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2014-02-10
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Does the Aminotransferase Aspartate to Platelet Ratio Index (APRi) value at the time of Kasai portoenterostomy show any relationship to long-term outcome in patients with Biliary Atresia

Andrew Grieve

A research report submitted for the degree of Master of Medicine in the Department of Surgery for the University of the Witwatersrand Health Sciences.

Declaration

I, Andrew Grieve, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the Department of Surgery at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signed: _  _____

Date: _____27 February 2013_____

Place: _____Johannesburg_____

Dedication

To my wife, Susan, who continues to encourage and motivate me in each endeavour.

Publications and presentations arising from this research

Presentations

1. Combined Paediatric and Paediatric Surgical journal club meeting Charlotte Maxeke Johannesburg Academic Hospital 6 May 2011. *Biliary atresia and the APRi.*
2. European Paediatric Surgical association congress June 2011 (Barcelona, Spain). *Prognostic value of APRi in Biliary atresia.*
3. British Association of Paediatric Surgeons congress July 2011 (Belfast, Ireland). *APRi is a useful adjunt in prediction of cirrhosis in biliary atresia evident at the time of Kasai portoenterostomy.*
4. University of the Witwatersrand Department of Surgery Bert Myburgh Research forum November 2012. *APRi in infants with biliary atresia: prognostic value at presentation.*

Publications (please see appendix):

Grieve AD, Makin E, Davenport M. Aspartate Aminotransferase-to-Platelet Ratio index (APRi) in infants with biliary atresia: prognostic value at presentation. Journal of Pediatric Surgery (accepted October 2012).

Acknowledgements

Supervisors: Professor Mark Davenport (NHS, London UK) and whom initiated the study idea.

Dr Deirdré Kruger (WITS, Johannesburg, RSA)

Abstract

Background: Biliary atresia (BA) is characterised by a progressive obliterative cholangiopathy. If surgical treatment by a Kasai Portoenterostomy (KP) is undertaken early on in life there is the potential for successful bile drainage. The natural disease progression without intervention results in fibrosis and cirrhosis, necessitating liver transplantation before two years of life. Despite the advances in the management of biliary atresia over the recent decades we still do not have a good indicator of which patients will do well after surgery and which will require further intervention for their liver dysfunction. There are many clinical and serological indicators that suggest liver failure, but liver histology remains the gold standard indicating the extent of liver damage. This is, however, being slowly replaced by various new less-invasive biological markers, including the Aminotransferase Aspartate to Platelet Ratio Index (APRi).

This study looks at this biological marker for patients with biliary atresia with reference to their level of disease at the time of surgery and whether it is a prognostic tool for long-term outcomes in this group of patients.

Methods: This study was conducted as a retrospective data analysis of histologically proven patients with biliary atresia who underwent Kasai portoenterostomy at King's College Hospital in London (UK) from 1999 to 2010. Initial APRi values were related to other biochemical indices and liver appearance at the time of Kasai portoenterostomy. These APRi values were then analysed in relation to clearance of jaundice, development of significant oesophageal varices and native liver survival. Data are expressed as median (interquartile range). Non-parametric comparison was performed and a P-value of ≤ 0.05 was regarded as significant.

Results: Overall 260 infants were included in the study. Median APRi was 0.67 (0.43-1.12) at a median age of surgery of 58 (range 14 - 209) days. APRi correlated with age

(r_s=0.44; P<0.0001), spleen size (r_s=0.48; P<0.0001) and bilirubin (r_s=0.45; P<0.0001). Liver assessment at operation was divided into cirrhosis [n=28 (10.8%)] or non-cirrhosis. Using a cut-off value of 1.22 [AUC 0.83 (95% CI 0.73-0.90)] showed a sensitivity of 75% and a specificity of 84% for macroscopic cirrhosis.

Native liver survival was significantly different but improved only for those in the lowest APRi quartile (<0.43; P<0.009). APRi values at presentation had no significant association with later development of significant oesophageal varices.

Conclusion: APRi at the time of KP is a useful adjunct in evaluating severity of liver disease in BA at presentation.

