

Dr Elizabeth Forrest
Queensland Liver Transplant Service
Princess Alexandra Hospital
Brisbane, Queensland, Australia

19th September 2017

Dear Ms Cui,

I am writing in response to an email dated 4th September 2017, regarding the review of our manuscript "Risk factors and clinical indicators for the development of biliary strictures post liver transplant: the significance of bilirubin." (Manuscript number 35881).

We appreciate the comments of the reviewers and we have addressed each of these in the comments listed below. In view of these comments we have made several changes to our manuscript. We have uploaded updated manuscripts for your viewing, both tracked and clean versions, using the manuscript central site.

Thank you in advance for considering our revisions and please informed me if there is any further required documentation.

Kind regards,

Elizabeth Forrest, MD, BSc, BA (on behalf of all authors)

Queensland Liver Transplant Service
Princess Alexandra Hospital
Care of - Gold Coast Hospital and Health Service, Department of Surgery, 1
Hospital Blvd Southport, Gold Coast 4215, QLD, Australia.
Email: elizabethforrest89@gmail.com

Reviewers' Comments to Author:

Reviewer 1 (02454185):

1. The objective criteria for defining stricture should be described in the method section, especially the inner diameter used for the diagnosis.

We thank the reviewer for this comment. Biliary strictures were identified by an experienced liver transplant surgeon (JF) for the purposes of this study. A stricture was defined as a narrowing of the bile duct with dilatation of the proximal biliary duct. No strict diameter cut-offs were used to define the structure.

The following sentence was included in the methods section (page 9, paragraph 1): a biliary stricture was defined as a narrowing of the bile duct with dilatation of the proximal biliary system.

2. "All continuous variables are expressed as median (interquartile range) and all categorical variables as frequency (percentage). "---insert a reference after the sentence (Ann Transl Med. 2016 Mar;4(5):91. doi: 10.21037/atm.2016.02.11.).

Thank you for this comment. We have inserted the reference in the statistical analysis section of the manuscript (Page 9, paragraph 2).

3. Table 3: why not perform statistical testing for the three groups? Some characteristics might be different among the groups.

The authors would like to thank the reviewer for their suggestion. The authors strongly believe that comparing these three group is inappropriate as certain individuals are represented in more than one group. For example, some patients underwent reoperation of the biliary anastomosis and then proceeded to retransplant.

The follow sentence was inserted in Table 3 and on page 11, paragraph 1 “some patients were represented more than once as they underwent two interventions for biliary stricture formation”.

4. The causal relationship between stricture and elevated bilirubin was important. the former may be the reason and the latter is a consequence of the stricture.

We agree with the reviewer that a bilirubin rise post transplantation could be a consequence of the biliary stricture, however imaging at this early time point is not routinely performed. Another possibility is that impaired bile transporter function within the first week post transplantation, with associated increased in bile toxicity, ultimately results in stricture formation later down the track. Previous studies have identified a more toxic bile composition in recipients developing non-anastomotic biliary strictures. Furthermore, prolonged graft ischaemia was found to cause an unparalleled impairment of bile acid transporter expression in cholangiocytes leading to prolonged biliary transit time of bile acids inducing apoptosis.

