Utility of real-time US-guided percutaneous liver biopsy in pediatric liver transplant recipients

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Authors: S. Mandal\textsuperscript{1}, R. Miraglia\textsuperscript{2}, L. Maruzzelli\textsuperscript{2}, R. Liotta\textsuperscript{2}, K. Cortis\textsuperscript{2}, A. Luca\textsuperscript{2}; \textsuperscript{1}Pittsburgh, PA/US, \textsuperscript{2}Palermo/IT
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Aims and objectives

This study assesses the safety of US-guided PLB within pediatric liver allograft recipients, describes the pathological results according to early (#12 months) and late (>12 months) post-transplantation periods, and analyzes the value of liver function tests (LFT) and Doppler US variables in determining these results.

Introduction

The use of ultrasound (US)-guided percutaneous liver biopsies (PLB) in pediatric liver transplant recipients is routinely motivated by the possibility of allograft rejection. Acute rejection is reported to occur in 20%-40% of patients within the first months post-operatively, while chronic rejection is typically seen with 2% of patients anytime during their lifetime [1].

Because of the invasive nature of US-guided PLB, there has been interest in obviating its use by relying on the value of liver function tests to predict histological outcomes; in fact, some institutions have delayed the use of US-guided PLB until children fail to respond to therapy based on clinical judgment [2]. What is understood in adult cohorts is that standard liver function tests do not correlate well in many cases of grading the severity of liver graft dysfunction [13-15]. Similarly, the interest in US variables as a predictor of forms of rejection post liver-transplantation has existed, but this work has utilized mostly or exclusively adult cohorts, focused on the time period within 2 weeks or shortly after transplantation, and/or preceded a standardized classification of rejection [16-23].
Methods and materials

Patient Characteristics

Eighty five consecutive patients from March 2005 to May 2012 underwent US-guided PLB post liver-transplantation (mean age 7 years, range 6 months-18 years). Two hundred and nineteen biopsies were conducted in the early (n=92, 42%) and late (n=127, 58%) post-transplantation period. All patients underwent liver function testing and US evaluation on or the day before the biopsy.

The patient characteristics are shown in Figure 1.

![Table showing patient characteristics](image-url)
Fig. 1: Characteristics of 85 patients post LT who underwent 219 US guided PLB & graft/procedure characteristics (LT, liver transplantation; PLB, percutaneous liver biopsy, LFT, liver function test)

References: Diagnostic and Therapeutic Services, Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IsMeTT) - Palermo/IT

US and PLB Techniques

GE Logiq 7 and GE Logiq E9 machines (GE Healthcare, Milwaukee, WI) using a 3.5MHz sectorial transducer and a 6 to 15 MHz linear transducer were used for evaluation of the liver, biliary tree, and hepatic vasculature prior to or on the day of liver biopsy. All examinations were performed by 4 radiologists with more than 5 years of experience in Doppler US in pediatric liver transplant recipients. Images were reviewed and collected by a medical student under the supervision of a radiologist with 11 years of experience in abdominal interventional radiology, both of whom were blind to biochemical and histological results. Portal vein velocity peak and main hepatic arterial resistance index (the ratio of the difference of end-diastolic velocity and peak systolic velocity to the peak systolic velocity) were measured. Hepatic vein flow pattern (calculated by imaging during at least 2 cardiac cycles) and bile duct dilatation were quantitatively and qualitatively assessed.

Liver biopsies were performed by 2 abdominal interventional radiologists with 11 and 8 years of experience. All procedures were performed as inpatients. Procedures were performed with a percutaneous substernal approach using an 18 gauge automatic needle with a 19 mm sample notch (Tru-core II; Angiotech Pharmaceuticals Inc.,Gainesville,FL,USA) advanced under real-time US guidance using GE Logiq 7 or GE Logiq E9 machines using a 3.5MHz sectorial transducer or a 6 to 15 MHz linear transducer. In all procedures a sterile disposable needle guide (Ultra-Pro II Needle Guide, CIVCO,Kalona,Iowa,USA) was placed over a sterile cover-probe on the specific transducer bracket. The biopsy needle was followed, by the operator, within the US-screen software guidelines.