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List of abbreviations

ANOVA	Analysis of variance
APRi	Aspartate Aminotransferase-to-Platelet Ratio index
ALT	Alanine Aminotranserase
AST	Aspartate Aminotransferase
AUC	Area Under (the receiver operating characteristic) Curve
BA	Biliary atresia
BASM	Biliary atresia splenic malformation syndrome
CBA	Cystic biliary atresia
CBD	Common bile duct
CI	Confidence interval
CMV	Cytomegalovirus
GGT	Gama–glutamyl-transpeptidase
HREC	Human research ethics committee
IGM	Immunoglobulin M
IL1, IL-3, IL6, IL11:	Interleukin 1,3,6, and 11
INR	International Normalised Ratio
KP	Kasai portoenterostomy
KW	Kruskal Wallace
MD	Mark Davenport
NASH	Non-alcoholic steatohepatitis
ROC	Receiver Operator Curves
r_s	Spearman Rank Correlation
TGFβ1	Transforming Growth Factor β 1
TIMPS	Tissue Inhibitors of Matrix Metalloproteinases
US	Ultrasound
UK	United Kingdom

CHAPTER 1 - Introduction

Biliary atresia (BA) is a progressive disease affecting the extrahepatic biliary tree and resulting in a blockage of bile drainage from the liver. This progressive obliterative cholangiopathy results in liver fibrosis and cirrhosis. There are three major types of BA described by the Japanese society, with type I showing atresia of the common bile duct (CBD), type II showing atresia of the CBD and common hepatic ducts, and type III showing atresia of all of the extrahepatic biliary tree (Hartley, Davenport & Kelly 2009). BA can further be subdivided according to anatomical associations noted at the time of surgery. These include the Biliary Atresia Splenic Malformation Syndrome (BASM) in which BA has various associations which may include; asplenia, polysplenia, pre-duodenal portal vein and an interrupted retro-hepatic inferior vena cava (Davenport et al 2006). BA may also be associated with other congenital malformations such as anorectal anomalies and congenital cardiac anomalies. Cystic biliary atresia (CBA) is characterised by cystic dilatation of the extrahepatic ductal system seen at the time of surgery with atresia of distal CBD and an abnormal portal plate (Caponcelli, Knisely & Davenport 2008). Another potential subgroup of BA is seen in patients with Immunoglobulin M (IgM) positive Cytomegalovirus (CMV) serology (Zani et al 2010). Alternatively, and most commonly, BA is an isolated finding.

In the 1950's Kasai described a procedure that, when done early enough in childhood, may result in adequate liver drainage in approximately 50% of patients. In most infants an attempt is made to restore bile flow and preserve the native liver by excision of solid extrahepatic biliary remnants and reconstruction using a Roux loop [Kasai portoenterostomy (KP)] with primary liver transplantation reserved for those

where this is considered futile largely on the grounds of age and established end-stage liver disease and cirrhosis (Azarow et al 1997).

There are no current models that identify which patients with BA will benefit from early surgical intervention. Despite this Kasai portoentersotomy (KP) procedure, patients with BA continue to place a large burden on liver units worldwide and it is the most common indication for liver transplantation in children (Hartley, Davenport & Kelly 2009). Patients with non-drainage post KP have high levels of morbidity and mortality from the complications of liver failure. If we could determine which patients may benefit from early transplantation without attempted KP there would be potentially decreased morbidity and mortality secondary to liver failure in this subgroup of patients.

A number of factors evident at the time of initial surgery have been studied previously to try and determine outcome post-KP. These can be categorised as the effect of biliary remnant histology [e.g. portal plate remnant ductal size the effect of macroscopic sub-type of BA, the effect of age at surgery and the effect of circulating mediators of inflammation (Davenport et al 1993, Davenport et al 2005, Davenport et al 2008, Nio et al 2003, Langenburg et al 2000, Sanhai et al 2009, Schoen et al 2001, Tan et al 1994)]. Progressive cholestasis within the liver substance itself together with the development of fibrosis and cirrhosis are time-dependent consequences of BA and the prognostic value of liver histology itself has been also evaluated in a number of studies (Azarow et al 1997, Davenport et al 2001, Weerasooriya, White & Sheperd 2004).

