Early deterioration of PELD score in young children with biliary atresia predicts poor outcome

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Abstract

Background
The Pediatric End-stage Liver Disease (PELD) score is designed to prioritize children with biliary atresia for liver transplantation, based on the severity of chronic liver disease. High scores at listing predict poor outcome, including death. Periodic calculation of the PELD score in children awaiting liver transplantation may be an important additional predictor of pre-transplantation mortality. We aimed to determine the PELD change or cut-off score in young children with biliary atresia (BA) on the waiting list that could assist clinicians in identifying those at high risk of dying before transplantation.

Methods
A national cohort of children younger than 5 years with BA, screened for liver transplantation between 2000 and 2012 was retrospectively analyzed. PELD scores and change scores were calculated at listing and then bi-monthly until death or liver transplantation.

Results
A total of 71 children with BA-associated end-stage liver disease were included, of which 12 (17%) died before transplantation. At the time of listing the optimum PELD score cut point to differentiate high from low risk patients was 21 points. For children with a PELD score of 21 points or higher mortality risk before transplantation increased to 40% (10/25), whereas a score of 20 points or lower reduced this risk to 4% (2/46). Two months after listing, when 47 patients were still waiting for a donor liver, the optimum cut point to differentiate high from low risk patients was 24 points. Children with a score of 24 or higher had a 56% (9/16) risk of death, whereas a score of 23 or lower gave a 0% risk (0/31; 95% confidence interval (CI) 0 to 9%). A deterioration of PELD score of 5 points or more in the first two months after listing indicated a 50% (9/18) risk of death before transplantation, whereas a change score of 4 points or less reduced mortality to 0% (0/29; 95% CI 0 to 10%).

Conclusions
Periodic calculation of the PELD score in young children with BA-associated end-stage liver disease awaiting transplantation facilitates recognition of those with a high risk of pre-transplantation demise. Earlier listing of BA patients and/or adaptation of priority rules on the waiting list could help to decrease mortality and thus increase the prognosis of BA.
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1. Introduction

1.1 Biliary atresia
Biliary atresia is a neonatal cholestatic disease in which there is obstruction of bile flow because of fibrosis and obliteration of the intra- and particularly extrahepatic duct. It is the most common neonatal cholestatic disorder, occurring in approximately 1:17,000 to 1:19,000 live births a year in Western countries and 1:8000 in Asian countries. Until now the etiology of biliary atresia has been unclear, although there are some suggestions that viral infections, genetic susceptibility and/or immune dysregulation play an important role.3,4

Most patients have an isolated form of biliary atresia, but in approximately 20% of the cases there are one or more associated congenital anomalies. The most common anomaly is biliary atresia splenic malformation syndrome, which counts for 10% of congenital malformations. This includes polysplenia (90%), situs inversus (50%) and vascular anomalies, such as absent or interrupted vena cava inferior and vena portae aplasia.3 Some data suggest that children with biliary atresia splenic malformation syndrome might have a worse outcome compared to children with an isolated BA.5

Because of obstruction or absence of the common bile duct there is no drainage of bile. Waste products in bile, like bilirubin, will accumulate in the liver and cause damage to the liver cells. In 2-6 weeks after birth there will be a conjugated hyperbilirubinemia, which leads to jaundice, pale stool and dark urine. Next to that there is no resorption of fat and fat-soluble vitamins (A, D, E and K) because bile salts, responsible for this process, are not present in the duodenum. This leads to growth failure for a majority of the children. As the disease is progressing the patient will eventually develop portal hypertension, leading to splenomegaly, ascites (because of increased vascular permeability) and esophageal varices.

1.2 Operation by Kasai
Since 1950 the hepatopportoenterostomy (HPE) according to Kasai is the first step in the treatment of children with BA. During this operation the fibrotic biliary tracts are removed and a Roux-En-Y anastomosis of the bile ducts into the jejunum is created (figure 1).30 Before 1950, when there was no treatment, 50-80% of the patients died before the age of one year and 90-100% before the age of three years.1

Several studies showed that timing of the Kasai procedure is the most important factor for outcome.1,6 70-80% of the children with BA yield successful bile drainage if the Kasai procedure is performed within 60 days after birth, resulting in increased pigmentation of the stools and resolution of jaundice. The success rate declines when the delay until Kasai operation becomes longer, respectively 40-50% for a delay between 60 and 90 days, 25% for a delay between 90 and 120 days and

Figure 1. Kasai-procedure
10-20% after 120 days. So the HPE gives the best results if performed before eight weeks.¹ Timely diagnosis is hampered by the clinical overlap with breath milk associated jaundice, which is a harmless condition.⁷

Furthermore, outcome after Kasai is also improved by surgical experience and surgical techniques by centralization of HPE.⁸

1.3 Liver transplantation
Although a hepatopancreaticoenterostomy gives clinical improvement for most of the children, 75% of the children will still require orthotopic liver transplantation (OLT) within 10 years.⁴ This operation is performed in children since 1980. Biliary atresia is the most common indication, as it counts for 50%.¹,⁶ Transplantation should be considered when there is no decrease in total serum bilirubin after Kasai surgery or when the child is developing portal hypertension, recurrent cholangitis or growth failure.⁹

During the early years of pediatric liver transplantation only size matched whole graft livers could be transplanted. In 1989 the split liver technique and living donor transplantation made their entrance.¹⁰ In split liver transplantsations the donor liver is divided in two parts, whereby the biggest part (extended right lobe) is used for an adult recipient and the smallest part (left lateral lobe) is used for a pediatric recipient. During a living donor liver transplantation a child receives a part of the liver of a living relative.