All procedures were performed with monitored anesthesia with spontaneous respirations (Propofol intra-venous 125 to 300 mcg/kg/min; Fresinius Kabi,Verona,Italy), and additional local anesthesia at the puncture site (1% Lidocaine, Monico,Venezia,Italy).

If coagulation defects were present (platelet count between 30,000/ mm³ and 50,000/ mm³ and a International Normalized Ratio (INR) between 1.5 and 1.9) patients were infused with platelets and/or fresh frozen plasma before the procedures. In six children with severe coagulation impairments (platelet count <30,000/ mm³ and/or INR # 2.0) requiring liver biopsy, transjugular liver biopsy was preferred; those patients were not included in our analysis.
The number of needle passages was dependent on the integrity of the specimen obtained with each passage. A manual compression of 10 minutes at the puncture site was performed following each procedure. Track embolization was never performed.

**Histopathological & Laboratory Analysis**

Specimens from US-guided PLB were fixed with 10% phosphate buffered formalin and stained with hematoxylin and eosin. All samples were processed through routine histologic examination by a pathologist with 11 years of experience blind to other clinical, laboratory, or imaging findings. Some cases were posted for second-opinion consultations using a telepathology system. The diagnoses of various forms of rejection were obtained using the Banff criteria with a rejection activity index (RAI) less than 3 serving as indeterminate acute rejection and greater than or equal to 3 as acute rejection [24]. Chronic rejection was diagnosed through bile duct loss and obliterative arteriopathy [25]. Hepatitis and grading of inflammation and fibrosis were assessed according to the hepatitis activity index (HAI) [26, 27]. Laboratory data retrieved included levels of total bilirubin, amino alaninetransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase, platelet count, prothrombin time (PT), and INR.

**Statistical Analysis**

Means, medians, and standard deviations were used to describe continuous variables and multinomial logistic regression was used to evaluate relationships with nominal categorical variables. Kruskal-Wallis test was used to compare medians of non-normal distributions and ANOVA was used to compare means of liver function enzymes and US variables. Proportion and Fisher’s exact test were used to compare differences amongst pathologies within the early and late post-transplantation period and also the distribution between the two periods. \( x^2 \) was used in comparison of distributions of hepatic vein flow type. The timing of the biopsy post-transplantation was controlled for, and patients with no pathological findings post biopsy were used as a baseline measure in all multinomial logistic regression analyses. All statistical analyses were performed with Stata Version 12 by a statistician with 8 years of experience.
Results

Two patients (0.91%) had complications consisting of a 2-g or greater hematocrit drop requiring blood transfusion, none of the two patients had associated clinical signs of hemorrhage. No patients required surgical interventions or interventional radiological procedures secondary to biopsy complications.

All samples were deemed adequate for histological analysis. In the early post-transplantation stage, findings included cholestasis which often overlapped with ischemia (36%), no pathology or minimal changes which were not clinically relevant according to the HAI (16%), acute rejection (13%), inflammatory diseases comprising of hepatitis and cholangitis (15%), indeterminate acute rejection (11%), chronic rejection (4%), and fibrotic diseases including cirrhosis, severe fibrosis, and hepatoporal sclerosis (4%). Late stage biopsy results included cholestasis (18%), no pathology (24%), acute rejection (5%), inflammatory diseases (15%), indeterminate acute rejection (7%), chronic rejection (14%), fibrotic diseases (12%) and other diseases including steatosis (5%).

A significant difference existed between early and late pathology distribution (Fisher's exact=.001) (Figure 2). This effect was largely driven by the significant differences with cholestasis (p=.003), acute rejection (p=.027), and chronic rejection (p=.017) between the early and late stages post-transplantation.
Fig. 2: Distribution of pathologies before (n=92) and after (n=127) 12 months for 219 biopsies.