Ohi's group showed that the timing of portoentersotomy is important for long-term outcomes of BA with greater success the earlier surgery is undertaken, specifically under 120 days of life (Ohi 1990). Usually by 120 days of life there is established liver cirrhosis and established irreversible liver dysfunction that will not respond to surgical intervention. However, multiple studies have shown no benefit in long-term outcome regardless of timing of portoenterostomy if done within the first 100 days of life (Caponcelli, Knisely & Davenport 2008, Hartley, Davenport & Kelly 2009, Schoen et al 2001).

More important are the type and the subgroup of BA (as discussed above), and the level of fibrosis at the time of surgery (Weerasooriya, White & Shepherd 2004). CBA and BASM show poorer outcomes with increasing age at surgery even when performed under 100 days of life, however, CBA shows improved outcomes if operated on under 70 days of life (Davenport et al 2008, Davenport & Grieve 2012, Kang et al 1993).

Patients with BA show heterogeneous outcomes that are influenced by multiple factors of which some have more weight including the level of liver fibrosis at the time of KP. Weerasooriaya's group studied the relationship between the extent of liver fibrosis in BA and patient outcomes post KP (Weerasooriya, White & Shephard 2004). They demonstrated that the level of fibrosis and cirrhosis is more influential on outcome than the age at surgery in patients under 90 days of life. They also noted that the older the children with BA were when operated on, the more progressive the level of fibrosis and cirrhosis present.

In liver disease, histology has long been the gold standard for diagnosis, staging and follow-up of liver fibrosis. There is currently a move away from biopsies due to their

invasive nature and potential complication risks. In addition, core biopsies only sample a miniscule portion of the organ examined with variable intra-observer findings (Bedossa et al 1994, Peters & Rockstroh 2010, Shaheen & Meyers 2007, Wai et al 2003). Although core biopsies may be representative of the liver as a whole in diseases with homogenous pathological fibrosis such as viral hepatitis and autoimmune diseases, in diseases with heterogeneous fibrosis such as cystic fibrosis and BA, core biopsies may give misleading histological grading of fibrosis and cirrhosis (de Le'dinghen et al 2007).

Biochemical markers of liver fibrosis are thus being utilised with increasing frequency. They are most commonly used as a tool in monitoring liver fibrosis and disease progression (Mangus, 2010). Class I markers are specific and reflect the development of fibrosis in the liver. They are secretory products of hepato-stellar cells and myofibroblasts, such as hyaluronic acid and Transforming Growth Factor β1 (TGFβ1) (dos Santos de Oliveira et al 2010, Gressner, Weiskirchen & Gressner 2007). However, these are not routine laboratory investigations and thus are often unavailable and expensive. Class II markers make use of a combination of factors algorithmically manipulated to help indicate the current state of hepatic destruction. These factors often include common serological tests and biographical data, for example the Hepatoscore, King's score, APRi and the Fibrotest (Table 1)(Boursier et al 2009, Cross et al 2009, de Le'dinghen et al 2007, dos Santos de Oliveira et al 2010, Fabris et al 2006, Gressner, Weskirchen & Gressner 2007, Ngo et al 2006, Peters & Rockstroh 2010, Poynard et al 2010, Shaheen & Myers 2007, Wai et al 2003). It is difficult to compare all these tests to one another as they are evaluated on different levels of fibrosis and cirrhosis to one another. Adams has compared where

possible numerous biomarkers utilised in hepatitis C, alcoholic liver cirrhosis and non-alcoholic liver cirrhosis (Adams 2011). These AUC, sensitivity and specificity are added to the Table 1 below where present.

Fibrosis/Cirrhosis markers.	**Components required to calculate.**	**Diseases in which marker has been validated in as indicator of fibrosis/cirrhosis.**	**AUC (Sensitivity/Specificity)**
APRi	AST level Platelet count	Hepatitis C Biliary atresia	0.8 - 0.88 (41-91% / 47-95%)
AST/ALT ratio	ALT level AST level	Hepatitis C	
Bonacini's discriminate score	ALT AST INR	Hepatitis C	
Fibroscan	Specialised equipment	Hepatitis C	
Fibrotest	α2-macroglobulin ALT Apolipoprotein Haptoglobulin Bilirubin γ-glutamyl transpeptidase	Hepatitis C	
Forns fibrosis index	Age Cholesterol level GGT Platelet count	Hepatitis C	0.81 – 0.86 (30-94% / 51-95%)
Hepatoscore	α2 Macroglobulin Age Bilirubin GGT Hyaluronic acid Sex	Hepatitis C	0.82 – 0.85 (67% / 92%)
King's score	Age AST INR Platelets	Hepatitis C	0.75 – 0.83 (86% / 80%)
Transforming growth factor β 1	Transforming growth factor β 1	Hepatitis C Biliary atresia	

TABLE 1 Liver fibrosis/cirrhosis marker's components.