Hong et al. (2009) showed that the survival rates for children for segmental grafts from deceased and living donors are comparable with whole donor liver transplantation.¹¹ Another study with 567 participants showed an overall survival of all kinds of OLT of 90% after 6 months and 88% after 3 years.¹² Most deaths were caused by infection (37%).¹²

Unfortunately, death whilst on the waiting list for liver transplantation is still a considerable problem. The predicted mortality after 6 months of listing is 10% and 30% of the overall death in BA occurred in persons on the waiting list for OLT.¹²

1.4 Pediatric End-stage Liver Disease score
In 2002 the Pediatric End-stage Liver Disease (PELD) score is designed by the United Network for Organ Sharing to prioritize children with biliary atresia who are on the waiting list for liver transplantation, based on the severity of chronic liver disease.²¹ Before 2002, prioritizing was based on waiting list time. The score is calculated at listing and is based on a formula which consists of five parameters, namely total serum bilirubin, serum albumin, INR, age (for children under one year of age before transplantation) and growth retardation (figure 2).

\[
\text{PELD} = 10 \times (0.480 \times \ln(\text{bilirubin mg/dl}) + 1.875 \times \ln(\text{INR}) - 0.687 \times \ln(\text{albumin g/dL}) + 0.436 \times \text{age <1year}^* + 0.667 \times \text{growth failure (weight for age or height for age <-2SD)})
\]

* Patients listed for liver transplantation before the patients first birthday, continue to include the value assigned for age <1 year until the patient reached the age of 24 months.
As the score is only calculated at listing, one of gaps in the existing knowledge is the effect of change in PELD score whilst on the waiting list. Little is known about this, however, in 2005 Bourdeaux et al. concluded that higher changes in PELD whilst on the waiting list might be associated with lower survival post transplantation. Another gap in the existing knowledge is that there is no cut-off point in PELD score to distinguish between children with high risk and children with a low risk at mortality.

1.5 Objectives
Periodic calculation of the PELD score in children awaiting liver transplantation may be an important additional predictor of pre-transplantation mortality. We aimed to determine the PELD change or cut-off score in young children with biliary atresia on the waiting list that could assist clinicians in identifying those with a high risk of dying before transplantation.
2. Material and methods

2.1 Study design, setting and participants
We retrospectively analyzed a national cohort of children with biliary atresia, who were screened for OLT at the University Medical Centre of Groningen (UMCG) between the 1st of January 2000 and the 30th of June 2012, with an age younger than five years at the moment of screening. The primary outcome was death whilst on the waiting list.

2.2 Periods of recruitment
Post-Kasai, follow-up was done in the hospital where the surgery was performed. The hospital followed a standard protocol for referral to the transplant center in Groningen for screening for liver transplantation (figure 3).

![Figure 3. Referral to transplant center after HPE](image)
* TB = total serum bilirubin
** In case of fever, increase of infection parameters, increase of TB and/or gamma GT and/or clinically or laboratory recovery after intravenous antibiotics

After listing a child was seen at the outpatient department for pediatric liver transplantation once every four to six weeks. During this visit the previous period was evaluated, growth parameters were measured and the child went for laboratory investigation.

Data of the selected children were collected from the moment of listing until death or liver transplantation on a bi-monthly basis (figure 4). Change in PELD was calculated between every successive moment.
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Figure 4. Timeline from birth until transplantation
Focus of the study is colored in red. Collection of data started from the moment of listing and then bi-monthly until death or until transplantation.

2.3 Data
2.3.1 Variables
The collection of data was done by a single investigator. General data included date of birth, sex, blood type, reason of screening, associated congenital anomalies, if and where Kasai was done and results of liver histology from liver biopsy. Every two months the nutritional status and possible interventions (tube feeding or total parenteral nutrition), albumin, bilirubin, INR, signs of portal hypertension (ascites, splenomegaly, varices and/or retrograde flow with ultrasound), use of antibiotics, use of corticosteroids and presence of hepatorenal and/or hepatopulmonary syndrome was written down. Disease severity was expressed as PELD scores, using the equation given on page 6.

In case growth parameters (height, length or mid-upper arm circumference) or laboratory values were not measured during the outpatient visit, we used the measurements taken two weeks earlier or later than the study visit. In case of completely missing values we carried the last observation forward. For the use of antibiotics and/or corticosteroids we took a maximum of four weeks before the measured moment.

