Severe Unresolved Cholestasis Due to Unknown Etiology Leading to Early Allograft Failure Within the First 3 Months of Liver Transplantation

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Background. Causes of severe cholestasis after liver transplantation (LT) are multi-factorial. Although the etiology is predictable in some, others culminate in graft/patient loss without a definitive cause identified. Severe cholestasis is usually associated with overlapped histological findings of rejection and biliary features, and diagnostic interpretation may pose a challenge. **Methods.** This is 10-year retrospective analysis of patients with unexplained severe cholestasis resulting in death/graft loss within 90 days of LT. Of 1 583 LT during the study period, 90-day graft failure occurred in 129 (8%) cases; a total of 45 (3%) patients had unresolving severe cholestasis (bilirubin, >100 μmol/L; alkaline phosphatase, >400 UI/L after 15 days from LT), excluding those due to primary nonfunction/sepsis/vascular causes (n = 84). Demographics, allograft biopsies, radiological investigations, and clinical outcome were analyzed. **Results.** All patients had persistent abnormal liver biochemistry. Doppler ultrasound scan was normal in all cases. Thirty-five (78%) recipients had at least 1 allograft biopsy (2 [1-9]). On the first biopsy, 22 (63%) patients had acute rejection, 4 (18%) early-chronic rejection, 12 (34%) antibody-mediated rejection. In subsequent biopsies chronic rejection was evident in 5 (14%) cases. Donor-specific antibodies were detected in all patients tested. Biliary anatomy was studied in detail in 9 (20%) patients, all presenting biliary strictures. The majority (n = 39; 87%) died within 32 (10-91) days, only survivors were from retransplantation (n = 3;6.5%) and biliary intervention (n = 3;6.5%). **Conclusions.** Unresolving severe cholestasis after LT is a key parameter predicting patient/allograft outcome. Histologically, rejection seems to overlap with biliary strictures; hence, allograft biopsy with signs of rejection should not be a reason to overlook biliary problems, in particular when biliary features are present. Only extensive radiological investigation/intervention or retransplantation prevents patient/allograft loss.

(Transplantation 2018;102: 1307–1315)

Cholestasis is not uncommon after liver transplantation (LT) and well recognized etiologies include anastomotic or nonanastomotic biliary strictures, early allograft dysfunction (EAD), severe sepsis and viral hepatitis such as early hepatitis C recurrence or cytomegalovirus hepatitis or even small for size syndrome.[1,2] In addition, graft rejection may present as cholestasis; some cases of acute cellular rejection (ACR) progress to severe cholestatic and ductopenic chronic rejection within a very short period despite institution of salvage immunosuppression therapy.[3] Also antibody-mediated rejection (AMR) may have cholestatic biochemistry and biliary features on biopsy.[4-6]

Early graft loss within first 3 months after LT is a problem faced by the transplant community. This may be sporadic in low volume centers, but in high volume centers several cases may be encountered each year and the reported incidence

may even reach up to 5% of all transplant cases. Early allograft loss has a considerable added burden to transplant programs. The problem lies with difficulty in diagnosing the exact etiology. Histology may show overlapping biliary and rejection features, which may also overlap in etiology: the biliary features may be secondary to the rejection but obstruction cannot be excluded histologically and it is increasingly recognized that biliary obstruction may occur as a consequence of AMR.

Isolated severe cholestasis should be considered as a different clinical entity from traditionally described "delayed graft function" (DGF), also termed as "early allograft dysfunction" (EAD),[7-10] where severe early hepatocyte injury has been identified as the major cause of graft dysfunction. EAD is often secondary to a combination of donor and recipient characteristics[11] as well as surgical factors, such as length of total ischemia[12] and ischemia/reperfusion injury,[13] requiring often organ support before complete restitution of normal graft functions or succumbing to sequelae such as severe sepsis.

LT recipients with severe cholestasis in the present study are an entirely different cohort than those fulfilling the criteria for EAD. This group of patients recovers well within the early postoperative period and is fit enough to be transferred to the transplant ward from the intensive care unit, thus not requiring prolonged organ support and is free of sepsis, and presenting asymptomatic elevation of liver function tests (LFTs). Routine imaging usually rules out vascular causes and they often undergo liver biopsy.

Through this study we aim to resolve this diagnostic dilemma through retrospective review of the histology report of all LT recipients who developed unexplained severe cholestasis within 3 months in a large volume transplant center in Europe, with the aim to define causes and pitfalls in their management.

MATERIALS AND METHODS

Patient Population and Clinical Data

This is a retrospective review of all LT performed between January 2004 and January 2014 at The Liver Unit, Queen Elizabeth Hospital Birmingham, United Kingdom. All recipients with persistent severe cholestasis (defined as persistent bilirubin >100 µmol/L and alkaline phosphatase [ALP] >400 UI/L after 15 days from LT) and graft failure within 3 months from LT were included in the study. LT recipients with a definitive identifiable etiology or complication leading to graft failure such as primary nonfunction (PNF), hepatic artery thrombosis (HAT), other vascular complications (venous occlusion/portal venous thrombosis) and sepsis (proved by positive microbiology) were excluded. Clinical data were collated from the electronic clinical database including donor and recipient demographic characteristics, intra-operative details, postoperative LFTs, radiological investigation, treatment for graft rejection and postoperative clinical outcome and pathology report. The Donor Risk Index was calculated using the formula defined by Feng at al.[14] This study was approved by the clinical audit and research management system (CARMS) of the institution (registration number: CARMS-11425).

