Liver transplantation : chimerism, complications and matrix metalloproteinases

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Chapter 5

Sequential liver chemistry profiling and abdominal ultrasound assessments to predict biliary strictures after liver transplantation

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Abstract

Background

After orthotopic liver transplantation (OLT) early detection of biliary strictures is important. Our aim was to evaluate the predictive value of routine serum liver chemistry profiling and abdominal ultrasound as non-invasive diagnostic tools in detecting biliary strictures after OLT.

Methods

We performed a retrospective study in which 141 OLTs, performed between 1992 and 2007 with more than 1 year follow-up, were included. Routinely assessed serum levels of alkaline phosphatase, alanine-aminotransferase, aspartate-aminotransferase, gamma-glutamyl transpeptidase and bilirubin at 3, 6, 9 and 12 months, and abdominal ultrasounds performed at 3, 6 and 12 months after OLT were evaluated. Time-dependent Cox regression analysis was performed to identify predictive factors for the development of biliary strictures.

Results

Eighteen grafts developed non-anastomotic strictures (12.8%) and 18 grafts (12.8%) developed anastomotic strictures requiring intervention. An elevated gamma-glutamyl transpeptidase (HR 1.25 per 100 IU/L; $p = 0.04$) and dilated bile ducts on ultrasound (HR 3.54; $p < 0.01$) were found to have an independent predictive value for the development of biliary strictures requiring intervention. Bilirubin and the other studied liver enzymes were not independently predictive.

Conclusion

Dilated bile ducts on ultrasound and elevated gamma-glutamyltranspeptidase after OLT are independent predictive factors for the development of biliary strictures requiring intervention.

Introduction

Biliary complications are common after orthotopic liver transplantation (OLT), with a reported prevalence of 6% to 35%.[1-4] Biliary strictures occurring at the surgical anastomosis are classified as anastomotic strictures (AS), whereas strictures in the donor biliary tree are referred to as non-anastomotic strictures (NAS). Stricture formation is often insidious and usually only then detected when lead to clinical symptoms as cholestasis, with serum liver enzyme abnormalities, intrahepatic bile duct dilatation and/or infection.[5] The definite diagnosis is made with endoscopic retrograde cholangiopancreatography (ERCP), percutaneous transhepatic cholangiography (PTC) or by magnetic resonance cholangiopancreatography (MRCP).[6,7] Although ERCP and PTC are considered the gold standard, they are invasive procedures and associated with considerable morbidity. ERCP and PTC are often immediately performed when clinical symptoms such as jaundice or cholangitis are present.[8] Several uncontrolled series evaluated the efficacy of ERCP in predicting biliary complications.[8,9] Most of these were evaluated in settings where patients present themselves with symptoms such as cholangitis due to a biliary stricture. However, in many liver transplant programs liver chemistry and enzymes and abdominal ultrasound are routinely assessed in an outpatient clinic at certain intervals as a screening tool, often long before patients develop symptoms such as fever or abdominal pain. It is remarkable that only few studies addressed the predictive value of routinely assessed serum liver chemistry profiles and abdominal ultrasonography (US) after OLT as predictors for the occurrence of biliary strictures.[10] Although some studies did evaluate the prognostic value of liver chemistry and US, the usefulness of routinely assessing these diagnostic tools in clinical setting remains unclear in a post-transplantation population.[10,11] The risk of developing biliary strictures varies over time, probably in association with the liver chemistry profile and US findings. The aim of the present study was to evaluate the predictive value of routinely assessed serum liver chemistry and abdominal ultrasound as non-invasive predictors for the development of biliary strictures requiring intervention after OLT.

