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Post-liver transplant intrahepatic cholestasis: etiology, clinical presentation, therapy

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Abstract. – Post-liver transplant intrahepatic cholestasis is consequent to the impairment of bile flow or formation. It may develop in the early (within 6 months) or in the late (more than 6 months) post-liver transplant period and different causes may be recognized according to the time elapsed from a liver transplant. The raise at various degrees of serum bilirubin, alkaline phosphatase, and gamma-glutamyl transpeptidase, with or without increased transaminases levels, are common hematochemical findings. Liver histology is helpful for diagnostic assessment, and sometimes crucial to differentiate among possible causes of cholestasis. Although timely treatment of underling conditions as well as supportive care may resolve post-liver transplant intrahepatic cholestasis, the risk of graft loss and retransplantation are remarkable. For this reason, post-liver transplant intrahepatic cholestasis should be managed in collaboration with the LT center, and treatment should be devolved to expert hepatologists.

Key Words:
Cholestasis, Liver transplant, Ischemia reperfusion, Rejection, Small for size, Immunosuppression, Drug induced liver injury, DILI.

Introduction

Cholestasis is a condition characterized by defective bile flow or formation[1]. It may result from an altered uptake, transfer and secretion of bile components, mainly caused by liver injury in the absence of mechanical obstruction.

The histological features of cholestasis are bile stasis in liver parenchyma and bile ducts plugs; bilirubin accumulation into hepatocytes, Kupffer cells, and canaliculi in zone 3 as well as ductular proliferation in zone 1, or bile acid retention ("cholate stasis") may be present[1]. Blood examinations show the raise at various degrees of serum bilirubin, alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT), with or without increased transaminases levels.

In the liver transplant (LT) setting, cholestasis may be classified as extrahepatic, due to mechanical impairment of bile flow (e.g. anastomotic strictures, bile stones), or as intrahepatic, associated with impairment of liver cells or ductular dysfunction. Post LT cholestasis may develop early (within 6 months after LT) or late (more than 6 months after LT), with different etiology according to the time elapsed from LT (Table I).

In this review, the main causes of post LT intrahepatic cholestasis are discussed, focusing on clinical presentation and therapeutic approach.

Early Post-Liver Transplant Intrahepatic Cholestasis

In the early post LT period intrahepatic cholestasis is common, usually subclinical and self-limiting. However, some patients may develop prolonged cholestasis and irreversible liver injury, leading to re-LT.

Initial poor graft function (IPGF) and primary graft non-function (PNF) may manifest with early post LT cholestasis. PNF is an irreversible graft dysfunction requiring emergency liver replacement within the first 10 days after LT[2]. It is characterized by rapidly rising transaminases, absence of bile production, severe coagulation deficit, hypoglycemia, high lactate levels, and hemodynamic instability. According to the united network of organ sharing (UNOS) criteria, PNF is defined by the presence of aspartate transaminase (AST) $\geq$ 5000 IU/L, international normalized ratio (INR) $\geq$ 3.0, and acidosis (pH $\leq$ 7.3 and/or lactate concentration $\geq$ 2$\times$ normal). Conversely, there is no agreement on the diagnostic criteria of IPGF, which is mainly characterized by elevation of serum transaminases[3-5].

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The incidence rates are variable, up to 11.8% for PNF and to 36% for IPGF, respectively; PNF is responsible for 81% of re-transplantations during the first week after surgery[6].

The most frequent causes of early post LT cholestasis, which sometimes lead to PNF and IPGF, are mainly related to liver graft preservation and size match, infectious complications, acute rejection, and drugs.

Ischemia/Reperfusion Injury

Ischemia reperfusion injury (IRI) is the consequence of the inflammatory response triggered by the procedures of organ procurement and preservation. Thus, IRI is a kind of "sterile inflammation", which develops in the absence of any microorganism[7].

Two major types of IRI can be recognized. "Warm" IRI develops *in situ* during LT surgery when hepatic blood supply is temporarily interrupted, and is consequent to hepatocellular damage. "Cold" IRI, occurs *ex vivo* during liver graft preservation, is caused by hepatic sinusoidal endothelial cells damage and is coupled with warm IRI[8,9]. Other causes of IRI may be sepsis, shock, and trauma, which are pathological conditions eliciting systemic and liver hypoperfusion that may occur in the setting of LT.