Histological Data

Protocol time zero biopsy was defined as a biopsy taken in the immediate postreperfusion period, and comparisons were made when this information was present. Posttransplant

biopsies were performed at varied time points to investigate the cause of the cholestasis. All liver biopsies were reported by experienced LT histopathologists at the time of diagnostic biopsy and were re-reviewed by 1 histopathologists to look for features of AMR,[4] not defined in the earlier part of the study.

The histological features assessed were those of (1) ACR (portal infiltrate, bile duct inflammation and damage, venous inflammation and central perivenulitis),[15] (2) early chronic rejection (paucity of bile ducts and "dysplastic/sick" bile ducts),[15,16] (3) AMR (portal oedema, ductular reaction, portal microvasculitis, sinusoidal infiltrate, arteritis and eosinophilic venulitis).[15,17,18] The rejection activity index (RAI) was determined. The severity of ACR was classified as indeterminate, mild, moderate or severe according to standard criteria.[15] The severity of bilirubinostasis/cholestasis was also assessed. Donor-specific antibody (DSA) testing was not performed routinely, but in recent years, DSA testing has been performed in cases of histological or clinical suspicion. Hepatic steatosis was defined as microvesicular or macrovesicular steatosis and classified into mild (<30%), moderate (30-60%) or severe (>60%).[19]

Statistical Analysis

Data were obtained from a prospectively collected consecutive database (Infoflex; Chameleon Information Management Systems). Donor/recipient characteristics and clinical data are shown (wherever applicable) as either median with range or mean ± standard deviation. Univariate data were analyzed using the Mann-Whitney test and Fisher exact test. A P value less than 0.05 was considered significant. Normal distribution continuous data were analyzed by parametric test (Student t test). Survival rates were calculated using the Kaplan-Meier method for univariate analysis and Cox regression for multivariate analysis. The program used for statistical analysis was SPSS 13.0 (233 South Wacker Drive, Chicago, IL) for Windows.

RESULTS

Donor, Recipient, and Transplant Characteristics

A total of 1583 LT were performed during the study period. One hundred twenty-nine (8%) recipients had graft

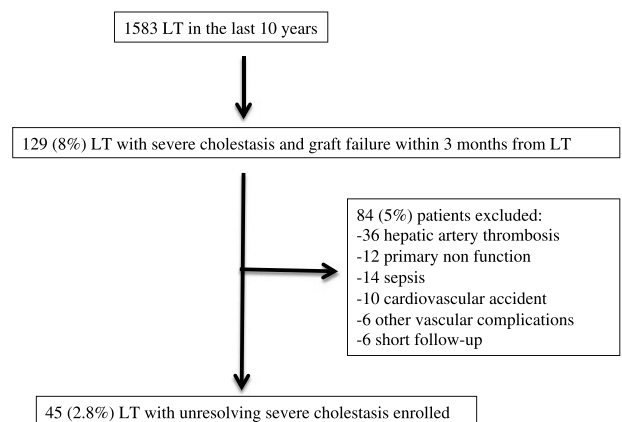**FIGURE 1.** Study population selection for unresolving severe cholestasis after LT. All patients undergoing LT in the last 10 years at our institution, who developed graft failure within 3 months from LT, associated with unresolving severe cholestasis and without a definitive cause, were included. Severe cholestasis was defined as persistent bilirubin >100 µmol/L or ALP > 400 UI/L after 15 days from LT.

TABLE 1.

Recipient, donor, and surgical characteristics of the study population

Recipient variables	Number (range)
No. patients	45
Sex (male/female)	33/12
Age, y	49 (17-71)
MELD score	18 (8.0-35.0)
UKELD score	54 (45.0-68.0)
Indication for LT	
Primary sclerosing cholangitis	3 (7%)
Alcoholic cirrhosis	12 (27%) (5 with HCC)
HCV-related cirrhosis	8 (18%) (1 with HCC)
HBV-related cirrhosis	1 (2%) (1 with HCC)
Primary biliary cirrhosis	4 (9%)
Secondary biliary cirrhosis	1 (2%)
Regraft	6 (14%) (4 PNF, 2 HAT)
Autoimmune hepatitis	2 (4%)
Seronegative hepatitis	1 (2%)
Caroli disease	1 (2%)
Cryptogenic cirrhosis	1 (2%)
Amyloidosis	1 (2%)
NASH	1 (2%)
Acute liver failure	3 (2%)
Comorbidities	
Circulatory disease	6 (13%)
Diabetes type II	6 (13%)
Renal impairment (not dialysis)	4 (9%)
Asthma	4 (9%)
Ulcerative colitis	2 (4%)
Cystic fibrosis	1 (2%)
Donor variables	
Donor age, y	46 (18-69)
Donor graft type (DBD/DCD)	39/6
DRI	1.7 (1.04-2.9)
Surgical variables	
Duration of surgery (range), h	5 (3-8)
CIT (range), min	492 (166-768)
WIT (range), min	41 (29-68)
Whole graft/split graft	38/7
Biliary anastomosis (duct-to-duct/HJ)	38/7
ITU stay, d	21 (1-49)