A decade ago, Davenport's group looked at the value of various biological (including age at surgery) and biochemical factors (including Aspartate Aminotransferase (AST) level, Gama–glutamyl-transpeptidase (GGT) level, Bilirubin level and platelet counts) in predicting whether patients would drain bile post KP, require early transplantation or die, or whether patients required delayed transplantation (Davenport, Betalli & Hadzic 2002). Using binary logistic regression and Receiver Operator Curves (ROC) they showed that a high platelet count ($p = 0.006$) and low AST levels ($p= 0.01$) showed predictive value in relation to a better two year prognosis in BA patients post KP.

Wai developed the Aspartate Aminotransferase-to-Platelet Ratio Index (APRi) for use in hepatitis C patients to establish the need for anti-retroviral therapy without the requirement of repeated liver biopsies (Wai et al 2003). APRi is calculated by the formula:

$$APRi = \left(\frac{AST / Upper\ value\ of\ normal\ AST}{Platelet\ count\ (10^{9}/L)} \right) x \quad 100$$

FIGURE 1. APRi calculation

Although multiple papers have been published on the use of biochemical markers in hepatitis C patients, it is only really the APRi that has been previously looked at as a reference marker for fibrosis and cirrhosis in patients with BA (dos Sandos de Oliveira et al 2010, Kim et al 2010, Mangus et al 2010).

Dos Santos de Oliveira *et al.*, from Brazil studied the relationship of APRi and serum TGF-β1 to liver collagen density liver histology in 17 patients (10 at time of KP and 7 at time of liver transplant) (dos Santos de Oliveira et al 2010). Although not in the

results, it is possible to calculate a correlation with collagen density (reflecting fibrosis) in the groups. As such, no correlation can be found either *ab initio* or using the entire BA group, but neither was there correlation with the class I marker TGF-β1 either. Kim *et al.* looked at APRi and liver fibrosis (assessed as Metavir index on wedge liver biopsy) in 35 infants. APRi values were calculated at the time of initial KP and compared to liver biopsies taken during KP (Kim et al 2010, Shaheen & Myers 2007). Eleven patients were classified as having "no - moderate fibrosis" and 24 patients "severe fibrosis – cirrhosis". A cutoff value of 1.42 separated these two groups with an AUC of 0.91. They also allude to worse long term outcomes in the group with the higher APRi values, but without specific details.

Following this, the APRi was chosen as a potential predictive marker in the patients of this retrospective study as it is a simple calculation using variables that are measured routinely in patients with BA, no additional costs would be incurred and it correlates well with the level of fibrosis and cirrhosis in patients with liver disease.

CHAPTER 2 – Study Objectives

Study Question

Does the APRi value at the time of KP show any relationship to long-term outcome in patients with BA?

Objectives

The objectives of this study were to determine the APRi in patients with BA and to investigate:

1. APRi's relationship to BA at the time of KP when looking at bilirubin level, age at surgery, macroscopic liver cirrhosis, type of biliary atresia, and spleen size.
2. APRi's prognostic value at the time of KP with regard to long term outcomes based on clearance of jaundice, development of oesophageal varices and end stage liver failure determined by either death or requirement of liver transplantation, and
3. APRi's value as an outcome measure indicating further management of these patients noted by requirement of intervention for oesophageal varices.

Hypothesis

APRi at the time of KP has long-term prognostic value for patients with BA determined by anicteric state, bleeding oesophageal varices and liver transplant requirement or death secondary to liver failure.

Ethical considerations

The study is was retrospective patient record review. Permission was granted by King's College Hospital to utilise their patient records. The Human Research Ethics Committee (HREC) of the University of the Witwatersrand also approved the study (HREC number: **M110933)**.