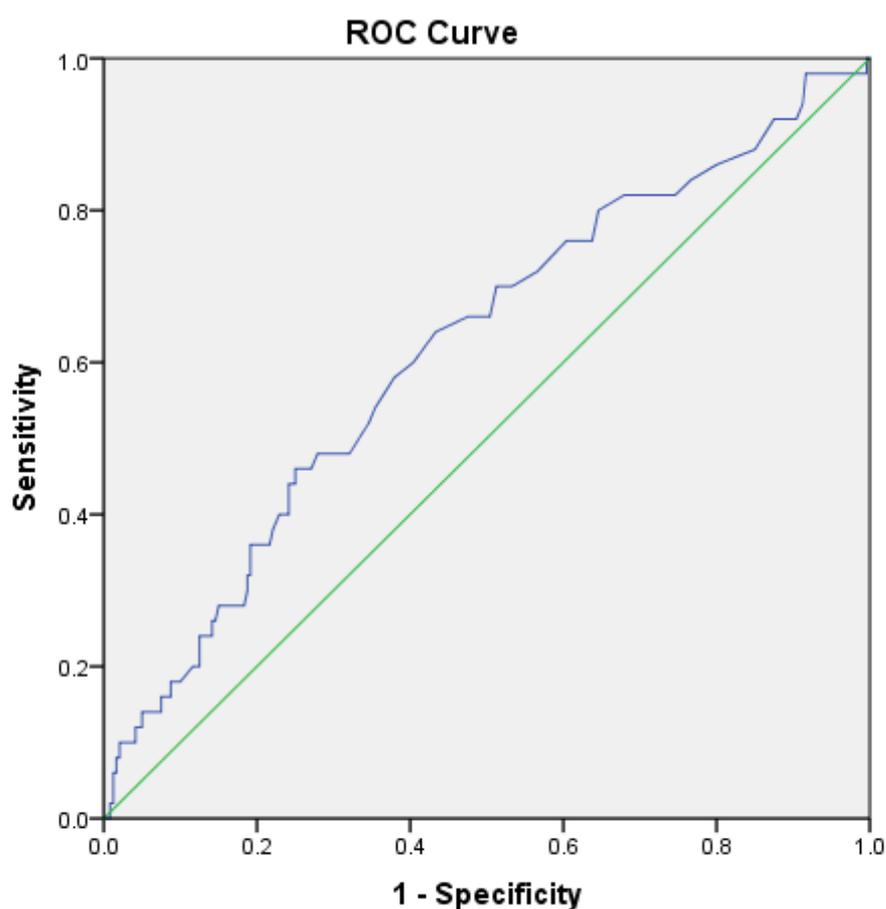
The above hypothesis was included in the discussion (page 13, paragraph 2).

5. "Multivariable logistical regression analysis was performed to determine the risk factors for biliary strictures following transplantation."---add a reference after this sentence (Ann Transl Med. 2016 Mar;4(6):111. doi: 10.21037/atm.2016.02.15).

Thank you for this comment. We have inserted the reference in the statistical analysis section of the manuscript (page 9, paragraph 2)

6. Further analysis was required to give the diagnostic accuracy of serum bilirubin for predicting stricture. Some statistics can be clinically relevant such as sensitivity, specificity and AUC.

Thank you for this comment. A statistical analysis was performed with a Receiver Operating Characteristic curve in SPSS. The following data was obtained for Day 7 total bilirubin ($\mu\text{mol/L}$) post liver transplant on the risk of developing biliary strictures. Area under the curve = 0.62 (Figure 1), indicating a result >0.5 and therefore not related to chance. Cross tabulation for Day 7 total bilirubin $> 55 \mu\text{mol/L}$ was used to calculate sensitivity and specificity for biliary stricture formation. Sensitivity for was 38.6% and specificity was 77.6%. These results have been inserted in the manuscript page 11, paragraph 2.



Diagonal segments are produced by ties.

Figure 1 ROC Curve for day 7 bilirubin as a continuous variable

Reviewer 2 (00504591):

1. Page 8, what is the indication of intervention for biliary stenosis?

The authors thank the reviewer for the question. At our liver transplant centre in Queensland, Australia, the current indication for intervention for biliary strictures is patient symptomatology (e.g. cholangitis, elevated bilirubin). When a biliary stricture is identified as an incidental finding and the patient is well, no intervention is indicated.

The following sentence was amended in the text (page 9, paragraph 1): Instead, imaging is guided by patient symptomatology.

2. Page 8, how often are the patients cared in the outpatient clinics?

After liver transplantation, patients spend a period of time as an inpatient for post-operative care and monitoring. Upon discharge patients are initially followed up daily in the outpatient department. When the direct post-operative period has passed the transplant recipients are seen in the outpatient clinic as follows:

1. From 3-4 weeks post transplantation: twice weekly
2. From 2-3 months post transplantation: weekly
3. From 3-12 months post transplantation: monthly
4. After 12 months: 3 monthly bloods, and review over 6 months to 1 year

The following sentence was included in the methods section, page 8, paragraph 1, "Patients were initially followed up daily in an outpatient clinic post hospital inpatient discharge, following this twice weekly if three week's post-transplant, weekly if two months post-transplant, monthly if three months post-transplant and finally with third monthly blood tests if 12-months post-transplant."

Reviewer 3 (02539179):

1. The article title is not appropriate because bilirubin level is not a risk factor for the development of biliary strictures post liver transplantation. Hyperbilirubinemia may be a good clinical indicator for the development of biliary strictures post transplant, if other causes of hyperbilirubinemia can be ruled out.