2.3.2 Definitions
Severity of fibrosis, based on liver histology pre- or peri Kasai, was evaluated by a three point scale, namely mild (no bridging), moderate (occasional bridging) or severe fibrosis (diffuse portal bridging). Ascites was confirmed by presence of shifting dullness at physical examination, by ultrasound examination or when diuretics drugs were used. Portal hypertension was confirmed when at least one of the following signs was present: splenomegaly, gastrointestinal bleeding or oesophageal varices, or when ultrasound examination of the portal vein revealed retrograde flow, increased diameter, decreased flow velocity, fluttered flow or thrombosis. Portal vein diameter was increased if the portal vein diameter was more than 5 mm in the period between birth until 1 year and more than 8 mm at the age from 1-4 years. Hepatopulmonary syndrome was
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considered present when the arterial oxygen saturation was less than 97\% and/or when ultrasound examination identified air micro-bubbles in the left atrium.\textsuperscript{18} Hepatorenal syndrome was defined as a plasma creatinine concentration above 133µmol/L (1.5mg/dL), in absence of any other apparent cause for this symptom (in particular spontaneous bacterial peritonitis) and with lack of improvement in renal function after volume expansion with intravenous albumin and withdrawal of diuretics for at least two days.\textsuperscript{19,20}

2.3.3 Measurement of PELD scores
The PELD scores were calculated by PASW (SPSS) for Windows, by entering a formula which is available at the website from the United Network for Organ Sharing.\textsuperscript{21} This formula consists of total serum bilirubin (mg/dL), serum albumin (g/dL), INR, age below 1 year and growth failure (figure 2). Growth failure is defined as weight for age or length for age more than 2SD below the mean of the WHO global growth curves. For calculating the z-scores (SD) we used WHO global growth curves.\textsuperscript{22} When values were missing we carried the last observation forward. In case of missing INR values, INR was reconstructed using the formula in figure 5.

\[
\text{INR} = \left( \frac{\text{Patient PT (sec)}}{\text{MN PT (sec)}} \right)^{\text{ISI}}
\]

\textbf{Figure 5.} Formula for calculation of the INR
\textit{MNPT = Mean Normal Plasma Time, ISI =International Sensitivity Index}

2.4 Statistical methods
All statistical analyses were performed using PASW (SPSS) for Windows, version 19.0 (SPSS Inc, Chicago, IL). The survival group and the death on waiting list group were compared with the Mann-Whitney U (for nonparametric continuous variables) and Chi-squared test (for nonparametric, nominal variables). Differences were considered statistically significant when the p-value was < 0.05. This level was chosen because of the limited number of patients in the analysis.

In order to determine a clinical relevant cut-off for PELD score we used a frequency table, in combination with a sensitivity analysis (ROC curve). Finally we used cox regression survival analysis with backward elimination to identify predictors of mortality at the moment of screening. Candidate predictors with a p < 0.10 in bivariate analysis were selected for use in multivariate analysis.

To prevent confounding, we excluded the variables with a lot of missing values in the survival analysis. There was no loss to follow up, other than to transplantation or to death.

2.5 Ethical considerations
As this study concerned collection of data by routine medical care, it was exempted from institutional review board approval. The data was collected and recorded in such a manner that subjects could not be identified, directly or through identifiers linked to the subjects.
2.6 Reporting

We used the Strengthening the Reporting of Observation Studies in Epidemiology (STROBE) guidelines to write this report. STROBE is a checklist of 22 items that is designed to improve reporting of observational studies.\textsuperscript{23}
3. Results

3.1 Patient characteristics
A total of 71 children with BA-associated end-stage liver disease fulfilled the criteria to be included in this cohort study, and 12 (17%) died before transplantation (table 1). From the 71 patients, 27 were man (38%). The median age at listing was 7 (range 3-58) months. 19 patients had blood type A (27%), 6 blood type B (8%), 2 blood type AB (3%) and 44 patients blood type O (62%). 67 (94%) children had Kasai surgery before screening in one of the eight centers in the Netherlands. In the remaining 4 patients Kasai was considered not possible or not indicated, because of surgical or clinical reasons. The median age at time of HPE was 61 days (range 22-132). In 4 patients HPE was not performed (6%). 11 of the patients who had a liver biopsy (n=60) had mild fibrosis (18%), 11 of them had moderate fibrosis (18%) and 37 had severe fibrosis (62%).

At the moment of listing 29 patients (41%) were admitted to the hospital, 47 had nutritional intervention (66%) and 22 patients had growth failure (31%). In 68 patients there were symptoms of portal hypertension (96%), in which 46 persons had ascites (65%), 65 had splenomegaly (92%) and 13 had varices (18%). Antibiotics was used in 66 persons (93%), 1 patient used corticosteroids (1%). Hepatorenal was found in 4 persons (6%), hepatopulmonary syndrome was found in 1 person (1%).