CHAPTER 3 – Methodology

Kings College Hospital is the largest tertiary institution for the treatment of paediatric hepatobiliary disease in the United Kingdom (UK) and derives most of its patients from the South-East of England. King's College Hospital is one of three centres in the UK that treat paediatric hepatobiliary surgical conditions. This is a retrospective study of patients with biliary atresia. Data was obtained for this review using the Biliary Atresia Registry of prospectively collected data at King's College Hospital in London, the King's College theatre database, Electronic Patient records and Patient files.

Patients: We included all those infants with histologically-proven BA coming to laparotomy at King's College Hospital, London in the period Jan 1999 - December 2010. All surgical procedures were performed by a single surgeon (MD).
A total of 260 patients are included in the study. Patients not resident in the UK were excluded from the study for follow-up reasons. All patients were children.

Data collection: Data collection was divided into two parts:

1) Data from the time of KP and

2) Data from patient follow-up visits.

The end points of follow-up were death, liver transplant or most recent out-patient clinic visit.

Data collected at the time of KP included: biochemical and haematology values [platelet count, AST, γ-glutamyl transpeptidase (GGT), total bilirubin; cytomegalovirus (CMV) serology (where available); age at surgery and spleen size. Spleen size was assessed by two paediatric radiologists looking at the maximum diameter as

measured at pre-operative ultrasound (US). Infants were also subdivided into four sub-groups depending on putative aetiological features. These sub-groups therefore included Isolated BA, Anomaly BA (typically BASM), Cystic BA (as noted at laparotomy) and CMV IgM+ve BA (at time of presentation). During the laparotomy a macroscopic assessment was made of macronodular cirrhosis being present or not. This was made by a single observer (MD).

Recently published studies of the normal range of platelet counts in infants were used to compare with observed values (Christensen 2009, McPherson 2005, Weidmeier 2009).

Routine follow-up data was collected which included liver biochemisty and clinical signs of portal hypertension including evidence of upper gastrointestinal bleeding. Upper gastrointestinal endoscopy was performed following clinically evident bleeding or for evaluation of splenomegaly etc. Endoscopy was done by MD with a grading system of 1, 2, or 3. Grade 3 varices being; large tortuous veins intruding into the oesophageal lumen greater than 1/3 of the lumen diameter, having evidence of recent bleeding such as a cherry red spot, or actively bleeding. Grade 3 varices underwent endoscopic intervention. Clinical outcome was defined by clearance of jaundice (to bilirubin ≤ 20μmol/L) and actuarial native liver survival.

Data analysis: Data are quoted as median (interquartile range) unless otherwise indicated. Statistical comparison of groups was done using non-parametric tests (e.g. Kruskal-Wallace ANOVA and Mann-Whitney test) because of predominantly skewed nature of the data (as assessed by the D'Agostino-Pearson normality test). Actuarial

native liver survival was compared using log-rank tests (Mantel-Cox). Principle statistical analysis was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). A P value of ≤ 0.05 was regarded as significant.

CHAPTER 4 – Results

During the period Jan. 1999 – Dec. 2010, 260 (147 female) infants underwent a KP for biliary atresia. The median age at surgery was 58 (45-71) days with a range of 14 - 209 days. Table 2 illustrates the type and variant of BA in the cohort, and as expected the majority were isolated type 3 BA (n = 255). CMV serological results were available for 111 (43%) infants and 15 (13.5%) proved to be CMV IgM +ve. US measured spleen size was available in 198 (76.1 %) infants with a median value 5.9 (5.2 - 6.6) cms and a range of 4 - 10.2 cms (Table 3).

Biliary atresia characteristic	N (%)	Age at surgery Median (IQ range)	Clinical cirrhosis
Macroscopic type			
1	2 (0.7%)	58 (45 – 71)	0
2	3 (1.2%)		1
3	255 (98.1%)		27
Clinical Variant			
Isolated	171 (65.7%)	60 (47 - 72)	17
CMV IgM +ve	15 (5.8%)	70 (61 - 81)	3
Cystic biliary atresia	27 (10.4%)	47 (35 – 54)	2
Anomaly associated (including BASM)	47 (18.1%)	53 (44 – 65)	6
Anomaly associated (excluding BASM)	10 (3.8%)		1

TABLE 2 Characteristics of biliary atresia (n = 260)

Median platelet count and serum AST levels were 536 (402 - 691) x 10^9 /L and 188 (130 -277) IU/L respectively. There was a low, but significant degree of intercorrelation (r_S = -0.14; P=0.02) and neither set of values passed a test of normality (P < 0.0001). Thrombocytosis is difficult to define however, if the traditional upper limit of >450 x 10^9/L were used then 176 (67.7%) could be so defined.

