable benefit at 12 mo. Cumulative rates of graft loss or death were not significantly different, at 6.6% for the SIR group vs 5.6% for the CNI group. However, rates of acute rejection and discontinuation due to side effects were higher for patients treated with SRL. Then, one must consider the characteristics of the individual patient when designing an immunosuppressive regimen. For patients who are at great risk of end-stage renal disease (ESRD), the risk of acute rejection that is posed by conversion to SRL may be outweighed by the nephrotoxicity associated with the use of CNIs. For patients who are more concerned about acute rejection than nephrotoxicity, it makes sense to use CNI therapy.

MALIGNANT COMPLICATIONS POST-OLT

Recurrent and *de novo* malignancies are the top nonhepatic causes of late death in liver transplant patients, often listed alongside CVD. Some of the increased incidence in *de novo* malignancies in liver transplant recipients compared to the general population can be attributed to the use of exogenous immunosuppression^[80,81].

The greatest incidence of post-transplant malignancies is associated with chronic viral infection. Specifically, Epstein-Barr virus-associated post-transplant lymphoproliferative disease, skin cancers, squamous cell carcinoma, and Kaposi's sarcoma are associated with status post-OLT^[82]. Baccarani *et al.*^[82] (2009) find that 42 (12.8%) of patients undergoing OLT, out of 330, developed *de novo* cancers. Further, these patients had a lower 10-year survival rate than those who did not develop *de novo* cancer.

In hopes of reducing malignancy, current immunosuppression strategies focus on minimizing TAC with optional use of mTOR inhibitors or MMF. mTOR inhibitors are known for their antineoplastic activity^[83], and CNI use can be associated with increased development of malignancy^[84], making CNI reduction and replacement with mTOR inhibitors particularly favorable for patients at risk for malignancy.

MS

MS constitutes a number of symptoms that, when occurring simultaneously, indicate a primary clinical outcome of CVD. The criteria for MS is that a patient meet three of five components: Abdominal obesity

and visceral fat, increased triglyceride (TG), decreased serum high-density lipoprotein, high blood pressure (HTN), insulin resistance and/or glucose intolerance^[85]. Despite the wide range of systemic effects, each of these symptoms converges towards provoking CVD^[86].

The Framingham study^[87] found that 25% of all new-onset CVD could be predicted by presence of MS. More recently, Watt *et al.*^[4] (2010) report that causes of death more than 1 year after OLT have the following etiologies: 28% hepatic, 22% malignancy, 11% cardiovascular, 9% infection, and 6% renal failure. They conclude that modifiable risk factors such as diabetes, hypertension, and renal insufficiency can be used to improve long-term outcomes. Table 1 describes some of the interplay between MS components and immunosuppression. Because immunosuppressive strategies can sometimes be altered, relative risks and benefits should be weighed on a case-by-case basis.

Obesity: The boss of MS

Abdominal obesity is a prevalent characteristic of MS, and adipocyte dysfunction is hypothesized to underlie many metabolic disorders. Some explanations of adipocyte metabolism focus on the location of fat as a determinant of metabolic properties, such that visceral or abdominal fat might contribute more to MS than subcutaneous fat^[88]; other explanations emphasize immunological modulation of adipocyte metabolism^[89]. Whatever the underlying cause, obesity is a major public health issue^[90], one that may be an environmental hit to a genetic predisposition.

Stegall *et al.*^[91] (1995) report that the incidence of obesity for adult liver transplant recipients 1 year after transplant was 41.9% for women, and 39.3% in men. In a more recent article, Richards *et al.*^[92] (2005) found that by one and 3 years after liver transplant, 24% and 31% of patients met criteria for obesity as body mass index (BMI) > 30 kg/m².

Despite clear evidence that obesity reduces graft and patient survival^[93,94], there are studies that dispute its independent predictive power. Leonard *et al.*^[95] (2008) found that in a cohort of 1313 patients, obesity does not independently correlate to risk of graft or patient survival. Perhaps a measure of obesity is not enough, and there are more specific characteristics of excess adipose tissue that lead to it being a risk factor.