Values reported as medians and ranges.
DBD, donor after brain death; DCD, donor after circulatory death; DRI, donor risk index; MELD, Model for End-Stage Liver Disease; UKELD, United Kingdom Model for End-Stage Liver Disease; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HBV, hepatitis B virus; NASH, nonalcoholic steatohepatitis; CIT, cold ischemic time; WIT, warm ischemic time; HJ, hepaticojejunostomy; ITU, intensive therapy unit.

failure associated with persistent severe cholestasis (as defined above) within 3 months from LT. Of these, 84 (5%) patients with known causes of graft failure, including HAT (n = 36;2%), PNF (n = 12;0.7%), definitive diagnosis of sepsis (n = 14;0.9%), postoperative cardiovascular accident (n = 10;0.6%), other vascular complications (n = 6;0.4%) and short postoperative follow-up ([6; 0.4%], median, 7 days [3-11]) were excluded (Figure 1).

This resulted in 45 (3%) LT recipients (male/female, 33/12; median age, 49 [17.4-70.5] years) with severe cholestasis of unknown cause leading to graft loss within a median time of 32 days (15-91 days) from LT included in this study (Table 1). During the study period, the average number

of patients with cholestasis of unknown etiology was 4 cases per year (range, 2-6 cases). Eight (18%) recipients had a split LT, and 1 (2%) patient received a combined liver-kidney transplant. Biliary anastomosis was performed as duct-to-duct biliary reconstruction in 38 (84%) cases, whereas 7 (16%) recipients had Roux-en Y choledochojejunostomy. All split recipients had a T-tube kept in place up to 3 months, as is local practice.

Thirty-nine (87%) patients received a graft from a donor after brain death and 6 (13%) patients from a donor after circulatory death. Recipient, donor, and surgical variables are reported in Table 1.

The baseline variables of the study population were compared with 1538 adult LT recipients who did not experience unresolved cholestasis, transplanted in the same period at our institution. The study cohort showed similar baseline characteristics to these of LT recipients without unresolved cholestasis including recipient age (49 [17.4-70.5] years vs 52 [16.4-73.4] years, P = n.s.), Model for End-Stage Liver Disease at the time of LT (18 [8.0-35.0] vs 17 [6-40], P = n.s.), donor age (46 [18-69] years vs 48 [6-83] years; P = n.s.), whereas only the recipient sex was different in the 2 groups (male, n = 33 [73.3%] vs n = 882 [57.3%], P = 0.043).

Biochemical Findings and Immunosuppression Trough Levels

The median LFT levels during the observational period are reported in Figure 2. The bilirubin level increased from 88.5 (19-317) µmol/L on day 1 to 135 (101-573) µmol/L on day 7 (P = 0.003). At 15, 30, 60, and 90 days after LT, bilirubin levels were 170 (102-573) µmol/L, 239 (58-615) µmol/L,
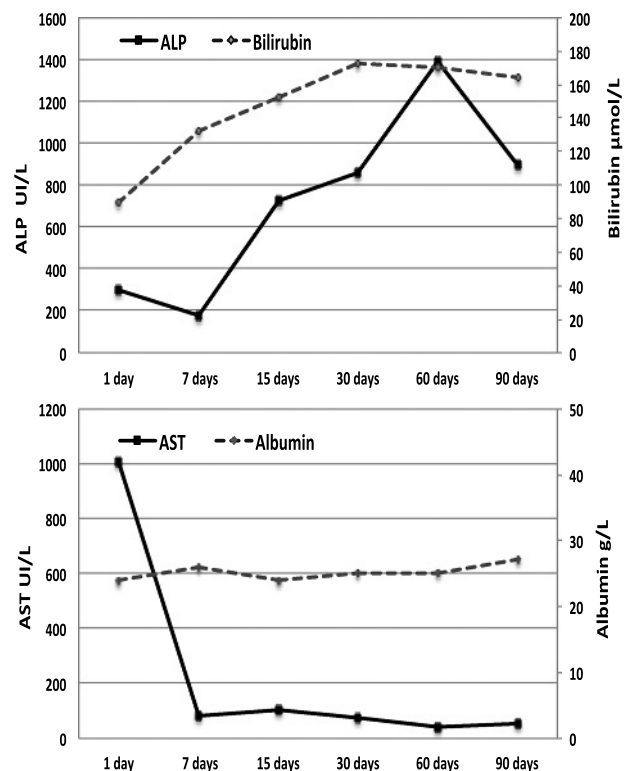

FIGURE 2. The biochemical course within 3 months from LT. Values reported as medians. ALP, UI/L; AST, UI/L; bilirubin, µmol/L; albumin, g/L.

245 (45-706) μmol/L, and 115 (69-250) μmol/L, respectively (*P* = n.s.).