References: Diagnostic and Therapeutic Services, Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IsMeTT) - Palermo/IT

Significant differences in liver function enzymes amongst pathologies existed with total bilirubin (p=.001), AST (p=.0004), ALT (p=.0023), and GGT (p=.0001) (Figure 3).
Fig. 3: Mean liver function test values by pathological diagnosis found to be significantly different for 219 biopsies.

**References:** Diagnostic and Therapeutic Services, Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IsMeTT) - Palermo/IT

Using the biopsy results that demonstrated no pathological findings (n=46, 21%) as a baseline measure, multinomial logistic regression analysis revealed relative risk ratios (RRR) for each functional parameter in relation to a specific pathological outcome. Within liver function enzymes, GGT demonstrated a significant relationship with cholestasis (p=.011, CI 1.00-1.01) and inflammatory diseases (p=.017, CI 1.00-1.01); however, the RRR was 1.00 indicating no increased associated risk. The coagulation-related parameters of platelet count and PT demonstrated significance with fibrotic and inflammatory diseases and indeterminate acute rejection; however, the RRR was 0.99 indicating almost no relationship. Elevations in INR was associated with a 1.64 times likelihood development of fibrotic diseases (p=.005, CI 1.2-2.3).

The differences amongst pathologies in terms of the US variables of portal vein velocity, main hepatic arterial resistance index, hepatic vein flow type, and bile duct dilatation as well as the RRR associated with each pathology using the biopsies with
no pathological findings as a baseline measure showed that significant differences in bile duct dilatation (p=.003) and hepatic vein flow type (p=.01) existed amongst pathologies; however, multinomial logistic regression analysis only showed significant relationships with increases in hepatic arterial resistance index (p=.011, CI 1.14-2.76) and the presence of bile duct dilatation (p=.002, CI 2-29) with the development of cholestasis (Figures 4 & 5).

Fig. 4: Mean US parameters by pathological diagnosis for 219 biopsies (MHA RI, main hepatic artery resistance index, † statistically significant).

References: Diagnostic and Therapeutic Services, Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IsMeTT) - Palermo/IT
Fig. 5: Relative risk ratios and p values by US parameters of pathological biopsies compared against those biopsies revealing no pathology (MHA RI, main hepatic artery resistance index, † statistically significant).

References: Diagnostic and Therapeutic Services, Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IsMeTT) - Palermo/IT

Discussion

The main goal of our study was to investigate whether US-guided PLB is appropriate for pediatric liver transplant recipients during the early and particularly late post-transplant period and whether more non-invasive techniques of liver function testing and US imaging could obviate the need for US-guided PLB. Since there is a likelihood that patients can develop various diseases other than rejection (Figure 2), we explored whether non-invasive tests could correlate to these particular pathological outcomes. Specifically, we used multinomial logistic regression to observe what the RRR would be between these non-invasive measures and a specific pathology in relation to a baseline of those biopsies demonstrating no pathology. Because this cohort of "no pathology" was often correlated to clinically pathological indications (e.g. elevations in liver function enzymes), it is important to note that no pathology does not equate to no histological abnormalities. If the abnormality was not relevant enough according to the HAI, then the outcome was designated as no pathology.

Liver function tests showed poor predictive value in determining the pathological outcomes demonstrated in this study. This result is in line with a pediatric series which evaluated the incidence of certain pathologies including hepatitis B to show the weak diagnostic value of liver function tests with a sensitivity and specificity of 75% and 54%, respectively [30]. In our cohort, while the median concentration of liver enzymes were significantly different amongst various pathologies (Figure 3), further exploration of the liver enzymes through multinomial logistic regression against a baseline of no pathology did not reveal any meaningful relationships. Furthermore, while it appears there is an associated risk between elevations in INR and fibrotic diseases, the lack of a relationship between PT and fibrotic diseases is inconsistent with a definitive conclusion.