Després *et al.*^[96] (2006) explain that the deposition of visceral fat, as opposed to normal subcutaneous fat, can lead to adipose tissue overflow and hormonal imbalance. The net effect of these factors is an increase in ectopic fat to muscle, liver, and epicardial tissue. Compounding this issue, visceral adipocytes are less responsive to insulin, and thus not subject to the antilipolytic effects of insulin^[97].

While the anatomical location of an adipocyte may be illuminating, physiologic factors also demonstrate the heterogeneity of metabolic dysfunction: Abnormal fat can accumulate as a result of defects in nuclear receptor genes involved in lipid sensing, synthesis, and

Table 1 Summary of immunosuppressant effects on metabolic syndrome

	Calcineurin inhibitors	Mycophenolate mofetil	mTOR inhibitors	Steroids
Body mass	Increase ^[92,103]	No change ^[105]	Less weight gain than CNIs ^[37]	Increase ^[144,145]
Dyslipidemia	Increase ^[104,121]	Less than CNIs ^[115]	Increase, but anti-atherosclerotic ^[144]	Increase ^[145]
Hypertension	Increase ^[107]	Less than CNIs ^[115,144]	No difference from CNI ^[146]	Increase ^[145]
Insulin resistance	Increase ^[128]	Potential benefit ^[145,146]	Potential benefit ^[149,150]	Increase ^[145]
Renal damage	Increase ^[112,113]	Less than both CNIs and mTOR inhibitors ^[147,151]	Decrease compared to CNIs ^[152]	Not significant
Note	Neoplastic ^[84]	Leukopenic ^[147,153,154]	Antineoplastic ^[155]	

CNIs: Calcineurin inhibitors; mTOR: Mechanistic target of rapamycin.

oxidation^[98]. Supporting the ectopic fat hypothesis, Porter *et al.*^[99] (2009) report that analysis of 3000 Framingham study participants indicated that abdominal subcutaneous fat had no corresponding linear increase of obesity-associated risk factors.

Further complicating the issue is what type of lipids are most abundant in a dysfunctional metabolic state. In the context of non-alcoholic steatohepatitis/nonalcoholic fatty liver diseases, it has been suggested that hepatocyte TG accumulation may be protective against free fatty acid (FFA)-induced oxidative lipotoxicity^[100]. As far as dietary fats go, there is evidence that unsaturated fats contribute to this lipotoxicity, whereas saturated fats are actually hepatoprotective^[101].

Each of these issues - lipid distribution and lipid metabolism - is a qualitative issue that is outside the measure of BMI. Then, a more specific measure of adipose metabolism is necessary to further individualize treatment options for post-OLT patients. Given the heterogeneity of adipose metabolism, some authors suggest adipocyte transplantation as a treatment for metabolic issues such as diabetes, atherosclerosis, and nonalcoholic steatohepatitis^[102].

Not only is obesity incident to the population of liver transplant recipients, it is exacerbated by the effects of immunosuppression^[92]. A mainstay of immunosuppression, steroids, are well-known for effects on weight gain^[103]. CNIs are also associated with weight gain: Ersoy *et al.*^[103] (2008) report that weight gain at 12 mo for renal transplant recipients prescribed TAC and CsA was 3.5 and 8.0 kg, respectively. As far as changing immunosuppressive regimens, it appears that using the mTOR inhibitor EVR in combination with a reduced dose of TAC can cause less weight gain than full-dose TAC immunosuppression^[104]. If mTOR inhibitors are inappropriate for the specific patient, MMF is a different potential substitute that is not associated with post-transplant weight gain^[105].

Hypertension and renal insufficiency

HTN and subsequent renal insufficiency are major concerns for post-OLT patients. Increased blood pressure and systemic vascular resistance is pathological, and can lead to hepatorenal syndrome. The use of immunosuppression, specifically CNIs, compounds the metabolic issues already present in liver transplant recipients^[106,107].