All patients received the local standard immunosuppression regimen, comprising: prednisolone 20 mg daily (gradually reduced by 5 mg/day every 3 weeks and stopped after 12 weeks), Azathioprine (1-2 mg/kg per day) or mychophenolate (1 g twice/day), Tacrolimus (0.1 mg/kg per day). At 7, 15, 30, 60, and 90 days after LT, the tacrolimus trough levels were 9.1 (1.1-17.1) ng/mL, 7.05 (1.1-16.2) ng/mL, 8.01 (1.1-12.1) ng/mL, 8.15 (1.1-14.1) ng/mL, and 6.7 (1.1-12.6) mg/mL, respectively (*P* = n.s.). No significant variations in tacrolimus blood level were observed during follow-up.

Radiological Investigations

During the postoperative period, the median number of Doppler ultrasound (US) scans for each patient was 5 (range, 2-14), which in all cases were negative for intrahepatic biliary dilatation and vascular complications. Twenty-six (58%) patients had a computed tomography scan, and in 9 cases, an abdominal collection amenable to percutaneous drainage was identified. Biliary anatomy was studied in detail in 9 (20%) cases by magnetic resonance cholangiopancreatography (MRCP, [n = 5]), endoscopic retrograde cholangiopancreatography (ERCP) (n = 7) and/or percutaneous transhepatic cholangiography (PTC) (n = 4). All specific biliary imaging performed suggested that the cause of persistent cholestasis was of biliary origin as in mechanical obstruction; a total of 9 (100%) cases reported biliary strictures, which were associated with anastomotic bile leak in 3 cases (all of them received a split graft). Of 5 patients who underwent MRCP, 2 patients were treated with PTC biliary stent, 2 cases had combined PTC and ERCP biliary stenting, whereas 1 case had conservative management. Endoscopic retrograde cholangiopancreatography was performed in another 5 patients, but a biliary stent was placed in only in 3 of them. Of 7 LT recipients who received a biliary interventional treatment, the biochemical cholestasis resolved in only 3 cases.

Histological Findings

Thirty-four (76%) recipients had a time zero liver biopsy, graft reperfusion injury was observed in 30 (88%) biopsies (20 mild, 9 moderate, 1 severe), and features of graft steatosis were reported in 20 (59%) cases (19 mild, 1 moderate).

After LT, 35 (78%) recipients underwent at least 1 allograft biopsy as an investigative tool for persistent cholestasis. The median number of biopsies per patient was 2 (range, 1-9) (20 having 1 biopsy, 5 having 2 biopsies, 3 having 3 biopsies, 3 having 4 biopsies [1 also with an explant], 1 having 5

FIGURE 3. Severity of cellular rejection components in the allograft biopsies after LT. The RAI was used to score liver allograft biopsies with acute rejection. The RAI score was defined by the presence of portal inflammation (score, 1-3), venous endothelial inflammation (score, 1-3) and biliary duct inflammation (score, 1-3) at biopsy numbers 1, 2, and 3 performed during the observational period. Values are reported as medians.

biopsies and 1 having 9 biopsies and graft explant during the follow-up period). Histological findings are summarized in Table 2.

On the first biopsy, 22 (63%) of 35 patients had ACR (8 mild, 7 moderate, 7 severe), whereas 2 cases were indeterminate for rejection. The median RAI score was 4 (0-9) with histological inflammations score (portal 1.5 [0-3], bile duct 2 [0-3], venous endothelial 1 [0-3]) (Figure 3). Four biopsies had features of early chronic rejection. Twelve (34%) biopsies had features suspicious for AMR (5 highly suspicious), all but 1 with concurrent ACR, the other developed ACR in subsequent biopsies. Two patients had a donor-derived steatohepatitis.

Over subsequent biopsies, chronic rejection was developed in 5 (14%) patients, and 1 patient showed venous outflow obstruction. Histological features suspicious of AMR became more striking in 1 patient but did not develop in any when not present in the first biopsy, with the exception of finding a fibrinoid arteritis in conjunction with a foam cell arteriopathy in the explant of 1 patient transplanted for chronic rejection. C4d staining was done in at least 1 biopsy of 12 patients and was positive in 4 patients.

Three of those highly suspicious for AMR did not have a C4d stain and 3 had a variably positive C4d stain by immunoperoxidase. Of the 3 more recent cases in which the C4d stain had been performed, all had a DSA detected. Four of the 5 slightly suspicious for AMR cases did not have a C4d stain, whereas 1 was negative by immunoperoxidase. The patients with established chronic rejection did not have histological features suspicious for AMR. Bilirubinostasis (24 severe, 8 moderate, 2 mild) were present in all biopsies (Figure 4).

Twelve (27%) patients with persistent abnormal LFTs underwent second and third biopsies, and in 9 cases, features of ACR (4 mild, 4 moderate, 4 severe) persisted, and in 2 cases, features evolved to chronic rejection, and DSA were detected in both cases.

Clinical Outcome and Survival

Postoperative complications included renal impairment (n = 31 [69%], of which 2 cases [4%] required long-term dialysis), gastric bleeding (n = 1), bowel perforation (n = 1), and

TABLE 2.