Patients and Methods

Patients

We examined 141 consecutive OLTs with at least one year of follow-up and complete data on serum liver chemistry and enzymes and upper

abdominal ultrasonography (US) performed between September 1992 and April 2007 performed at the Leiden University Medical Centre. Re-transplantations (n=31) were excluded. Clinical data were obtained from the medical digital records, the hepatological and surgical patient charts, and endoscopy reports. Follow-up was up to August 2008 with a median of 5.2 years (range 1.0 -15.6).
OLT was performed according to standard procedures with cavo-caval, porto-portal, and hepatic artery to hepatic artery anastomosis. A duct-to-duct biliary anastomosis over an 8-12 Ch stent was performed, if possible. The biliary stent was removed after 6 weeks or removed earlier as indicated. In some cases the hepatic artery was anastomosed to the aorta via an iliac conduit. All patients received immunosuppressive agents according to protocol: cyclosporin A or tacrolimus, prednisone during the first half-year and patients with renal impairment received azathioprine before 2001 or mycophenolate mofetil from 2001 on. From 2001 on, basiliximab was given post OLT. In some cases sirolimus was used after month 3 in which case the calcineurin-inhibitor was lowered or discontinued. All patients received ursodeoxycholic acid in the first 3 months after transplantation.

Biochemical variables
Serum liver enzymes levels of alkaline phosphatase (ALP), alanine-aminotransferase (ALAT), aspartate-aminotransferase (ASAT) and gamma-glutamyltranspeptidase (GGT) were determined daily during the first two weeks and weekly for two months, after that at 3, 6, 9, and 12 months post-operatively. The same was done for bilirubin. Only the latter 4 time points were included in the study because in the first three months after liver transplantation liver enzymes are very susceptible to change due to procedure-related causes such as ischemia-reperfusion damage, rejection and infections. Therefore, the first three months of liver chemistry assessments, ultrasounds and biliary strictures after transplantation were excluded.
The upper limit of normal serum level was for ALP 120 IU/L, for ASAT, ALAT and GGT the upper limits of normal were 40 IU/L, 40 IU/L and 51 IU/L, respectively. The upper limit for bilirubin (total) was 17 µmol/L.

Imaging variables
US was performed routinely on day 0, 1 and 7, and subsequently at 3, 6 and 12 months after OLT. The US performed at 3, 6 and 12 months were included in this study. These US were performed by different experienced radiologists. A bile duct of >7 mm on ultrasound was considered dilated

and prompted either direct intervention by ERCP or PTC or additional MRCP which in turn might prompt ERCP or PTC. Routine abdominal CT scan was performed after 3 to 7 days post-OLT and routine liver biopsies were performed at 6 months after transplantation. Additional liver biopsies were taken on indication. Virology monitoring, including CMV-DNA, was performed frequently in the first year.

Clinical variables

Presentation of a biliary stricture (anastomotic and non-anastomotic) was with clinical symptoms such as cholangitis, pruritus or jaundice and/or abnormal liver chemistry. Diagnostic tests to confirm the diagnosis were performed on indication but not included in the present analyses. Only strictures occurring more than three months after OLT that required intervention by ERCP, PTC or surgery were included in this study. From 2001 on, routine ERCP with stent-removal was performed at week 6 post-OLT in case of a duct-to-duct anastomosis. Strictures were treated endoscopically with ERCP and dilation and/or stenting, and percutaneously with percutaneous transhepatic cholangiodrainage (PTCD) or with surgical intervention.

Statistical analyses

We have used a time-dependent Cox regression model to evaluate the diagnostic value of liver enzymes and routine US assessments in predicting biliary strictures. The Cox proportional-hazards regression model for time-to-event data (in this case the development of biliary strictures) takes into account the changes of variables over time, in this study changes of liver enzymes and bile duct dilatation on US. Time dependent predictors (covariates) for stricture development in this study were liver chemistry variables ALP, GGT, ASAT, ALAT and bilirubin, obtained with an interval of three months at 3, 6, 9 and 12 months post OLT and US performed 3, 6 and 12 months post OLT. Recipient characteristics, variables on etiology of liver disease and procedure-related variables were baseline characteristics. Coefficients were considered significant when $p<0.05$. The reported hazard ratios and p-values are *per* 100 international unit elevation for the liver enzymes. Bilirubin levels are reported per 10 µmol/L elevation.

Ethical committee

All data were obtained as part of patient care according to a strict protocol after OLT. There was permission from the local ethics committee to use these data.