Table 1. Characteristics of included patients at the moment of listing

<table>
<thead>
<tr>
<th></th>
<th>Children surviving until transplantation n = 59</th>
<th>Children who died before transplantation n = 12</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>23 (39%)</td>
<td>6 (43%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Age in months, median (range)</td>
<td>7 (3 – 58)</td>
<td>5 (5-8)</td>
<td><strong>0.020</strong></td>
</tr>
<tr>
<td>Kasai, n (%)</td>
<td>57 (97%)</td>
<td>10 (83%)</td>
<td>0.130</td>
</tr>
<tr>
<td>Time between Kasai and listing in months, median (range)*</td>
<td>5 (1-56)</td>
<td>4 (1-6)</td>
<td><strong>0.049</strong></td>
</tr>
<tr>
<td>Associated congenital anomaly, n (%)</td>
<td>6 (10%)</td>
<td>4 (33%)</td>
<td>0.058</td>
</tr>
<tr>
<td>Health status requiring hospital admission, n (%)</td>
<td>23 (39%)</td>
<td>6 (50%)</td>
<td>0.531</td>
</tr>
<tr>
<td>Growth failure**, n (%)</td>
<td>15 (25%)</td>
<td>7 (58%)</td>
<td><strong>0.039</strong></td>
</tr>
<tr>
<td>Nutritional intervention started, n (%)</td>
<td>36 (61%)</td>
<td>11 (92%)</td>
<td><strong>0.049</strong></td>
</tr>
<tr>
<td>Use of antibiotics, n (%)</td>
<td>57 (97%)</td>
<td>9 (75%)</td>
<td><strong>0.031</strong></td>
</tr>
<tr>
<td>Ascites, n (%)</td>
<td>38 (64%)</td>
<td>8 (67%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Splenomegaly, n (%)</td>
<td>55 (93%)</td>
<td>10 (83%)</td>
<td>0.266</td>
</tr>
<tr>
<td>Hepatorenal syndrome, n (%)</td>
<td>2 (3%)</td>
<td>2 (17%)</td>
<td>0.130</td>
</tr>
</tbody>
</table>

* 2 missing values in both groups
** Growth failure = weight for age, height for age and/or mid-upper arm circumference for age < - 2SD

3.2 Primary outcome
Table 1 compared survivors on the waiting list and children who died pre-transplantation. The median age of the children who died before transplantation was significantly lower compared to the survivors (respectively 5 and 7 months, p 0.020).
The group of children with poor outcome was further characterized by a significantly higher prevalence of faltering growth requiring nutritional intervention and a smaller proportion was treated with antibiotics before listing. In 11 of 12 (92%) children who died on the waiting list, nutritional intervention was started at the moment of listing, while in the survivors 36 of 59 (61%) received tube feeding. The remaining baseline characteristics were not different. Table 2 gives more background information about the children that died on the waiting list.

**Table 2. Characteristics of patients that died on the waiting list**

<table>
<thead>
<tr>
<th>ID</th>
<th>Blood type</th>
<th>Associated congenital anomaly</th>
<th>Status at listing</th>
<th>Growth failure at listing*</th>
<th>Nutritional intervention at listing</th>
<th>PELD at listing</th>
<th>PELD at death</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>-</td>
<td>Home</td>
<td>No</td>
<td>NG tube</td>
<td>12</td>
<td>43</td>
<td>MOF**, sepsis</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>-</td>
<td>Hospital</td>
<td>No</td>
<td>NG tube</td>
<td>25</td>
<td>25</td>
<td>MOF</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>Arthrogryposis</td>
<td>Home</td>
<td>Yes</td>
<td>NG tube</td>
<td>22</td>
<td>31</td>
<td>MOF</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>-</td>
<td>Hospital</td>
<td>Yes</td>
<td>NG tube</td>
<td>23</td>
<td>31</td>
<td>Sepsis</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>-</td>
<td>Hospital</td>
<td>Yes</td>
<td>NG tube</td>
<td>24</td>
<td>29</td>
<td>Unclassified</td>
</tr>
<tr>
<td>6</td>
<td>B</td>
<td>Jejunum atresia, absent vena cava inferior</td>
<td>Home</td>
<td>Yes</td>
<td>NG tube</td>
<td>24</td>
<td>31</td>
<td>MOF</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>Absent vena portae</td>
<td>Home</td>
<td>No</td>
<td>NG tube</td>
<td>15</td>
<td>23</td>
<td>MOF</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>-</td>
<td>Hospital</td>
<td>Yes</td>
<td>NG tube</td>
<td>21</td>
<td>26</td>
<td>Unclassified</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>Polysplenia syndrome, situs inversus</td>
<td>Home</td>
<td>Yes</td>
<td>Oral</td>
<td>24</td>
<td>55</td>
<td>MOF, GI hemorrhage</td>
</tr>
<tr>
<td>10</td>
<td>B</td>
<td>-</td>
<td>Hospital</td>
<td>No</td>
<td>NG tube</td>
<td>25</td>
<td>32</td>
<td>Unclassified</td>
</tr>
<tr>
<td>11</td>
<td>A</td>
<td>-</td>
<td>Hospital</td>
<td>Yes</td>
<td>NG tube</td>
<td>32</td>
<td>32</td>
<td>MOF</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>-</td>
<td>Home</td>
<td>No</td>
<td>NG tube</td>
<td>22</td>
<td>22</td>
<td>GI hemorrhage</td>
</tr>
</tbody>
</table>