In both warm and cold IRI, two stages of liver damage can be recognized. The first one is the ischemic injury phase, which initiates the process of cell death and is characterized by glycogen consumption, lack of oxygen, and ATP depletion. The subsequent reperfusion injury phase is the consequence of the metabolic derangement associated with inflammatory damage. Reperfusion injury phase can be further distinguished in early (or acute), occurring within the first 3 to 6 hours post-reperfusion and characterized by the activation of Kupffer cells, and late (or subacute), with massive neutrophil infiltration beginning at 18-24 hours post-reperfusion[9-12].

IRI is caused by the innate immune response mediated by pattern recognition receptors (PRR)[13-16], including Toll-like receptor (TLR) 4, TLR9 and the inflammasome[17]. Different cells of the immune system (T lymphocytes and natural killer lymphocytes, polymorphonuclear cells) are involved in the promotion of IRI, and recent data have reported a stimulation of adaptive immune response. The "no reflow" phenomenon observed after liver reperfusion is a common feature of IRI resulting from inflammation; in particular, blood flow mechanical obstruction is caused by adhesion of inflammatory cells to the endothelium, increased interstitial fluid and endothelial vasoconstriction[18]. The activation of the immune system leads to a massive production of cytokines, chemokines, adhesion molecules, reactive oxygen species (ROS), to the activation of the complement system and to the promotion of autoimmune injury, as well as of mitochondrial dysfunction triggering cell death programs[17]. This sustains the pro-inflammatory process and produces liver damage.

Treatment

Liver susceptibility to IRI depends on organ preservation techniques and is increased by donor starvation, age, and graft steatosis[19]. Several measures can be adopted to reduce the risk of IRI in predisposed organs[20]. Surgical interventions, pharmacological agents, and gene therapy are the main treatment strategies, which have been extensively reviewed elsewhere[19]. On the whole, the use of a vasoprotectors, modulators of the renin-angiotensin system, β-blockers, antioxidants, growth factors, tyrosine kinase inhibitors, angiotensin II blockers, hydroxy-methylglutaryl (HMG)-Coenzyme A (CoA) reductase inhibitors, calcium channel blockers, peroxisome proliferator-activated receptors (PPAR-α) agonists are the most innovative approaches reported in literature, to be distinguished form surgical measures widely used in everyday practice (e.g. minimization of cold and warm ischemia time, intermittent clamping, ischemic preconditioning, the use of preservation solutions with specific additives).

Small-For-Size Syndrome

Living donor LT (LDLT) is a common practice for Asian transplant Centers. It has been implemented in selected cases to increase the number of available organs in Western Countries and to reduce the waiting list period. In practice, donor's liver is splitted in two parts, one of them being used as transplantable graft. This procedure may be adopted for deceased donors too. To obtain an adequate equilibrium between the amount of transplanted (recipient) and residual (donor) liver tissue, in adult-to-adult LDLT graft size should cover 30-40% of the expected recipient's liver volume or 0.8-1.0% of the recipient's body weight[21]. Therefore, the right liver lobe is usually preferred. Living donor liver volumes are calculated based on three-dimensional CT scan; the most useful estimated parameters are graft volume to standard liver volume (SLV) ratio, and graft wei-

ght-to-recipient body weight ratio (GWBWR)[22-25]. A GWBWR of 0.8% is a widely accepted cut-off for minimizing the risk of graft failure[26]. In the case of deceased donors, the body surface area index (BSAi) is adopted[27].

Small-for-size syndrome (SFSS) occurs when the partial liver graft fails to fulfill the functional demand of the recipient[28]. If the hepatocyte cellular mass is inadequate for the recipient size, the small vascular network of the graft causes a discrepancy between accelerated liver regeneration and inadequate supply of oxygen and growth factors. This leads to hyperafflux in the portal system (portal hyperperfusion), reduction in arterial perfusion (hepatic arterial buffer response) and sinusoidal microcirculatory disturbances causing liver damage and inflammation[29-32].