Results

Patients and biliary strictures

Baseline characteristics of recipients, etiology of liver disease and procedure-related variables are presented in Table 1. Non-anastomotic biliary strictures requiring intervention developed in 18 of the 141 grafts (12.8%). Median time from OLT to NAS was 8.5 months (range 3-29). Median follow-up after the diagnosis of NAS was 5.5 years (range 0.0 – 11.6). Anastomotic strictures developed in 18 out of 141 grafts (12.8%). Median time from OLT to AS was 5.5 months (range 3-72). Median follow-up after the diagnosis of an anastomotic stricture was 2.8 years (range 0.6-15.3). A total of twenty-one ERCPs and twelve PTCs for the management of strictures were performed in the included cases. In three cases a surgical intervention to resolve the stricture was necessary. There was no difference between the duct-to-duct type anastomosis and the Roux-en-Y anastomosis in the occurrence of biliary strictures ($p = 0.88$). Other potential risk factors were calculated and are listed in Table 2.

Liver chemistry and abdominal ultrasound

Elevation of serum liver enzymes ALP, GGT, ASAT and ALAT above the upper limit of normal occurred in respectively 57.4%, 71.6%, 53.9%, and 61.0% of the patients at 3, 6, 9 or 12 months after OLT. Bilirubin was elevated in 41.6% of the cases. There was a significant relationship between the level of GGT and the development of biliary strictures requiring intervention, both in the univariate and in the multivariate analysis (hazard ratios 1.35 and 1.25, $p < 0.001$ and $p = 0.04$, respectively), as presented in Tables 2 and 3. AF above the upper limit of normal was also found to be a significant indicator for the development of biliary strictures in the univariate analysis ($p < 0.001$), but not in the multivariate analysis ($p = 0.23$). Elevated ALAT and ASAT were not associated with biliary strictures in both univariate and multivariate analyses (ALAT; $p = 0.61$. and $p = 0.81$ respectively, ASAT; $p = 0.62$ and $p = 0.42$ respectively). Elevation of bilirubin was not significant in both univariate and multivariate analysis for the prediction of the development of biliary strictures ($p = 0.08$ and $p = 0.33$ respectively (Table 3) Regarding the US assessments a significant relationship was found between dilated bile ducts on abdominal ultrasound and the successive development of a biliary stricture requiring intervention in both the univariate (hazard ratio = 4.48, $p < 0.001$) and multivariate analysis (hazard ratio = 3.54, $p < 0.01$). (Table 3)

Table 1. Baseline characteristics of 141 orthotopic liver transplants.

Recipient data	
- Male/ Female	91/50
- Median age (years) (range)	50 (16 – 70)
Etiology of liver disease	
Hepatitis B/C cirrhosis	10/22
Biliary cirrhosis (PSC/PBC)	30 (22/8)
Alcoholic cirrhosis	25
Hepatocellular carcinoma	19
Other	35
Donor and OLT procedure data	
DBD / DCD donor	135/6
Choledochocholedochostomy (duct-to-duct)/ Roux-en-Y hepaticojejunostomy	132/9
Donor warm ischemic time (DCD donors) (minutes) (range)	17 (11 – 23)
Cold ischemic time (minutes) (range)	605 (268 – 1095)
Recipient warm ischemic time (minutes) (range)	35 (16-90)

Table 2.

Univariate analysis of potential predictors for the development of biliary strictures (BS). Time-dependent analysis was used to calculate the predictive value of routinely performed liver chemistry profile assessments and dilated bile ducts on abdominal ultrasound (US) for detecting BS requiring intervention after OLT (n=141). The hazard ratios for liver enzymes are shown per 100 IU/L increase. The hazard ratio for bilirubine is shown per 10 µmol/L increase.

Clinical Variables	**Hazard Ratio (95% CI)**	**P-Value**
Dilated bile ducts on US	4.48 (1.97 -10.12)	< 0.001
GGT	1.35 (1.22 - 1.49)	< 0.001
ALP	1.55 (1.22 -1.89)	< 0.001
ALAT	1.13 (0.74-1.82)	0.61
ASAT	1.19 (0.61 -2.45)	0.62
Bilirubin	1.07 (0.99 – 1.16)	0.08
Gender	0.64	0.23
Type of surgical anastomosis (duct-to-duct / Roux-en-Y)	0.90	0.88
Age (at OLT)	0.98	0.14

Table 3.