* Growth failure = weight for age, height for age and/or MUAC for age < - 2SD
** MOF = multi organ failure

**3.3 PELD score**

3.3.1 **PELD score and its individual components at listing**

The median PELD score in the total cohort at the moment of listing was 17 (range -9 – 51). The median PELD score in the group of children that died on the waiting list was 7 points higher than in the survival group. All non-survivors were younger than 12 months at the moment of listing, compared to 47 (80%) persons among survivors. Furthermore, in children that died on the waiting list bilirubin was almost 7 mg/dL higher than in survivors and a larger proportion was classified as having growth failure at the moment of listing. In the non-survivors group, 7 children (58%) had faltering growth when listed for OLT, compared to 15 children (25%) in the survival group. Albumin and INR were not significantly different between both groups (table 3).
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Table 3. PELD score and individual components per group at listing

<table>
<thead>
<tr>
<th></th>
<th>Children surviving until transplantation (n = 59)</th>
<th>Children who died on the waiting list (n = 12)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PELD score, median (range)</td>
<td>16 (-9 – 51)</td>
<td>23 (12-32)</td>
<td>0.004</td>
</tr>
<tr>
<td>Age &lt;1, n (%)</td>
<td>47 (80%)</td>
<td>12 (100%)</td>
<td>0.020</td>
</tr>
<tr>
<td>Albumin g/dL, median (range)</td>
<td>2.9 (1.8-4.4)</td>
<td>2.5 (2.0-3.6)</td>
<td>0.053</td>
</tr>
<tr>
<td>Bilirubin μmol/L</td>
<td>218 (15-633)</td>
<td>338 (201-492)</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>12.8 (0.9-37)</td>
<td>19.7 (11.8-28.8)</td>
<td></td>
</tr>
<tr>
<td>INR, median (range)</td>
<td>1.3 (0.9-6.7)</td>
<td>1.5 (1.0-1.9)</td>
<td>0.211</td>
</tr>
<tr>
<td>Growth failure*, n (%)</td>
<td>15 (25%)</td>
<td>7 (58%)</td>
<td>0.039</td>
</tr>
</tbody>
</table>

* Growth failure = weight for age and/or height for age < - 2SD

3.3.2 PELD scores during follow-up

While on the waiting list, PELD scores were significantly higher in the group of children that did not survive until transplantation. For both the survivors and non-survivors the median PELD score per follow-up visit is shown in figure 6. A deteriorating health was observed within the first two months after listing in the non-survivors group, when the median PELD score increased from 23 to 30 points, while the score in survivors stayed the same (p = 0.001). At the remaining observed moments the change in PELD score was not seen significantly different between both groups. Furthermore, after four months of listing change could not be compared because the group that died on the waiting list was too small (n=1).

3.3.3 Cut-off PELD scores

At the moment of listing, the most significant difference in PELD scores between the group that died on the waiting list and survivors was seen when the PELD was 21 or higher (p=0.000) (table 4). In children with a PELD score of 21 points or higher (n=25) mortality risk before transplantation increased to 40% (10/25), whereas a score of 20 points or below (n=46) reduced the risk to 4% (2/46).

Two months after listing, when 47 patients were still waiting for a donor liver, the most optimum cut-off for identifying those at high risk of dying before transplantation was a PELD score of 24 or higher (p=0.000) (table 4).
Children with a score of 24 points or more (n=16) had a 56% (9/16) risk of death, whereas a score of 23 or below gave a 0% risk (0/31; 95% confidence interval (CI) 0 to 9%).

Figure 7 shows the probability of survival in time for a PELD score higher than 21 at listing (7a) and for a PELD score of 24 or more two months after listing (7b). The probability of survival in time was significantly lower in the group with a PELD score of 21 or higher at listing (p=0.000) and also in children with a PELD score of 24 or more two months after listing (p = 0.000). From two months after listing, the probability of survival was 100% during the entire waiting list period for children with a PELD score of 23 or lower.

3.3.4 Deterioration in PELD score
The most optimum change in PELD score in the first two months after listing to differentiate between high and low risk at mortality while on the waiting list was a deterioration in PELD score of 5 points or more (table 4). A deterioration of 5 points or more in the first two months after listing indicated a 50% (9/18) risk of death before transplantation, whereas a change in score of 4 points or less reduced mortality to 0% (0/29; 95% CI 0 to 10%).

Figure 8 shows the probability of survival in time when the PELD score deteriorated 5 points or more in the first two months after listing. All children with deterioration less than 5 points stayed alive until transplantation.