SFSS encompasses various clinical presentations, ranging from mild hepatic dysfunction and isolated hyperbilirubinemia, to coagulopathy, ascites, portal hypertension, prolonged cholestasis, encephalopathy, and irreversible graft failure leading to death of the patient in the absence of an available organ for re-transplantation.

Liver biopsy shows diffuse ischemic damage, cellular ballooning, and features of cholestasis[33].

Treatment

Treatment is mainly aimed at reducing portal hyperperfusion by mechanic procedures (splenic artery ligation, splenectomy, portosystemic shunts, extracorporeal continuous portal diversion [ECPD]), or medical therapy (splanchnic vasoconstrictors such as: terlipressin, somatostatin, octreotide)[34].

Infections

Infectious complications are a common cause of morbidity and mortality among LT recipients. Bacterial infections are the most frequent (80%), followed by viral (20%) and fungal (8%) ones[35-37]. Both the reactivation of previous infections or the exposition to new infectious agents is possible. Concomitant factors may contribute to the onset and the evolution of the infective episode; they can be recipient-related (advanced age, MELD score >30 at LT, acute liver failure, malnutrition, > 48 hours stay in intensive care unit [ICU], prolonged hospital stay, previous infections, diabetes), donor-related (prolonged ICU stay, previous infections, marginal graft), surgery-related (choledochojejunostomy, prolonged surgery [> 12 h], re-operation or re-transplantation, transfusion of more than 15 blood units), and related to other post-LT factors (mechanical ventilation, level and type of immunosuppression [e.g. monoclonal and polyclonal antibodies], PNF, vascular complications [e.g. hepatic artery thrombosis and portal vein thrombosis], biliary complications [e.g. ischaemic cholangitis, biliary strictures and fistulae])[36-43].

The type of infection is associated with the time from LT. In the first month after surgery (early period) opportunistic, donor-derived and surgical site infections are usual, whereas at 2-12 months after LT (intermediate period) opportunistic (Mycobacterium tuberculosis, Pneumocystis, Listeria, Cryptococcus, Toxoplasma, Rhodococcus, Nocardia, Legionella, etc.) and community acquired infections (flu, urinary tract infections) are more frequent. After the first year post LT (late period) community acquired infections are more likely to occur, whereas opportunistic infections are generally rare[44].

Cytomegalovirus (CMV) infection is one of the most common viral complications of the early-intermediate post LT period, involving about 30-50% of LT recipients although a delayed onset can also be observed[44]. Either new or reactivated infection may occur. It is characterized by virus replication in blood, which can be defined as "CMV disease" in the presence of the following associated manifestations: fever >38°C, for at least 2 days within a 4-day period, neutropenia or thrombocytopenia, and tissue invasion with organ dysfunction, including lung, liver, kidney and central nervous system[45]. According to donor and recipient previous contact with CMV, the infection can be defined as primary (donor positive/recipient negative) or as superinfection (donor positive/recipient negative). In the case of primary infection, in the absence of prophylaxis, over 90% of the recipients develop CMV infection compared to about 25% of the recipients in the case of superinfection; in the case of reactivation of previous infection (donor positive/recipient negative), around 15% of the recipients become ill.

The liver is a regulatory organ in the host defense system, acting as a firewall against systemic diffusion of bacteria and pathogens; during infections, several proinflammatory cytokines (tumor necrosis factor alpha [TNF-α], interleukin [IL]-1, IL-6, and IL-8) are released, altering bile acid transport at the sinusoidal and canalicular membrane domains[46-49].

Cholestasis may precede the development of septic complications; bacterial infections usually

present with high fever, cholestasis, and positive blood cultures. Liver biopsy specimens show biliary tract inflammation with neutrophil infiltrates, bile duct proliferation, and bile plugs[50].

CMV hepatitis usually presents with fever, jaundice and increased cholestasis blood test. Liver histology shows cholestatic features, microabscesses and typical intranuclear "owl's eye" inclusions[51]. Persistence of CMV has been demonstrated in liver grafts developing vanishing bile duct syndrome and chronic rejection[52]. Although CMV infection may increase alloantigens expression, making bile ducts more vulnerable to immunologic damage[53], large studies have failed to demonstrate any significant association between CMV infection and the development of graft cholangiopathy[54].