Multivariate time-dependent Cox regression analysis for liver enzymes and dilated bile ducts on abdominal ultrasound (US) for detecting presence of BS requiring intervention after OLT (n=141). Gamma-glutamyltranspeptidase (GGT) and US remain significant predictors for the development of BS. The Hazard ratio for GGT shows the risk of having a stricture with each 100 1U/l increase. The hazard ratio for bilirubin was calculated for each 10 µmol/L increase.

Clinical Variables	**Hazard Ratio (95% CI)**	**P- value**
Dilated bile ducts on US	3.54 (1.47 – 8.49)	< 0.01
GGT	1.25 (1.02 – 1.55)	0.04
ALP	1.46 (0.93 – 2.24)	0.10
ALAT	0.92 (0.46 – 1.85)	0.81
ASAT	0.55 (0.13 – 2.33)	0.42
Bilirubin	0.99 (0.80 – 1.08)	0.33

Discussion

Biliary strictures frequently complicate orthotopic liver transplantation and lead to significant morbidity, graft loss and mortality. Early diagnosis and prompt intervention is therefore of great clinical importance. Cholangiography remains the most sensitive and specific assessment in diagnosing biliary strictures but is invasive. The most commonly used and least invasive diagnostic modalities after OLT are routine serum liver chemistry profile determinations and abdominal ultrasound. Surprisingly, there are only few studies on the prognostic value of these routinely performed laboratory tests and ultrasound. Our aim was to evaluate the predictive value of these routine diagnostic tests, often performed in an outpatient clinic setting, for the development of clinically relevant biliary strictures in a time-dependent model. NAS and AS represent different entities and have different etiologies. However, the tools for diagnosing and treating NAS and AS are generally similar. Therefore, in our study, these data were pooled. We found that after OLT an elevated serum gamma-glutamyltranspeptidase level and bile duct dilatation on abdominal ultrasound are independently highly predictive for development of biliary strictures requiring intervention.

The current findings are in accordance with non-transplantation studies in which GGT corresponds with the presence of biliary strictures, while mixed data are reported on the predictive value of liver chemistry and ultrasound for the presence of biliary complications after OLT.[8,10,12,18] Some studies showed no relationship between an aberrant serum liver chemistry profile and biliary complications post-OLT, whereas other studies did.[5,7,10,11,18]

Abdominal ultrasound is a non-invasive, readily available and economic diagnostic tool. However, several studies observed that ultrasound is not very sensitive in detecting biliary strictures in a post transplant population, in contrast to a non-transplant population, whereas few studies reported the opposite.[3,8,11,13] We found bile duct dilatation on abdominal ultrasound to be a powerful predictor of subsequent development of biliary strictures requiring therapy, exemplified by the high hazard ratio of 3.54 in the multivariate analysis. The use of abdominal ultrasound remains one of the safest and cheapest diagnostic tools for early detection of biliary strictures.

Although it may be not as sensitive as MRCP, these data show that dilated bile ducts on US after OLT often precedes biliary strictures requiring intervention. Sensitivity may be even better if strictures are clinically suspected. Dilated bile ducts on abdominal US after OLT

should prompt cholangiography. Whether this should be a MRCP first or immediate ERCP or PTC is a matter of debate: With ERCP and PTC the contrast flow across the stenosis can be assessed dynamically (and the use of an air-inflated balloon can be of additional value). MRCP is more expensive than US and access to MRCP is often more difficult. Quick action is warranted to prevent cholangitis in the immunosuppressed patient. MRCP can be considered first if there is less urgency.

Apart from a dilated bile duct on ultrasound there was an independent association between the increased serum level of gamma-glutamytranspeptidase and the risk of a biliary stricture requiring intervention. The accompanying hazard ratio (HR=1.25) is given per 100 units increase of GGT above the upper limit of normal (i.e., 151 U/L). To put these figures in the appropriate perspective: a GGT of 151 U/l (100 U/l above normal range) would result in a 25% increased risk of having a stricture requiring therapy. In fact, the hazard ratio for any elevation of GGT can be calculated. The given hazard ratios in table 2 and table 3 were also calculated per 100 units of elevation for illustrating purposes. Time-dependent analysis calculates the hazard ratio per 1 IU/L of elevated GGT, which in our study was 1.0022. Thus, an elevation of 100 IU/L would result in $1.0022^{100} = 1.25$ or a 25% increased risk. In formula terms a GGT elevation results in a hazard ratio of $1.0022^{(\text{elevation above upper limit in IU/L})}$ for the development of a biliary stricture.