### Table 4. Scenario analysis

<table>
<thead>
<tr>
<th>Optimum cut point to distinguish children with low and high risk at mortality</th>
<th>Patients</th>
<th>Death on waiting list, n</th>
<th>Proportion of patients (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At listing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cohort at risk</td>
<td>71</td>
<td>12</td>
<td>17% (9-27%)</td>
</tr>
<tr>
<td>PELD score ≤ 20</td>
<td>46</td>
<td>2</td>
<td>4% (1-14%)</td>
</tr>
<tr>
<td>PELD score ≥ 21</td>
<td>25</td>
<td>10</td>
<td>40% (22-60%)</td>
</tr>
<tr>
<td><strong>Two months after listing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cohort at risk</td>
<td>47</td>
<td>9</td>
<td>19% (10-32%)</td>
</tr>
<tr>
<td>PELD score ≤ 23</td>
<td>31</td>
<td>0</td>
<td>0% (0-9%)</td>
</tr>
<tr>
<td>PELD score ≥ 24</td>
<td>16</td>
<td>9</td>
<td>56% (32-78%)</td>
</tr>
<tr>
<td>Deterioration in PELD ≤ 4</td>
<td>29</td>
<td>0</td>
<td>0% (0-10%)</td>
</tr>
<tr>
<td>Deterioration in PELD ≥ 5</td>
<td>18</td>
<td>9</td>
<td>50% (28-72%)</td>
</tr>
</tbody>
</table>

3.4. Scenario analysis of the key results
Ten (83%) persons of the mortality group (n=12) were identified as high risk of mortality when the cut-off PELD score of 21 or more at the moment of listing was used. Two months after listing, three of them already died. The remaining nine persons were all classified as high risk when the cut-off PELD score of 24 or higher or a deterioration of 5 or more in the first two months after listing was used.
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7a.

**Figure 7.** Probability of survival in time for children with a PELD score $\geq 21$ at listing (7a) and for a PELD score $\geq 24$ two months after listing (7b)

7b.

**Figure 8.** Probability of survival in time for children with a deterioration in PELD score of $\geq 5$ in the first two months after listing
3.5 Risk analysis of waiting list mortality

Table 5 shows the risk analysis for waiting list mortality at the moment of listing. By univariate analysis, median age (in months), nutritional intervention, use of antibiotics and PELD score were significant predictors of waiting list mortality. In the multivariate model, only age at listing and nutritional intervention stayed significant predictors.

**Table 5. Predictors at the moment of listing for death whilst on waiting list**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Regression coefficient</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analysis (n=71)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age in months</td>
<td>-0.365</td>
<td>0.69</td>
<td>0.47-1.04</td>
<td>0.075</td>
</tr>
<tr>
<td>Time between HPE and listing (n=67)</td>
<td>-0.474</td>
<td>0.62</td>
<td>0.36-1.08</td>
<td>0.622</td>
</tr>
<tr>
<td>Associated congenital anomaly</td>
<td>0.836</td>
<td>2.31</td>
<td>0.69-7.69</td>
<td>0.174</td>
</tr>
<tr>
<td>Nutritional intervention</td>
<td>1.767</td>
<td>5.85</td>
<td>0.75-45.4</td>
<td>0.091</td>
</tr>
<tr>
<td>Underweighted* (n=105)</td>
<td>0.428</td>
<td>1.54</td>
<td>0.41-5.74</td>
<td>0.524</td>
</tr>
<tr>
<td>Growth failure</td>
<td>0.904</td>
<td>2.47</td>
<td>0.78-7.86</td>
<td>0.126</td>
</tr>
<tr>
<td>Use of antibiotics</td>
<td>-1.366</td>
<td>0.26</td>
<td>0.07-0.95</td>
<td>0.041</td>
</tr>
<tr>
<td>PELD score</td>
<td>0.076</td>
<td>1.08</td>
<td>1.02-1.14</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age in months</td>
<td>-0.547</td>
<td>0.58</td>
<td>0.34-0.99</td>
<td>0.045</td>
</tr>
<tr>
<td>Nutritional intervention</td>
<td>1.892</td>
<td>6.63</td>
<td>0.78-56.7</td>
<td>0.081</td>
</tr>
</tbody>
</table>

* Underweighted = weight for age < -2 SD  
** Growth failure = weight for age and/or height for age < -2SD
4. Discussion

4.1 Key results
We aimed to determine the optimum PELD change or cut-off score in young children with biliary atresia on the waiting list that could help clinicians in identifying those at high risk of dying before transplantation. Predictors of high risk at mortality on the waiting list were a PELD score of 21 or higher at the moment of listing, a score of 24 points or more two months after listing and deterioration in PELD score of 5 points or more in the first two months after listing. These results support the assumption that periodic calculation of the PELD score in young children with BA-associated end-stage liver disease awaiting transplantation facilitates recognition of those with a high risk of pre-transplantation demise.

4.2 Limitations
As all Dutch children with biliary atresia are screened for OLT in one single center, we are confident that we included all children who had a screening below the age of five years between 2000 and 2012. The national character and long time span largely exclude chance and regional variation.

The PELD score was calculated by using the formula from the UNOS website, in which growth failure was defined as weight or height for age < -2SD below the mean of the reference population. As weight for age overestimates nutritional status in children with edema it is likely that part of the patients with faltering growth was overlooked. We therefore suggest to adjust the definition of growth failure in the PELD formula. All children that fulfill at least one of the following criteria should be regarded as growth retarded: weight for height < -2SD; height for age < -2SD; height for age below parental-based target height; or Mid Upper Arm Circumference (MUAC) < -2SD. In our own cohort we might have overestimated the proportion of growth retarded children, as we frequently carried the last measurement of length forward.