Treatment

The treatment of the infectious complication usually resolves cholestasis.

Acute Cellular Rejection

Although the prevalence of acute cellular rejection (ACR) is declining, the current incidence of clinically significant acute rejection is 10-40%[55].

ACR may be early, usually occurring within 3 months post LT, or late, occurring after 3 months post LT[56]. In any case, it is triggered by a T cells-driven immune response against major histocompatibility complex (MHC) alloantigens in the liver graft. CD4+ and CD8+ T cells but also myeloid cells and innate lymphoid cells are initially involved, but hepatic inflammation leads to further recruitment of leukocytes from circulation[55,57]. Inflammation results in hepatocytes, endothelial cells, and bile ducts damage[58-62].

The association between antibody-mediated rejection and ACR has also been a controversial issue in the LT setting. Antibody-mediated rejection is a hyperacute rejection characterized by graft endothelial damage by complement activation, with direct injury to the capillary endothelium and activation of inflammatory cells, such as natural killer (NK) lymphocytes, macrophages, and neutrophils[63].

ACR should be distinguished from hyperactue rejection. Donor-specific human alloantibodies (DSAs) against leukocyte antigen (HLA) and ABO antibodies are involved in hyperacute rejection. DSAs are a well-known risk factor for decreased graft survival after kidney and heart transplantation. A positive "cross-match", intended as the detection of antibodies in the recipient's serum binding to the surface of donor's lymphocytes, is associated with an increased risk of hyperacute rejection of the kidney allograft[64].

The liver is relatively resistant to antibodies mediated injury due to its wide sinusoidal endothelial surface allowing the absorption of circulating antibodies, to its intrinsic ability to regenerate and also for the secretion of soluble HLA I that binds and inactivate circulating antibodies[65-68]. These mechanisms of protection allow the clearance of preexisting DSAs in 90% of presensitized LT-recipients[69]. However, in patients with rejection or inflammatory conditions involving the liver the exposure of alloantigen secondary to tissue injury may trigger the production of "de novo" DSAs, similary to what reported for kidney transplant recipients[70,71].

The association between a positive cross-matching and ACR in LT recipients is not constant among published studies and is, therefore, difficult to draw firm conclusions[69,72-77]. Although the presence of inflammation in biopsy specimens from patients with a positive cross-match has been reported[78], no significant difference in transaminases and bilirubin levels, as well as in graft and recipient's survival, was proven compared to cross-match negative patients[78-81].

Histologically, ACR is classified according to Banff criteria[82,83]. Portal, bile duct and venous endothelial inflammation are the three main features of ACR, and at least two of them are required for the diagnosis. Inflammatory cells in the portal spaces are represented by a mixed population of lymphocytes (T cells), blast cells, macrophages, neutrophils, and eosinophils. Bile ducts appear inflamed, with degenerative changes or focal luminal disruption. Centrilobular perivenulitis consists in hepatic venous and perivenular inflammation with perivenular hepatocyte loss. In late ACR this "typical" picture is less prominent, but central perivenulitis is more marked[56,84,85].

ACR is as always asymptomatic, but jaundice can be present. The diagnosis is supposed based on the increase in transaminases, bilirubin and cholestasis enzymes levels and is confirmed by liver biopsy findings.

Treatment

Advances in immunosuppressive therapy have significantly increased the success of LT, minimizing the risk of ACR. Histological evidence of ACR without any biochemical alteration does not

require treatment; on the other hand, when ACR is suspected liver biopsy is mandatory to grade its severity and to exclude other causes of liver damage (e.g. drugs, IRI, infections, recurrent disease) before treatment. The increase of immunosuppression alone is usually effective to treat mild ACR; the maintenance of increased levels of immunosuppressive drugs associated with corticosteroid boluses is recommended in moderate-severe ACR[86].

Drug Induced Liver Injury

Despite data on the general population are scarce, the prevalence of hepatotoxicity among patients hospitalized for jaundice has been estimated to range between 2% and 10%[87-91] and an incidence as high as 2% has been reported in LT recipients[92].

Drug induced liver injury (DILI) is an uncommon cause of cholestasis, even if large studies confirm that among patients with DILI 20-40% may have a cholestatic histological pattern and 12-20% a mixed hepatocellular/cholestatic pattern[93-96].