Major histological findings at the graft biopsies after LT

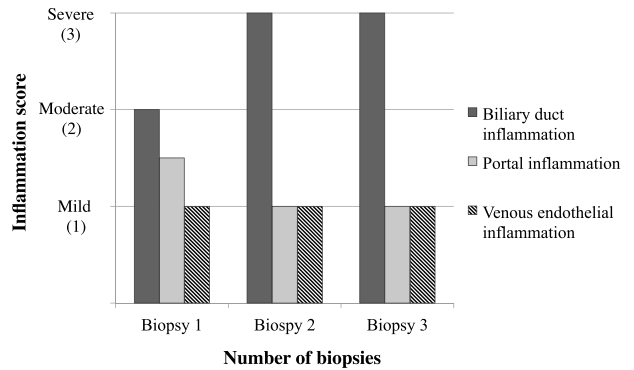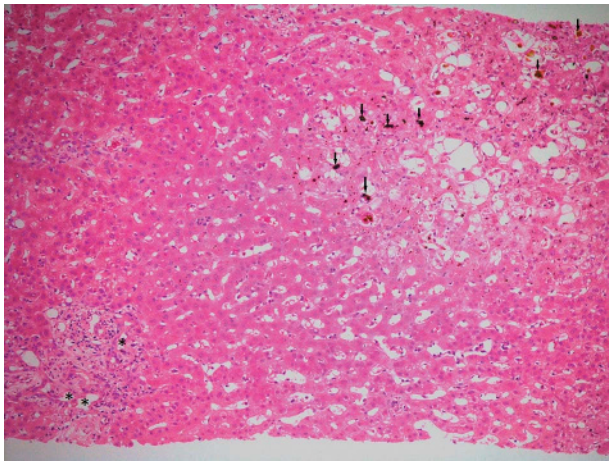| Histological findings	Number (range, %)
Recipients underwent graft biopsy	35 (78%)
Graft biopsies for patient	2 (1-9)
ACR	22 (63%)
Mild	8
Moderate	7
Severe	7
AMR	12 (34%)
Early chronic rejection	4 (18%)
Chronic rejection	5 (14%)

FIGURE 4. Histological features of cholestasis and acute rejection in the early period after LT. Hematoxylin and eosin–stained section showing a portal tract in the bottom left with dilated bile ductules (*) and mild portal inflammation. There is severe bilirubinostasis with numerous bile plugs, predominantly in a centrilobular position (arrows) (original magnification, ×10).

enteric leak (n = 1) requiring second surgery for bowel resection; cerebrovascular accident (n = 1).

Twenty-six (74%) patients were treated for ACR with high doses of steroids (prednisolone 200 mg for 3 days), and the median number of treatments was 2 (1-4). All patients with biliary strictures (n = 9) had previously histological prove of ACR and severe cholestasis. In particular, 7 recipients had a first liver biopsy showing mild (n = 4) and moderate (n = 3) ACR and were treated with standard doses of steroid boluses

without resolution of cholestasis; the second liver biopsy did not show any signs of rejection (but persistent severe cholestasis), thus later, they underwent biliary anatomy investigations and were treated with biliary stents (n = 5) or conservative management (n = 2). The remaining 2 patients were treated with steroids boluses twice for ACR (after histological proven in 2 consecutive graft biopsies); however, the biochemical cholestasis was not resolved, therefore, both underwent ERCP with biliary stent. Despite failure to respond to antirejection treatment and/or ongoing cholestasis without obvious cause, 17 of the remaining patients were not specifically investigated for a biliary cause.

The majority of recipients (n = 39; 87%) died within 32 (10-91) days. The only survivors (n = 6; 13%) were 3 (7%) patients who underwent retransplantation (2 for chronic rejection and 1 for acute on chronic AMR) and 3 (7%) recipients who underwent intervention for biliary strictures. The diagnostic and therapeutic management and patient outcomes are summarized in Figure 5. On univariate and multivariate analyses, there was no significant association of all other variables with patient survival. The survival rate at 3 months in this group, therefore, from LT was 13% (Figure 6).