The authors agree with the reviewer that hyperbilirubinemia is not a risk factor for the development of biliary strictures.

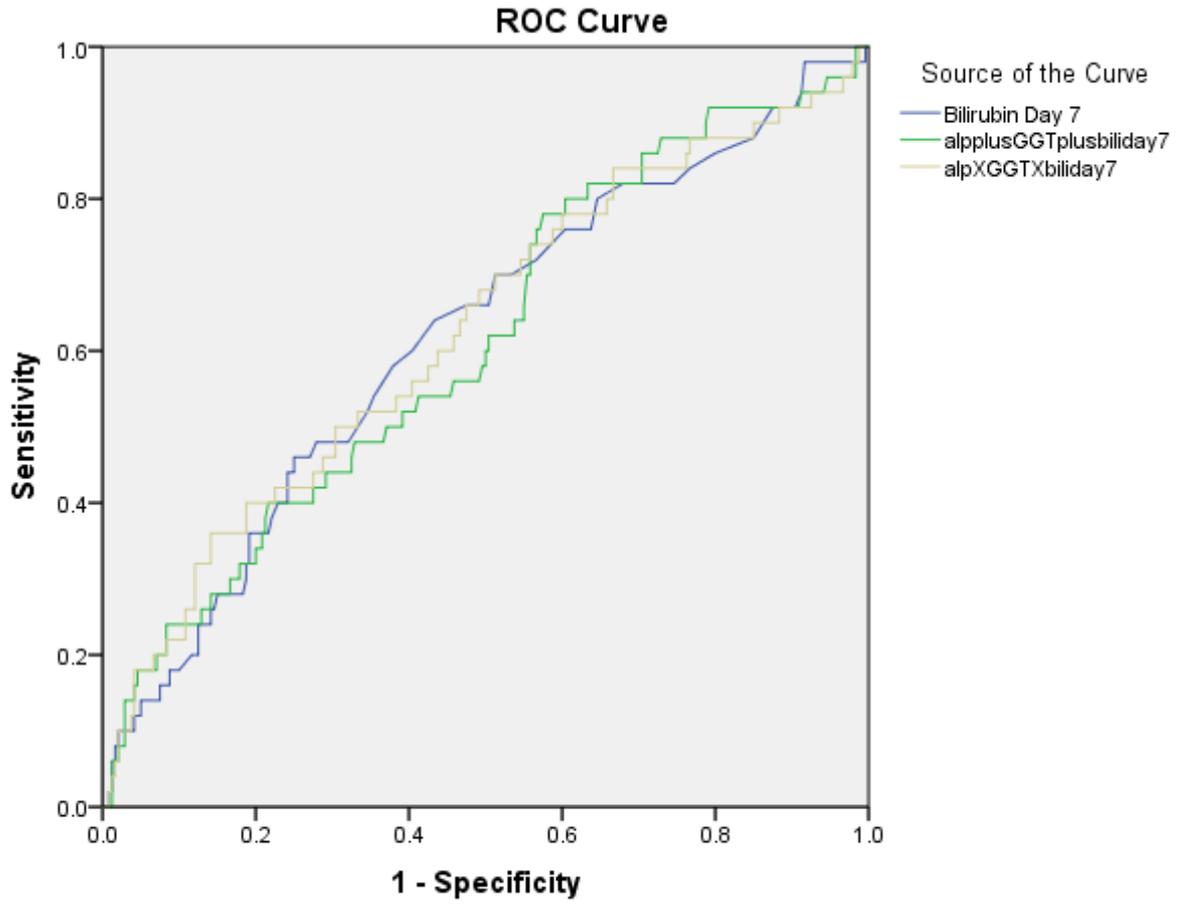
We therefore amended the manuscripts title: Risk factors and clinical indicators for the development of biliary strictures post liver transplant: the significance of bilirubin.

2.. What kind of serum bilirubin - Direct, indirect or total bilirubin was studied should be specified.

Total bilirubin was studied in this study. Several changes have made throughout the manuscript to reflect this.

3. The combination of serum direct bilirubin, serum alkaline phosphatase (ALP) and serum Y-Glutamyl transferase (GGT) status may have better performance than bilirubin level alone in predicting biliary stricture, which should be analyzed.