Several drugs necessary for LT recipients may have hepatotoxic effects and may lead to cholestasis. Therefore, drug induced liver injury (DILI) may be a cause of cholestatic damage in both the early and the late post LT period.

The most common clinical presentation of cholestatic DILI is the increase in ALP with or without jaundice and pruritus. Fever and abdominal pain may be present. Diagnosis of DILI is often difficult after LT, especially in the early period, due to the coexistence of other causes of liver damage.

Histology is helpful in the differential diagnostic process and in the classification of cholestatic DILI, which can be distinguished as follows[93-95]:

- Acute pure cholestasis, presenting with hepatocyte cholestasis, canalicular dilation, and bile plugs in the absence of relevant inflammation;
- Acute cholestatic hepatitis, characterized by the association of cholestasis, inflammation and sometimes hepatocellular necrosis;
- Cholestasis with bile duct injury, when ductular, cholangiolar, or cholangiolytic damage is prevalent and hepatocellular injury is minimal;
- Vanishing bile duct syndrome.

Cholestatic DILI pattern is defined as an increase in ALP >2 × the upper limit of normal and/or with an alanine aminotransferase (ALT)/ALP ratio <2, whereas a mixed (citonecrotic and cholestatic) DILI pattern as an ALT/alkaline phosphatase ratio greater than 2 and less than 5. However, these features are common in the early post LT period[97,98]. The Roussel Uclaf Assessment model (RUCAM) may be useful to investigate the likelihood of DILI[97,98]. Furthermore, some drugs have a "signature pattern," as a typical pattern of liver injury may be recognized after a similar duration of drug intake. In the diagnostic workup, online archives and web sites are useful tools to investigate drugs hepatoxicity[99].

It is usually difficult to recognize DILI in the LT setting due to the multiple factors potentially responsible for liver damage. However, in presence of unexplained cholestasis, after the exclusion of common causes of liver injury and after extensive testing including liver biopsy, DILI should be considered as a possible cause.

In the same way as in the general population, histological assessment of DILI is challenging in LT recipients and may be confounding, due to the influence of several other factors, sometimes delaying DILI diagnosis. Moreover, the investigation of medication history, which is mandatory to estimate the time to DILI onset, may be difficult due to variations in treatment doses and duration as well as interruptions/re-start. Conversely, the strict biochemical monitoring to which LT recipients are subjected is a potential advantage in the early detection of DILI.

Table I. Main causes of post LT intrahepatic cholestasis according to post LT period.

Early post LT period (<6 months)	Late post LT period (≥6 months)
Primary non function	Chronic rejection
Ischemia/reperfusion injury	Infections
Small for size graft	Drug induced liver injury
Infections	Recurrence of the original disease
Acute rejecton	
Antibodies-mediated rejection	
Drug induced liver injury	

Table II. Classification of medications associated with drug induced liver injury according to the number of reports and to the likelihood of use in liver transplant recipients[98,123].