The incidence of severe renal dysfunction can

reach up to 18.1% at a mean of 13 years post-OLT^[108]. Longenecker *et al.*^[109] (2015) recently reviewed renal function before and after OLT, finding that the overall rate of progression to ESRD is strongly associated with estimated GFR (eGFR) less than 60 mL/min × 1.73 m² and diabetes, but find that eGFR at OLT is not associated with 12-mo mortality.

For patients presenting with proteinuria as a result of HTN due to renal-insufficiency, treatment includes standard approaches such as angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers^[86,110].

Aside from the standard of care for MS, the specific treatment of post-OLT patients with renal insufficiency should include reduction or withdrawal of CNI therapy as soon as possible because of the nephrotoxicity associated with CNIs^[111,112]. Masetti *et al.*^[113] (2010) evaluated whether early withdrawal of CsA followed by initiation of EVR monotherapy preserves kidney function compared to their standard CsA regimen. The study found that incidence of stage 3 chronic kidney disease (< 60 mL/min GFR) at 1 year was significantly higher in the standard CsA group (55%) than in the group treated with EVR monotherapy (15.4%). Further, there was no difference in patient survival between the two groups.

Saliba *et al.*^[104] (2013) found that TAC reduction with addition of EVR were associated with increased estimated GFR, demonstrating a significant benefit to renal function. Tsai *et al.*^[114] (2009) confirm that, for renal allograft recipients, CNI minimization with the introduction of SRL reduces acute rejection and improves renal function and survival.

CNI withdrawal and replacement with MMF is another promising approach to post-OLT immunosuppressive management in patients who have renal insufficiency. Orlando *et al.*^[115] (2007) report success with MMF monotherapy as a means of reducing the toxic effects of CNIs. Of 42 post-OLT individuals who were weaned off of CNI therapy and placed on subsequent MMF monotherapy, renal function improved in 89% and arterial hypertension decreased in 80% of cases. In a separate study examining post-OLT patients with severe side effects from CNI therapy, Dharancy *et al.*^[116] (2009) found that a switch to MMF monotherapy could lead to increased eGFR without significant increase in rejection. Several studies have concluded that MMF is

less nephrotoxic, indicating that MMF could be used preferentially in patients with renal dysfunction^[117,118].

Dyslipidemia

Incidence of dyslipidemia exceeds 70% and 40% for patients with and without pre-transplant MS, respectively^[119]. Because MS affects a such great proportion of OLT patients^[7], methods of preventing or reducing dyslipidemia could benefit a preponderance of patients.

In an evaluation of the metabolic impact of OLT, Luzi *et al.*^[120] (1996) reported that liver transplant recipients had abnormal FFA levels at 5 mo post-OLT. A follow-up at 26 mo found reduction in abnormal lipid and protein metabolism - in fact, plasma free fatty acids were reduced for transplant recipients with respect to the control group.

CNI therapy is associated with dyslipidemia post-OLT; but the level of dyslipidemia might be reduced upon use of mTOR inhibitors in combination with TAC^[121]. Saliba *et al.*^[104] (2013) report that hyperlipidemia was more frequent in patients on EVR + reduced dose TAC compared to patients on only full dose TAC. In spite of an increase in dyslipidemia, mTOR inhibitors do seem to decrease arteriovascular plaque formation^[122].

Orlando *et al.*^[115] (2007) found that CNI withdrawal and subsequent replacement with MMF improved dyslipidemia. Out of 41 patients, blood cholesterol decreased in 76% and blood TG decreased in 89%. Further supporting the use of MMF in patients who are at-risk for complications of atherosclerosis, Romero *et al.*^[123] (2000) also reported that MMF specifically reduces atherosclerosis in rabbits.

For patients with hyperlipidemia who are resistant to lifestyle changes, hydroxymethylglutaryl-CoA reductase inhibitors (statins) should be considered. Even with the potential for hepatotoxicity, the use of statins to counter immunosuppressive side effects can benefit patients^[124]. Martin *et al.*^[125] (2007), in a retrospective review of 69 liver transplant patients, explain that there is a general tolerability and low incidence of adverse events in patients treated with lipid-lowering agents. Indeed, they report that there is no change in liver function tests.