Additionally, there were a lack of meaningful associations between US variables and the pathological findings in our cohort. Prior studies have failed to demonstrate correlations between changes in hepatic arterial resistive indices and acute rejection [16, 17-19]. Our study has demonstrated a similar lack of results in children for both the early and late post-transplantation period for not only rejection, but also for all pathological findings except cholestasis. The significance with cholestasis was further explored by performing the multinomial logistic regression of hepatic arterial resistance of less than 0.55 and greater than 0.81 against the baseline of a normal index range of 0.55 to 0.81 [31, 32]; however, no relationships between cholestasis and hepatic arterial resistive index in the context of normal and abnormal ranges existed (less than 0.55 p value =0.60, greater than 0.81
p value=0.99, data not shown). Unsurprisingly, bile duct dilatation and cholestasis were correlated; however, due to the large confidence interval, the result is not very precise.

Some studies have demonstrated relationships between US parameters, mainly hepatic vein waveforms and portal blood velocity, and acute rejection typically within 2 weeks after transplantation [18, 20, 22, 23]; however, the contrary has also been reported [21]. Study methods and definitions, cohorts, and timing of biopsy post transplantation likely account for variability in these results and the lack of results within our study. We demonstrated differences in hepatic vein flow patterns by pathology; however, as seen in Figures 4 & 5, these differences did not result in any associated risk in developing any specific pathology.
Fig. 2: Distribution of pathologies before (n=92) and after (n=127) 12 months for 219 biopsies.

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Fig. 3: Mean liver function test values by pathological diagnosis found to be significantly different for 219 biopsies.

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**Fig. 4:** Mean US parameters by pathological diagnosis for 219 biopsies (MHA RI, main hepatic artery resistance index, † statistically significant).

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<table>
<thead>
<tr>
<th>Pathological Diagnosis</th>
<th>Portal Vein Velocity (cm/s)</th>
<th>MHA RI</th>
<th>Hepatic Vein Type of flow (% of each pathology)</th>
<th>Bile Duct Dilatation (% yes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non-phasic</td>
<td>Phasic</td>
</tr>
<tr>
<td>Fibrotic Diseases</td>
<td>42.38±12.99</td>
<td>0.68±0.08</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Inflammatory Diseases</td>
<td>61.26±38.52</td>
<td>0.66±0.10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Mechanical Cholestasis</td>
<td>55.95±34.20</td>
<td>0.70±0.11</td>
<td>18</td>
<td>82</td>
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<tr>
<td>No Pathology</td>
<td>48.43±16.13</td>
<td>0.66±0.09</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>Other</td>
<td>46.20±28.25</td>
<td>0.61±0.09</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Chronic Rejection</td>
<td>38.00±16.11</td>
<td>0.68±0.07</td>
<td>11</td>
<td>89</td>
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<tr>
<td>Indeterminate Rejection</td>
<td>59.82±23.01</td>
<td>0.63±0.16</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>Acute Rejection</td>
<td>71.18±41.92</td>
<td>0.65±0.09</td>
<td>6</td>
<td>94</td>
</tr>
<tr>
<td>p value</td>
<td>0.1778</td>
<td>0.0557</td>
<td>0.010†</td>
<td>0.003†</td>
</tr>
</tbody>
</table>

**Fig. 5:** Relative risk ratios and p values by US parameters of pathological biopsies compared against those biopsies revealing no pathology (MHA RI, main hepatic artery resistance index, † statistically significant).

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Conclusion

- The rate of complications following PLB using an 18 gauge automatic needle is low.

- A wide range of overlapping pathological outcomes exists between early and late liver biopsy.

- LFT and US findings are not predictors of pathological outcomes.

- The information provided by PLB justify the use of this invasive procedure.
Personal information

The authors declare that they have no conflicts of interest or financial disclosures.
References