Drugs category A (≥ 50 Cases)	Drugs category B (18-49 Cases)	Drugs category C (4-11 Cases)	Drugs category D (1-3 Cases)
Frequently used in LT:	Frequently used in LT:	Frequently used in LT:	Frequently used in LT:
*Allopurinol**	*Celecoxib**	*Amlodipine**	*Acyclovir*
*Atorvastatin**	*Clopidogrel**	*Candesartan**	*Atenolol**
Diclofenac	*Enalapril**	*Gemfibrozil**	*Carvedilol**
*Ibuprofen**	*Esomeprazole*	*Gilipizide**	*Clofibrate**
*Ticlopidine**	*Fenofibrate**	*Glimepiride**	*Enoxaparin*
	*Fluvastatin**	*Irbesartan**	*Fondaparinux*
Uncommonly/occasionally used in LT:	*Glibenclamide**	*Ketoprofen**	*Hydrochlorotiazide**
*Amiodarone**	*Lisinopril*	*Losartan**	*Glicazide**
*Amoxicillin+calvulanate**	*Lovastatin**	*Lansoprazole**	*Propofol*
	*Metformin**	*Metronidazole*	*Rabeprazole**
Rarely used in LT:	*Naproxen**	*Pravastatin**	*Raltegravir*
*Busulfan**	*Nifedipine*	*Pantoprazole*	*Repaglinide*
*Carbamazepin**	*Omeprazole*	*Ramipril**	*Spironolactone**
Dantrolene	*Ranitidine**		*Tamsulosin*
*Efavirenz**	*Rosuvastatin**	Uncommonly/occasionally used in LT:	*Valsartan**
*Erythromycin**	Uncommonly/occasionally used in LT:	*Ampicillin**	Uncommonly/Occasionally used in LT:
*Floxuridine**	*Amoxicillin**	*Alfuzosin**	*Cefaclor*
Halothane	*Azithromycin**	*Diltiazem**	*Cefadroxi*
*Infliximab**	*Captopril**	*Levocetirizine**	*Cefnidir*
*Ketoconazole**	*Cefazolin*	*Vancomycin*	*Cefepime*
*Nevirapine**	*Ceftriaxone**	*Verapamil**	*Cefoperazone*
*Nimesulide**	*Ciprofloxacin**	*Warfarin**	*Cefotaxime*
*Phenytoin**	*Clarithromycin**	Rarely used in LT:	*Cefprozil*
*Quinidine**	*Fluconazole*	*Amphotericin B**	*Ceftazidime*
*Rifampin**	*Levofloxacin**	*Albendazole**	*Cefuroxime*
*Simvastatin**	*Propafenone**	*Bosentan**	*Flecainide*
*Sulfasalazine**		*Cephalexin*	*Fosfomycin**
*Sulfonamides**	Rarely used in LT:	*Citalopram**	*Imipenem**
*Sulindac**	*Clindamycin*	*Cytarabine**	*Meropenem**
*Thioguanine**	*Clozapine**	*Daptomycin*	*Sitagliptin*
*Valproic Acid**	*Doxorubicin*	*Doxycycline**	*Valacyclovir**
	*Duloxetine**	*Escitalopram**	
	*Etanercept**	*Fluoxetine**	Rarely used in LT:
	Heparin	*Erlotinib*	*Acetazolamide**
	*Imatinib**	*Famotidine*	*Aliskiren**
	Irinotecan	*Flavocoxid**	*Alosetron*
	*Itraconazole**	*Fluorouracil**	*Alprazolam**
	*Moxifloxacin**	*Gabapentin**	*Anastrozole*
	*Olanzapine**	*Gemcitabine**	*Atazanavir**
	*Ofloxacin**	*Hydroxyurea*	*Bortezomib**
	*Paroxetine**	*Indomethacin**	*Carbenicillin**
	*Phenobarbital**	*Isotretinoin*	*Carboplatin*
	*Quinine**	*Labetalol*	*Chlorambucil**
	Rivaroxaban	*Levetiracetam*	*Cisplatin*
	*Sertraline**	*Linezolid**	*Clomipramine**
	*Tamoxifen**	*Mesalamines**	*Clonazepam**
	*Thiabendazole**	*Mirtazapine**	*Dabigatran*
	*Venlafaxine**	*Mitomycin*	*Dalteparin*
	*Voriconazole**	*Montelukast**	*Darbepoetin alfa*
		Nafcillin	*Dasatinib*
		*Natalizumab**	*Deferoxamine*
		*Nefazodone**	*Donepezil*
		*Norfloxacin**	*Entacapone**
		Orlistat	*Ethambutol**
		*Penicillin G**	
		*Pioglitazone**	
		*Pregabalin**	
		*Procainamide**	

Table continued

Table II. *Continued.* Classification of medications associated with drug induced liver injury according to the number of reports and to the likelihood of use in liver transplant recipients[98,123].