In clinical practice the calculated hazard ratios of both bile duct dilatation on US and the elevation of GGT are multiplied. In our example GGT levels 100 IU/L above the reference range together with dilated bile ducts on US result in a hazard ratio of 4.3 (3.54 x 1.25), which indicates that the risk of developing a biliary stricture requiring intervention in the next three months is 4.3 more likely compared to the standard risk.

It is remarkable that only few studies have addressed the usefulness of routinely assessing liver chemistry and performing ultrasound after OLT. Hussaini et al.[11], for example, showed that US was a valuable tool to diagnose biliary strictures with a sensitivity and specificity of 77% and 67%, respectively, whereas Zoepfi et al.[10] showed no significant relationship for both ultrasound and liver enzymes in the detection of biliary strictures. Que et al.[18] found US to detect biliary strictures with a sensitivity and specificity of 90% and 91% respectively, but reported GGT and ALP to be of poor diagnostic value even at 10-folds the upper limit of normal. However, these studies evaluated liver chemistry using sensitivity and specificity in relation to the presence of biliary strictures. Although this is a common way to evaluate the diagnostic value of clinical tools it has several limitations. Sensitivity and specificity are

used to determine the probability an aberrant result in case of present disease and vice versa. This method would applicable if the assessment of the liver chemistry profile and biliary strictures occur simultaneously as is the case in, for example, an emergency setting. However, routine assessments are often performed in an outpatient clinic setting where most patients have not yet developed symptoms due to biliary strictures. Routine assessments are used based on the idea that early detection of any aberrance of liver chemistry profile might prompt intervention to prevent complications due to biliary strictures such as cholangitis.

However, the time interval for occurrence of biliary strictures after routine assessment of liver chemistry varies for each patient, and the time for being at risk varies along. This means that the risk of developing a biliary stricture for each patient varies along with the changes in liver chemistry. Sensitivity and specificity are also based on dichotomized variables, that is: liver chemistry or enzymes are elevated or not. However, liver chemistry profiles are continuous variables and change over time. These data show that the level of elevation significantly impacts the hazard for development of biliary strictures.

We realize that our study has limitations. We decided to use a follow-up of one year after OLT, but excluded the first three months. The first three months postoperative months were not included because early after transplantation liver chemistry levels are influenced by many variables, such as ischemia-reperfusion damage, rejection and infection. A shorter follow-up would have been possible, but since liver chemistry and enzymes levels vary over time it is obligate to assess them over a longer period for their utility. A shorter follow-up would have weakened the statistical analysis. One might criticize that in this study patients were included over a long period of time and management of strictures may have changed over the years. Since we focused on the development of strictures rather than the treatment, this is not a relevant issue in interpreting our findings. Routine liver chemistry profiling and abdominal assessments were performed since our center started OLTs and strictures and their treatment were consistently reported. In a retrospective study such as this, one should be careful when using parameters that have not been defined beforehand, such as the ultrasound findings. All ultrasound procedures in our institute were performed by radiologists with expertise in liver transplantation and bile duct dilatation was reported using strict criteria. Other reported ultrasound findings of the biliary tree, such as sludge or a thickened biliary wall, were not taken into account in our analysis.

The evaluation of routinely assessed liver chemistry and abdominal

ultrasound in a time-dependent Cox regression model for predicting biliary strictures, as has been used for the first time in the present study, takes into account the variability of both changes of liver chemistry and bile duct dilatation on US, hence providing clinicians with common non-invasive tools for predicting biliary strictures requiring intervention. Based on these findings we conclude that routine assessments of gamma-glutamyltranspeptidase and abdominal ultrasound are useful for early detection of biliary strictures after OLT. We advocate that dilated bile ducts on ultrasound and elevated gamma-glutamyltranspeptidase more than three months after liver transplantation should prompt cholangiography for early diagnosis and therapeutic intervention of biliary strictures.

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