Another limitation in our study was the relative small sample size. In the Cox regression analysis there were 6 outcome events (death on waiting list) per independent variable, as there were 2 independent variables after multivariate analysis over a total of 12 events. The generally accepted ratio of multivariate survival analysis is 10 outcome events per independent variable. This means that overfitting of data could have played a role.

4.3 Interpretation
We followed a group of 71 patients with biliary atresia younger than 5 years, and report a pre-transplantation mortality rate of 17% (12/71). Earlier published pre-transplantation mortality studies, France 1999-2000: 7,5%; England and Wales 1999-2002: 4,2% and Canada 1995-2003: 3% focused on children with an age range of 0 to 18. The Dutch pre-transplantation mortality rate for the full age range is not different from these studies.

Several studies showed that waiting time for OLT has no relationship to death on the waiting list, except for acute liver failure. Our study confirmed this, as median waiting time and distribution of waiting time was not different between both groups. The PELD score was designed to prioritize children with biliary atresia for liver
transplantation, based on severity of chronic liver disease.\textsuperscript{12,14,26,27} High scores at listing have been associated with higher mortality on the waiting list and poor outcome post-OLT.\textsuperscript{12,27,28} This assumption was confirmed at our study, as the median PELD score at the moment of listing was 7 points higher in the group that died on the waiting list compared to the transplantation group (p=0.000). Mc. Diarmid et al. showed that the score has less prognostic value when it is used in the period before screening.\textsuperscript{27} Bordeaux et al. showed that absolute PELD scores had no influence on post-OLT outcome.\textsuperscript{13}

To our knowledge, this is the first research in children with biliary atresia that focused on the deterioration in PELD score at several moments on the waiting list and pre-transplant mortality. Earlier studies mainly focused on the PELD score at the moment of listing. In 2005, Bourdeaux et al. focused on change in PELD at the waiting list and post transplantation survival. They concluded that higher changes in PELD while on the waiting list might be associated with lower survival post transplantation.\textsuperscript{13} Our data revealed that early deterioration in PELD while on the waiting list is associated with higher mortality pre-transplant.

At the moment there is no cut-off PELD score used in the Netherlands, as referral for OLT assessment is still mainly based on each doctor’s individual judgment. One of the aims of this study was to determine a cut-off score in PELD score in children below age of five year with BA on the waiting list that could assist clinicians in identifying those with a high risk of dying before transplantation. Utterson et al. already showed that a cut-off PELD score of 20 or more at listing was a significant waiting list mortality risk factor for children at all ages.\textsuperscript{12} This suggest that the cut-off score in PELD after the age of 5 differs not much from the cut-off score before the age of 5, as this was only one point lower as our cut-off score for determining high risk at mortality at the moment of listing.

A cut-off score at other moments on the waiting list was not determined yet in other studies. With our data, the AUC ROC showed a very high predictive ability at mortality for children with a PELD score of 24 or more two months after listing. This was an important additional factor to differentiate children with a high risk of mortality pretransplantation, as 4% (2/46) of the children who were classified as low risk at the moment of listing, still died on the waiting list. These two children both had a PELD score of 24 or more two months after listing.

\textbf{4.4 Generalisability}

All children that died between listing and transplantation had high PELD scores at the time of listing, suggesting that they were in an advanced stage of chronic liver disease. These children may have shown a rapid deterioration of their condition, but late referral to the transplantation centre may have also played a role. Whichever was the case, advanced end-stage liver disease makes them vulnerable for infections, causes depletion of energy stores and severe growth retardation. Perhaps these children should have been kept under constant close monitoring. Children with an advanced end-stage liver disease or rapid deterioration may have better prognosis when transplanted early (e.g. within two months) after listing with a living related donor organ. Maintaining adequate growth is a challenging objective. Barshes et al. showed that both young age and poor growth are risk factors for unfavourable outcome.\textsuperscript{29} This dilemma makes the optimal timing for OLT
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very difficult. Serial PELD score calculations while on the waiting list – but even before referral to a transplantation center - can help clinicians to decide whether a fast track to transplantation is desirable.

The striking difference between survivors and non-survivors was already present at listing which supports the idea that lack of suitable donor organs did not contribute to pre-transplantation demise.

We used the cox regression survival analysis to determine predictors of poor outcome at the time of listing. Age and not initiating tube feeding remained significant predictors in the multivariate model. We recommend that future predictive research also incorporates pre-listing PELD scores.

4.5 Conclusion
This cohort study in young children with BA-associated end-stage liver disease awaiting transplantation suggests that late consideration for OLT and early deterioration while on the waiting list are important contributors to pre-transplantation mortality. Periodic calculation of the PELD score facilitates recognition of those with a high risk of pre-transplantation demise. Earlier listing of BA patients and/or adaptation of priority rules on the waiting list could help to decrease mortality and thus increase the prognosis of BA.
5. Acknowledgments

*I have no special talent. I am only passionately curious* – Albert Einstein

This statement from Albert Einstein describes in a few words why I have been able to accomplish this paper. Without my curiosity I could not have finished this work. After six months of hard work I am very proud to present you this paper.