Drugs category A (≥ 50 Cases)	Drugs category B (18-49 Cases)	Drugs category C (4-11 Cases)	Drugs category D (1-3 Cases)
	*Quetiapine**	*Hydroxychloroquine*	
	*Risperidone**	*Lopinavir**	
	*Ritonavir**	*Mebendazole*	
	*Rosiglitazone**	*Mefloquine**	
	Sorafenib	*Metoprolol*	
	*Thalidomide**	*Micafungin*	
	*Tolcapone**	*Nelfinavir*	
	Topiramate	*Ondasetron*	
	*Trazodone**	*Oxcarbazepine**	
	Vincristine	*Rifabutin**	
	Zafirlukast	*Rilpivirine*	
		Rivastigmine	
		*Saquinavir**	
		*Sildenafil**	
		Terbutaline	
		Thyroxine	
		Tobramycin	
		*Tolvaptan**	
		*Triamterene**	
		Zileuton	

**mixed or cholestatic pattern of drug induced liver injury.*

The optimization of tailored immunosuppressive drugs regimens have significantly minimized the incidence of DILI in LT recipients, but the risk is not completely absent and increases when complications or other concomitant medical conditions require treatment.

Cyclosporine may have cholestatic effects interfering with bile formation and leading to hyperbilirubinemia and to the formation of biliary sludge[100-108]. The inhibition of ATP-dependent export carriers and of the bile salts export pumps in the canalicular membrane of hepatocytes, together with impairment of biliary secretion of glutathione, are involved in cyclosporine-related impairment of canalicular bile flow[105-107]. Similarly to cyclosporine but at a lesser extent, tacrolimus has been reported to inhibit canalicular bile acids transport and glutathione secretion[105,109,110]. However, Ericzon et al. reported that tacrolimus was able to recover bile acid secretion after LT more rapidly than cyclosporine[111,112]. As regards other immunosuppressive agents, two cases of cholestatic liver damage have been reported in two renal transplant recipients receiving mycophenolate and sirolimus, respectively[112,113].

Azathioprine, a purine analogue rarely used as immunsosuppressant in the LT setting today, may cause damage to the hepatic sinusoidal and venular endothelial cells, resulting in a variety of clinical, biochemical, and histologic manifestations, including cholestasis[115-123].

Among other drugs that may cause a cholestatic syndrome in LT recipients, antifungal agents as well as antibiotics have been reported as the most common agents of DILI in LT recipients[92].

Pharmacological therapies for cardiovascular diseases are frequently employed in LT recipients as well as analgesics and antinflammatory antidiabetic neurologic, anticoagulant, antiaggregant, gastroenterologic and psychotropic medications. The main drugs potentially causing DILI, including the cholestatic type, are reported in Table II, and are stratified according to the frequency of hepatic adverse effects[124]. Medical therapies should not be avoided only for concerns regarding their potential hepatotoxicity, but thorough patient evaluation is mandatory to avoid underrecognized cases of DILI. Particular attention should be paid to the use of herbal and dietary supplements too, which may be hepatotoxic by itself or due to the presence of other ingredients and adulterants[125,126].

Only 6% of patients with DILI develop chronic liver injury[127]. However, patients with cholestatic DILI seem to be more prone to experience chronic liver disease; in a minor part of them, ductopenia and vanishing bile duct syndrome with the progression to biliary cirrhosis may also occur, especially in association with specific drugs[128-134]. Reversal of drug-related vanishing bile duct syndrome has also been reported[135,136].

DILI-related mortality ranges between 5% and 14%[93-95]. However, in a recent study focused only on LT recipients, DILI did not affect the post LT outcome as patients did not require re-transplantation and no death was reported[92].

Treatment

Immediate withdrawal of the suspected drug is the core principle of DILI management. In the case of immunosuppressive drugs, dose reduction or conversion to other regimens may be considered. Specific therapies may be adopted for few toxic agents, such as N-acetylcysteine for acetaminophen overdose[137]. N-acetylcysteine has been empirically administered to adults with DILI due to other agents too, reporting favorable results. Corticosteroids may be used in case of tyrosine kinase inhibitors-related DILI or in case of drug-induced autoimmune-like hepatitis, especially when liver injury does not resolve after withdrawal of the offending agent. Ursodeoxycholic acid administration has obtained beneficial results in the setting of cholestatic DILI, as well as cholestyramin alone or in association with antihistamines as regards pruritus relief. Silibinin use in the setting of DILI has obtained contrasting results, whereas few data on methionine and glutathione are available.

Gastric decontamination (aspiration by lavage, charcoal) should be considered in selected cases of DILI, such as those related to acteminophen overdose, toxic mushrooms, salicylate or to the ingestion of other toxic substances.