Diabetes

Post-transplant diabetes mellitus (PTDM) is a well-recognized issue, and minimization of immunosuppression is currently the best treatment option for PTDM patients. The diabetogenic properties of immunosuppressive therapies seem to be intimately related to the signaling processes that are shared between pancreatic islets and leukocytes. Moreover, in contrast to the physiological proliferative signaling mechanisms used by white blood cells, renal calcineurin mediates glomerular hypertrophy and extracellular matrix accumulation^[126].

Immunosuppressive regimens play a major role in new-onset diabetes, affecting patient and graft survival^[127]. Rostambeigi *et al.*^[128] (2011) explain that beta cells exposed to TAC and CsA decreased insulin

secretion and reduced mitochondrial density without affecting apoptosis rates, and posit that maybe there is a mitochondria-mediated dysfunction imposed by CNIs. Notably, the tacrolimus-exposed beta cells fared marginally better than their CsA counterparts. This is a reflection of the diabetogenic properties of TAC compared to CsA. On the other hand, some research finds no major metabolic differences between TAC and CsA post-OLT low-dose maintenance therapies^[129,130].

HCV: FROM INVARIABLE TO INCONSEQUENTIAL

HCV recurrence after liver transplantation is nearly universal, immediate, and has an accelerated natural history^[131]. However, the discovery of directly acting antiviral (DAA) protease inhibitors has dramatically reduced the impact of HCV. With SVR rates exceeding 90%^[132], high safety^[133], and a well-tolerated side-effects profile^[134], Hepatitis C treatment will hopefully become a non-issue.

Still, there are OLT patients who experience HCV recurrence, and these patients deserve special consideration. Notably, in contrast to patients who do not have HCV, an episode of early ACR in post-OLT HCV patients is associated with a higher risk for mortality^[135]. Despite the emphatic importance of treating ACR in this population, there has been a lack of consensus on the impact of using steroids - the front line of ACR treatment - in post-OLT HCV patients^[136,137].

At the Cleveland Clinic Foundation, ACR treatment protocol first considers RAI of the HCV-positive post-OLT patient. For patients with RAI less than six, there is an increase in CNI dose and further monitoring for rejection before a bolus of steroids is administered. In contrast, patients who have HCV with a RAI greater than or equal to six are treated the same as patients who do not have HCV: 1 g of methylprednisone is administered daily for 3 d followed by steroid taper. Antibody therapy is used for steroid-resistant rejection.

Besides treatment of ACR, maintenance therapy for HCV-positive post-OLT patients can be nuanced. For example, use of the monoclonal antibody OKT3 is associated with early and severe post-OLT HCV recurrence, and must be approached with caution^[138]. On the other hand, treatment with MMF and a 24-mo CNI taper appears to benefit liver function tests and presentation on histology for the hepatitis C patient^[139].

With the introduction of DAAs, however, focus has shifted from accommodating immunosuppression to early HCV treatment for potential liver transplant recipients. Achieving SVR before the time of transplantation is ideal, and helps reduce risk of HCV recurrence post-OLT^[140-142]. Still, the efficacy of recently-developed protease inhibitors must be evaluated specifically for post-OLT patients^[143].

CONCLUSION

While the landscape of immunosuppressive medications

remains steadfast, temperamental clinical weather demands that clinicians stay up to date on best practices. The good news is that HCV is nearly subdued as a post-transplant complication, increasing graft survival, perhaps even decreasing allocation of organs to retransplantation for HCV. The bad news is that MS is increasingly harming patients in ways that are exacerbated by immunosuppression - an issue in sore need of revolution like that in HCV treatment. Finally, given the confluence of MS and immunosuppressive side effects, treatment of early ACR could be excessive and must be reevaluated in light of today's average patient.

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