At first I had no idea where to start, mainly because I had no experience with doing scientific research at all. Nevertheless, mainly with a great deal of help from Dr. van Rheenen, I managed to make this research my own and I learned more about scientific research and statistics than I could ever have imagined before.

During working on this paper I learned that alongside curiosity, perseverance is one of the most important characteristics for being a good scientist. Every time when things didn’t work out the way I planned, I had to push myself to continue. There were times that I had to do things over and over again and times when the work I was doing wasn’t any use at all. I found out that this is all part of doing research and has made this paper what it is.

Most of all I want to thank Dr. van Rheenen, for wanting to be my supervisor and for his confidence in my ability to do this research. He taught me to make decisions on my own, by letting me work independently but at the same time he was always looking over my shoulder to make sure I was on the right path. Whenever I felt lost, he managed to point me the right direction. Dr. van Rheenen, thank you for your advice, patience and your ability to always put things into the right perspective.

Secondly I want to thank Professor Dr. Verkade, for bringing up the subject of this paper. Furthermore, I want to thank him for his constructively critical look at my work, his trust and enthusiasm.

Also, I want to thank Nicoline Kuiken for supplying her database. Without her, it would have been harder for me to make a start with this study. I want to thank Ellen Enninga, Tamara Lesimanoeaja and Anneke de Bruin for supplying the lists and files of the patients with biliary atresia. Also I want to thank Victor Bom, biochemicus, for the values which I needed to calculate the INR from the PT. Special thanks go to Peter and Mark, who supported me with the statistics. They helped me to understand the most difficult things, as they explained it in an understandable way. Finally, I want to thank Astrid, Marleen and Eske for listening to my frustrations or maybe too excited stories about the great results which I was so happy about.

I think this paper has given new insights in the use of the PELD scores in children with biliary atresia who are on the list for a liver transplantation. I hope it leads to further research, as it will generate new questions.
6. References


Early deterioration of PELD score in children with biliary atresia predicts poor outcome

M.I. Sijsling


7. Dutch abstract

Introductie
De Pediatric End-stage Liver Disease (PELD) score is ontworpen voor het prioriteren van kinderen met biliaire atresie (BA) die op de wachtlijst staan voor levertransplantatie, gebaseerd op de ernst van de chronische leverziekte. Hogere scores op het moment van plaatsing op de wachtlijst voorspellen een slechtere uitkomst, waaronder overlijden. Periodieke berekening van de PELD score bij kinderen die op de wachtlijst staan voor levertransplantatie kan een belangrijke additionele voorspeller zijn van pre-transplantatie mortaliteit. We hebben geprobeerd om het afkappunt of verandering in PELD score bij jonge kinderen met BA op verschillende momenten op de wachtlijst vast te stellen, wat clinici kan helpen om kinderen die een hoge kans hebben op overlijden op de wachtlijst te identificeren.

Materiaal en methoden
Een nationaal cohort van kinderen jonger dan vijf jaar met biliaire atresie die zijn gescreefd voor levertransplantatie tussen 2000 en 2012 is retrospectief geanalyseerd. PELD scores en verandering in scores zijn berekend op moment van plaatsing op de wachtlijst en vervolgens tweemaandelijks tot dood of tot levertransplantatie.

Resultaten
In totaal zijn er 73 kinderen geïncludeerd, waarvan 12 (17%) pre-transplantatie zijn overleden. Op het moment van plaatsing op de wachtlijst was het optimale afkappunt in PELD score voor het differentiëren tussen een hoge en een lage kans op mortaliteit 21 punten. Bij kinderen met een PELD score van 21 of hoger was de kans op overlijden 40% (10/25), terwijl een score van 20 punten of lager de kans deed afnemen naar 4% (2/46). Twee maanden na plaatsing, toen nog 47 patiënten wachten op een donor lever, was het optimale afkappunt voor het differentiëren tussen hoge en lage kans op mortaliteit 24 punten. Kinderen met een score van 24 of meer hadden 56% (9/16) kans op overlijden, terwijl kinderen met een score van 23 of lager een kans hadden van 0% (0/31; 95% betrouwbaarheidsinterval 0 tot 9%). Een verslechtering van de PELD score van 5 punten of meer in de eerste twee maanden na plaatsing op de wachtlijst indiceerde een 50% (9/18) kans op overlijden voor transplantatie, terwijl een verandering in de score van 4 punten of minder het overlijdensrisico verminderde tot 0% (0/29; 95% CI 0 to 10%).

Conclusie
Herhaaldelijke berekening van de PELD-score bij jonge kinderen met BA-geassocieerde eindstadium leverfalen die op de wachtlijst staan voor levertransplantatie vergemakkelijkt de herkenning van kinderen met een hoog risico op overlijden pre-transplantatie. Eerdere plaatsing van BA patiënten op de wachtlijst en/of adaptatie van de regels voor prioriteren op de wachtlijst kunnen bijdragen aan een afname van de mortaliteit en verhogen dus de prognose van BA.