From a clinical point of view, the primary cause of graft loss was defined as rejection (histologically proven) in 21 patients, biliary complications in 5 cases (of which 3 had a proven DSA and features of AMR on their biopsies before the development of the stricture) and unknown causes in 10 (26%) recipients, of which 2 had donor derived steatohepatitis. Unfortunately, testing for DSAs and C4d staining was not performed in the majority of these patients. During the
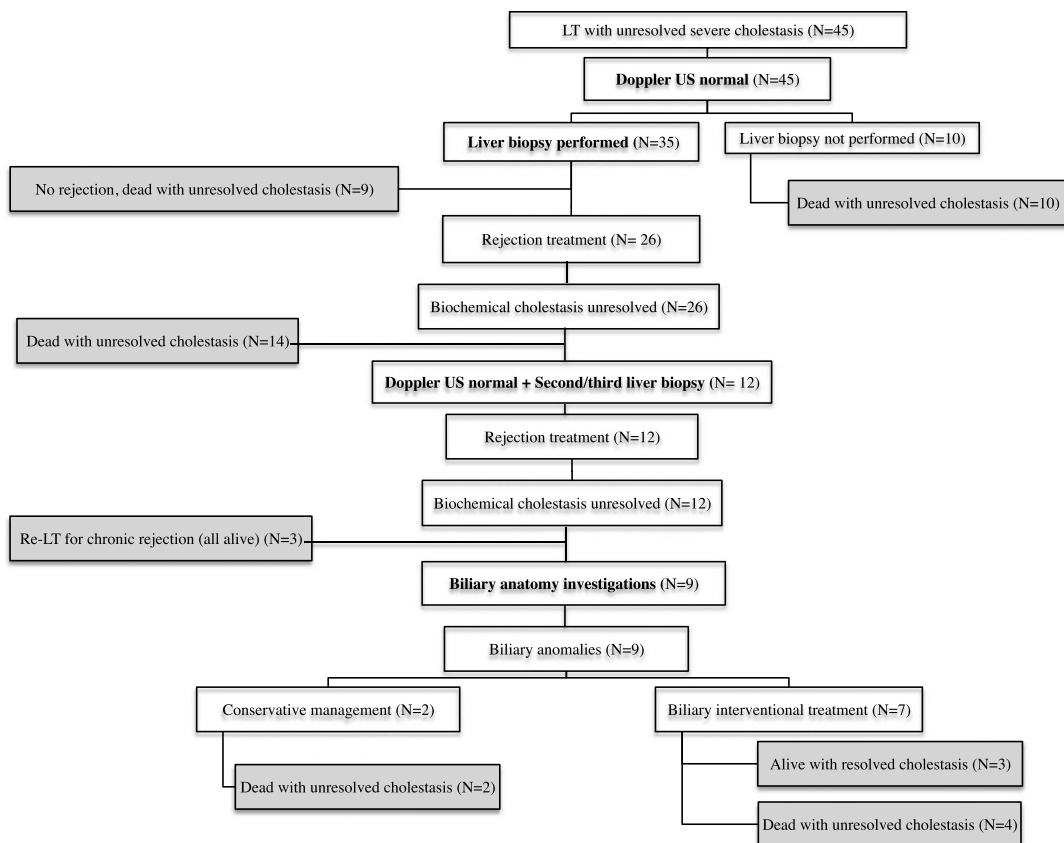

FIGURE 5. Diagnostic and therapeutic management and patient outcomes. N, number; re-LT, re-LT.
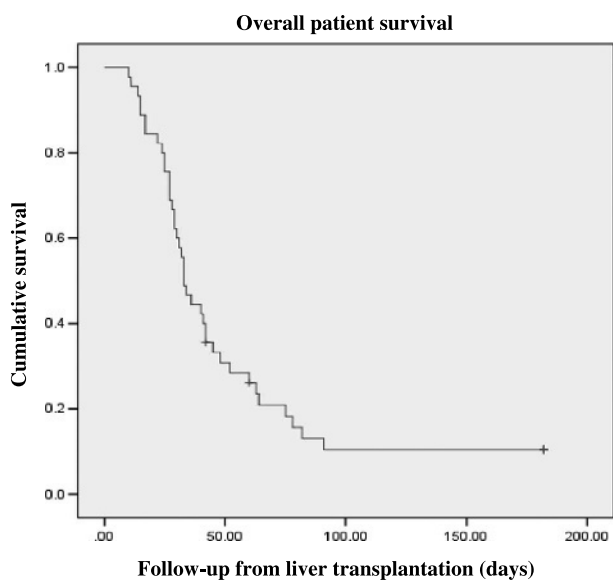
Overall patient survival

FIGURE 6. Survival rate after 3 months from LT of recipients who developed severe unresolving cholestasis.

observational period, the overall yearly graft dysfunction rate was 4.5%.

Overall rejection (histologically proven) may have been the predominant cause (60%), whereas rejection was not likely to have contributed in 14 (40%) patients.

In the 3 LT patients who underwent retransplantation, the liver grafts explanted at the time of the second transplant were analyzed; other patients did not have postmortem liver data. Of the 3 retransplanted patients, 1 underwent a second transplantation for chronic rejection, the diagnosis of which was confirmed on the explanted liver in association with severe ductopenia (C4d negative); the explant graft of the second patient who was retransplanted for chronic rejection showed features of chronic rejection, moderate ACR, severe centrilobular bilirubinostasis and focal microvesicular and macrovesicular steatosis. In the third patient, retransplanted for acute on chronic AMR, the histology of the explant graft showed signs of early cirrhosis and concomitant veno-occlusive lesions secondary to either AMR (Cd4 positive) or ACR.

DISCUSSION

Severe cholestasis is a frequent event early after LT as a consequence of a number of disparate disease processes with different mechanisms. In the immediate posttransplant period, severe intrahepatic cholestasis is mainly related to preservation reperfusion injury, which may cause damage to the bile canaliculi and is the major reason for EAD, contributing to a greater than 20% mortality rate after LT.[20] Other causes, which may present with cholestasis in the first weeks after LT, include vascular complications such as HAT and venous outflow obstruction, biliary anastomotic stricture or leak, and ACR.[1] Intrahepatic cholestasis may also be due to extrahepatic conditions such as sepsis,[21] cytomegalovirus infection,[22] and drugs, for example, cyclosporine.[23]

Transplant surgeons and physicians are routinely dealing with the management of such conditions to identify the major causes of cholestasis. Commonly, the initial diagnostic approach is by Doppler US followed by allograft percutaneous biopsy, when required. This pathway is considered sufficient to exclude the most common cause; however, a small proportion of recipients may develop persistent severe cholestasis during the first weeks after LT without apparent definitive cause detected by these routine investigations.