We thank the reviewer for the comment. The following analysis has been completed and did not show better performance, given the simplicity of bilirubin as a sole indicator, the authors think the combination of different factors it has no added benefit.



Diagonal segments are produced by ties.

Area Under the Curve

Test Result Variable(s)	Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Bilirubin Day 7	.618	.045	.009	.530	.705
alplusGGTplusbiliday7	.610	.044	.014	.523	.697
alpXGGTXbiliday7	.624	.046	.006	.534	.713

The test result variable(s): Bilirubin Day 7, alplusGGTplusbiliday7 has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Reviewer 4 (03538749)

Biliary stricture formation post liver transplantation is a frequent cause for patient morbidity and mortality and are referred to as the Achilles' heel of transplantation. This manuscript identified clearly risk factors associated with the formation of biliary strictures post orthotopic liver transplantation, and investigated potential post-transplant surveillance methods that could be used to identify patients at risk of biliary stricture formation. It is useful for our clinical work.

We would like to thank the review for the kind words and appreciate the positive feedback.

Reviewer 5 (01221925):

1. In patients with elevated bilirubin at day 7, what did the authors do? What studies were performed to identify a cause? How soon thereafter was a stricture recognized?

The authors thank the reviewer for this question. As this was a retrospective study over a 10-year period, the authors were not able to intervene on patient management. The study was conducted at our centre to investigate the presence of biliary stricture formation using imaging modalities such as ultrasonography of the liver, magnetic resonance cholangiopancreatography (MRCP) or Endoscopic Retrograde Cholangio-Pancreatography (ERCP). Imaging was only performed when the presence of a stricture was suspected. The median time between transplantation and stricture formation was four months.

2. Did patients with a T-tube undergo routine cholangiograms? If not routinely, when?

In the early years of our transplant program, T-tubes were routinely inserted. Prior to removal of the t-tube, a cholangiogram was routinely performed. Currently T-

tubes are no longer used in our centre and therefore an alternative clinical indicator of biliary stricture formation is urgently needed.

As only 23 patients included in the current study received a T-tube, the authors do not think that this had an impact on our findings.

3. How long were the T-tubes left in place?

In patients that did have T-tubes inserted in our cohort, a six-week check cholangiogram was performed prior to removal.

4. Why do the authors think that the incidence of biliary strictures in patients with hepaticojejunostomies was higher? Was it a technical issue or a matter of primary disease?

Although a small numerical difference was seen in the incidence of biliary stricture formation in the patients with hepaticojejunostomies, this was not found to be statistically significant $p = 0.24$.

The following sentences were removed from the results section (page 10, paragraph 3): In our cohort, recipients with biliary strictures were more likely to have had a Roux-en-Y anastomosis used ($n = 16, 35.6\%$). With most patients without biliary strictures using the duct to duct technique ($n = 184, 73.3\%$).

5. What kind of algorithm do the authors propose in patients with elevated bilirubin on day 7?

The authors suggest in patients with an elevated total bilirubin on Day 7 $>55 \mu\text{mol/L}$ an MRCP should be considered within the first 3-6-months post liver transplantation as they are at higher risk of developing biliary strictures. This algorithm is a suggestion and should be applied with appropriate clinical judgement.

6. What happened to the elevated bilirubin on day 7? Did it increase/decrease and how quickly?

It was noted in patients that developed biliary strictures, total bilirubin increased steadily in the seven post-operative days, with a steeper increase noted in between post-operative day five to seven (Figure 1). This is compared those patients without biliary stricture formation in which the total bilirubin levels on average decreased within the first seven post-operative days. Unfortunately, no further serum liver function tests results were collected after the first post-operative week. The authors however strongly believe that the bilirubin levels of these patients normalised as it would have otherwise resulted in earlier imaging of the biliary tree. The median time to detection of biliary stricture formation was 4 months.

7. How does this study differ from other studies in the literature looking at the association of biliary strictures and elevated bilirubin?

The authors would like to thank the reviewer for this comment as it brings up an important point. We believe the study's results add to the currently body of evidence on this topic, as it identifies a specific clinical parameter that clinicians can use as a guide to predict those at risk of biliary stricture formation post liver transplantation. In addition, instead of just analysing risk factors this study has developed a potential algorithm that clinicians can follow to assist these patients.