Late Post-Liver Transplant Intrahepatic Cholestasis

The main causes of intrahepatic cholestasis in the late post LT period are chronic rejection (CR), infections and DILI, which have been extensively discussed above.

Exclusion of the recurrence of the original liver disease (e.g. hepatitis C virus infection, primary sclerosing cholangitis, primary biliary cholangitis) and of extrahepatic conditions inducing cholestasis, such as hepatic artery thrombosis and mechanical obstruction, is mandatory to guide treatment.

Chronic Rejection

The prevalence of CR (otherwise called "ductopenic rejection") is 2% in LT recipients, although probably underestimated for the lack of protocols for routine histological assessment[138,139]. It tipically occurs during the first 12 months after LT but a delayed diagnosis is possible, since CR may have an indolent course over years[140]. CR may occur in patients with previous episodes of ACR, even if this is not a strict requirement[141]. As like as for ACR, a positive cross-matching has been demonstrated in patients with CR[80,142-144]. Notably, even if data from published studies are contrasting, circulating DSAs have been associated with an increased risk of developing rejection in patients who have weaned immunosuppression.

Loss of bile ducts (vanishing bile duct syndrome) at liver histology and obliteration of large and medium size arteries are the mainstay of CR. These pathological alterations are due to the infiltration of macrophages (foam cells) and inflammatory cells into the vessel wall, leading to fibrosis and, finally to the obliteration of arterial lumen with consequent ischemic damage.

Histologically, ductopenia should be present in more than 50% of portal tracts to make a reliable diagnosis of CR[145-147]. Bile ducts inflammation can be present in the early stages, subsiding during time, and bile ducts degeneration leads to dysplastic and athrophic features. In the centrilobular zone, bilirubinostasis, hepatocyte ballooning and loss may be present, evolving in centrilobular fibrosis and cirrhosis during time. Fibrosis pattern may be veno-centric, which is associated with the obliteration of hepatic and portal vein branches, periportal/biliary, when bile duct inflammation and loss is predominant, or centrilobular, which is associated with central perivenulitis. Bile duct proliferation and periportal fibrosis are more frequent in cases of late presentation, and are suggestive of a prolonged course of CR.

Microscopic vascular abnormalities can be present and the small portal tracts may show a reduced number of small arterial branches and of other microvascular channels[145-147].

CR may have a long asymptomatic course; progressive jaundice, with or without ascites, is the typical clinical manifestation.

Treatment

Switch of immunosuppressive therapy or its potentiation (combination of two or 3 drugs) are the most commonly used approaches. Although chronic rejection is sometimes irreversible, retransplantation is necessary in only 5% of cases[138,139].

Conclusions

Intrahepatic cholestasis in LT recipients is a challenge for clinicians, as multiple conditions may alter the clinical picture. In the early post LT period, cholestasis may be a manifestation of graft dyfunction, and a rapid assessment of the possible cause is mandatory to start the correct treatment rapidly and to avoid the loss of liver graft or its irreversible damage. The improvement in surgical techniques and organ preservation methods as well as the progressive shortening of both donor and recipient surgical procedures times, have significantly reduced the incidence of early post-LT cholestasis. Likewise, the optimization of immunosuppressive regimens has minimized the incidence of rejection, of early and late infections and of immunosuppressive drugs-related cholestasis.

In this scenario, DILI remains the most common cause of post LT cholestasis, especially in the late period. Thorough investigation of medical history is mandatory to exclude a possible DILI; while the identification of a drug responsible for cholestasis may be very difficult in the early postoperative period, it can be easier in the late phase, when pharmacological therapy is almost stable. However, most of the medications are necessary and treatment modifications should be done considering potential drug-to-drug interactions and under close clinical monitoring.

Liver biopsy maintains a crucial role in the differential diagnosis of cholestasis in the LT setting. However, as protocol biopsies in asymptomatic recipients with normal or near-normal liver tests after years from LT are not universally performed, liver histology is frequently obtained only when ematochemical parameters become abnormal.

For these reasons, the management of cholestasis in the post LT setting is a complex medical issue, and should be performed in collaboration with the LT center and devolved to expert hepatologists.

Conflict of interest

The authors declare no conflicts of interest.

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