Doppler US is the most economical and cost-effective imaging modality for evaluating postoperative fluid collections, vascular and biliary complications after LT. Usually, when bile duct dilatation, stones and/or leakage are identified by US, patients are referred for therapeutic ERCP or PTC.[24-26] In contrast, in cases with a normal abdominal US and persistent deranged LFTs, a liver biopsy is commonly performed to exclude rejection. The interpretation however is not always straightforward with a broad spectrum of pathological processes seen early posttransplant and often there is more than 1 pathological process occurring. During the majority of this study period, the histological features of AMR were not described and hence not looked for and the clinical relevance is only now starting to be appreciated.[15,17,18]

In the first few weeks after LT, ACR (often associated with superimposed changes secondary to preservation-reperfusion injury) is the most common histological findings.[9,27] Moreover, it is now recognized that concomitant AMR may also occur and has been recognized as a cause of early unexplained graft loss, often with cholestasis.[17] With modern immunosuppressive drugs, rejection is not considered as a significant cause of graft loss; however, in this cohort rejection, nonresponsive to standard treatment, appears to play a major role. AMR is a recognized cause of graft loss in other solid organ transplants, with the liver traditionally being thought as resistant to AMR; however, there is accumulating evidence that AMR may cause graft dysfunction in liver allografts.[28-31]

Cholestasis of varying degrees is a frequent histological finding in liver allograft biopsies and is seen in bile duct obstruction, acute and chronic rejection, preservation-reperfusion injury, recurrent hepatitis C, sepsis, and drug reactions.[32,33] There are subtle differences between the histological features present in addition to the bilirubinostasis, which are associated with the clinical details often allow differentiation of the most likely cause.

In our series, histological findings of ACR overlapping with biliary features were the most common pattern found and have been the predominant driver for the management of these patients. Thus, despite the failure of antirejection treatment and the presence of histological findings that could not exclude a concomitant biliary process, the assessment of the biliary tree has been overlooked or severely delayed.

It is recognized that ACR may be seen in up to 32% of protocol biopsies, without biochemical evidence of graft dysfunction.[34] In addition, the incidence of biliary complications after LT is reported to range from 5% to 25%,[35] increasing with risk factors, such as donor after circulatory death grafts, advanced donor age, prolonged warm ischemic time, graft steatosis, hepatic artery complications, and T tube use.[36,37] Taken together, the presence of ACR on a biopsy, particularly if there is a failure to respond to treatment, should not be used as reassurance that there is not a biliary process occurring, and there should be an early trigger to investigate the biliary tree. From the findings of O'Leary, supported by some of our cases, the possibility of AMR should also be

considered as contributing to the failure to respond to standard rejection treatment. Therefore, DSAs should be looked for and plasma exchange, rituximab, or other therapies for AMR considered. It is recognized that DSAs are associated with the development of biliary and vascular strictures,[38] so the presence of a stricture does not exclude rejection as the underlying cause. Recently, an analysis of the incidence, costs, and outcomes of posttransplant biliary complications showed that the occurrence varies widely among transplant centers and that higher rates are a strong marker for increased risk of death, graft failure and healthcare spending.[39]

It is well known that macrovesicular steatosis greater than 30% is a predictor factor for reduced 1-year graft survival.[19] In our cohort, donor-origin steatosis was found in 20 (59%) of 34 postreperfusion liver biopsies; this was mild degree in 19 cases and moderate in 1 case. However, although graft steatosis might represent a risk factor contributing to persistent cholestasis, we believe that in our study population, the presence of mild steatosis did not significantly impact on graft outcomes. In contrast, the outcome of the patient who received the graft with moderate steatosis could have been influenced by the presence of the higher degree of steatosis, as a cofactor for persistent cholestasis.

This study is limited because of being a retrospective observational study, with a moderate number of recipients, limited radiological investigations to map out the biliary tree, interpretation of pathological findings, and recognition of AMR in the background where C4d and DSA tests are lacking. However, from our analysis, we believe that recipients with ACR, in whom biochemical cholestasis does not resolve after conventional antirejection treatment, should be investigated for an additional antibody mediated component to the

rejection and would benefit from early investigation for biliary complications; this should be the case even if the US does not show biliary features. Concomitant biliary pathology, possibly secondary to rejection related injury to the peribiliary plexus, may lead to graft loss.[40,41]

In clinical practice, invasive assessment of the biliary tract by ERCP is still difficult in the first weeks after LT due to the risk of iatrogenic injury to "fresh" anastomosis, perforation, infection, bleeding, pancreatitis, and sedation-related complications.[42-44] An alternative is MRCP, which has a sensitivity and specificity of 97% and 98%, respectively, for diagnosing biliary obstruction in the nontransplant setting.[45] A recent meta-analysis compared the accuracy of MRCP with ERCP in diagnosing biliary complications after LT[46]: MRCP seems to have an excellent accuracy for detecting biliary obstruction after transplantation, but only if performed at centers with well-developed expertise in MRCP.[47-49] Consequently, in our experience, the MRCP may be an appropriate test in patients with low to moderate suspicion of biliary obstruction, and its use could potentially avoid the unnecessary risks of ERCP in this clinical scenario (Figure 7).

Thus, in the last years, several authors proposed potential alternative approaches for the posttransplant cholestasis management. Recently, Lingala et al[50] suggested using a hepatobiliary iminodiacetic acid scan and peak aspartate aminotransferase (AST) to identify which patients with post-LT cholestasis would benefit from ERCP, showing that a normal hepatobiliary iminodiacetic acid along with combination of peak AST and bilirubin levels offer a valuable tool in identifying patients who will not benefit from ERCP. Gilroy et al[51] retrospectively analyzed all tests performed in LT recipients with biliary strictures and reported that
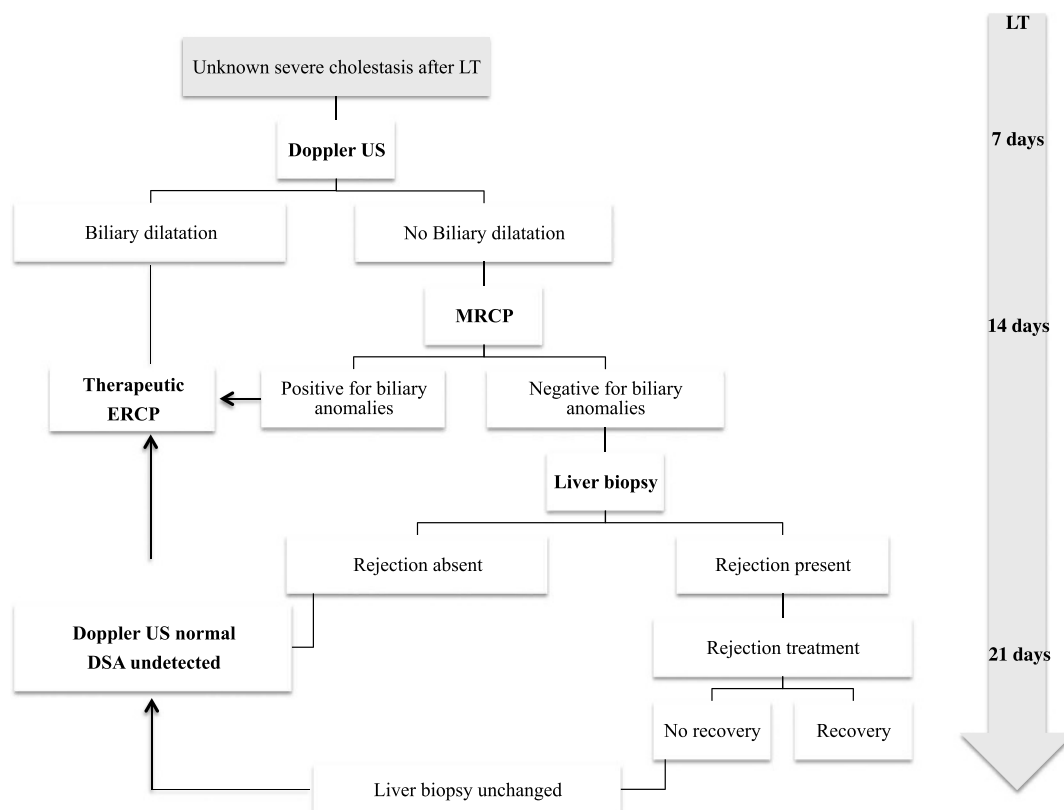

FIGURE 7. Proposed management of unresolved severe cholestasis after LT.

US scanning is very insensitive for identifying biliary complications while liver histological findings of pericholangitis and lobular acute inflammation are strong predictors of biliary strictures. They suggest performing a graft biopsy before considering ERCP as diagnostic approach in patients without biliary dilatation on US scan. Moreover, a great interest in the relationship between the increased rate of biliary strictures/cholestasis after LT and the ischemic injury caused by low hepatic artery flow has recently been shown.[52] This seems to be physiopathologically related not only to the ischemic bile duct damage but also to the functional impairment of bile secretion as previously described.[53]

In conclusion, unresolved severe cholestasis after transplantation influences patient/allograft outcome and its management is challenging. During the first weeks after LT, the role of US is unreliable in excluding early intrahepatic or extrahepatic biliary anomalies. Specific biliary radiological investigations are essential to rule out a biliary cause. Histologically, ACR in the presence of biliary features should trigger investigation of both biliary anastomotic complications and an AMR component, particularly, if there is no or limited response to standard rejection treatment. Close clinicopathological correlation and a multidisciplinary team comprising pathologists, hepatologists, and surgeons are necessary for the best outcome.

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