However, a recent American study looked at platelet counts obtained from a population of over 47,000 neonates up to 90 days of age and noted two peaks; one at 2 to 3 weeks and a second at 6 to 7 weeks. The 95th percentile during these peaks were as high as 750 x 10^9 /L. Using this limit, then 47 (18%) infants could be defined as thrombocytosis. The platelet count also varied significantly according to underlying sub-group (Fig. 2) with CMV BA having the lowest counts and Anomaly BA the highest (P = 0.0002).

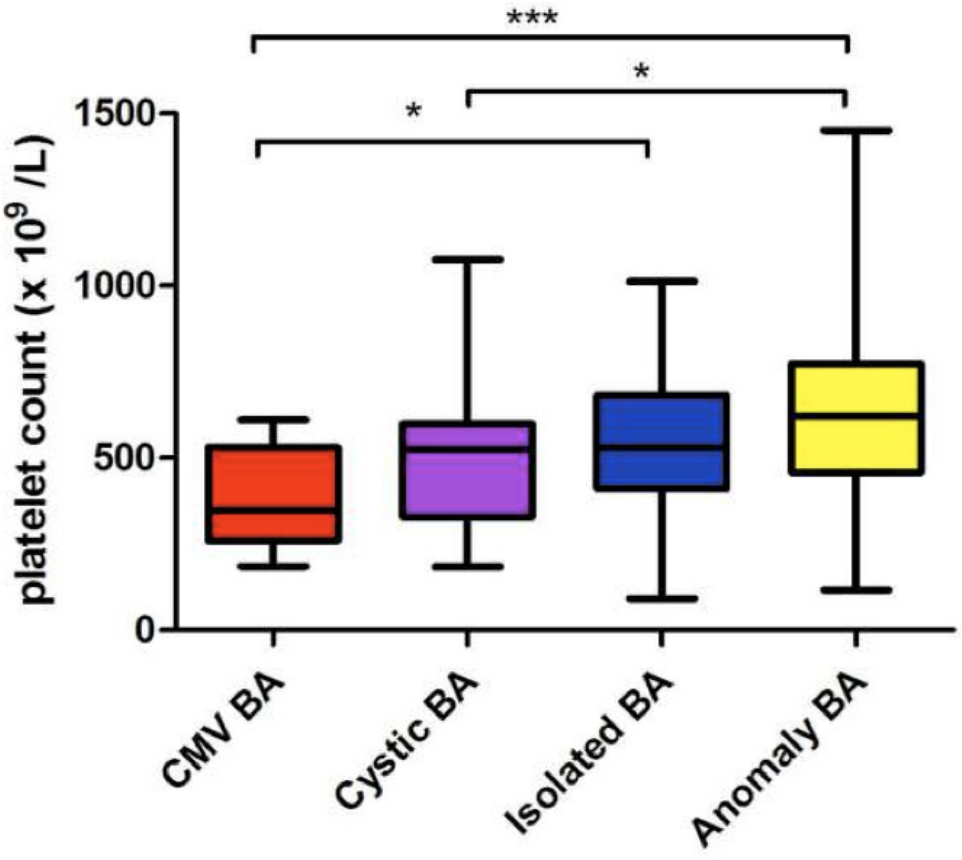


FIGURE 2 Platelet count in biliary atresia (n = 260), by sub-group.
[* P < 0.05, *** P < 0.0001]

Median APRi was 0.67 (0.43-1.12) with a range of 0.11 - 10.81 but was not uniform across the various sub-groups (KW = 17; P = 0.007). Specifically, higher values were found in the CMV IgM+ve BA sub-group compared to the two developmental sub-groups Anomaly BA (P = 0.01) and Cystic BA (P< 0.01) (Fig. 3).

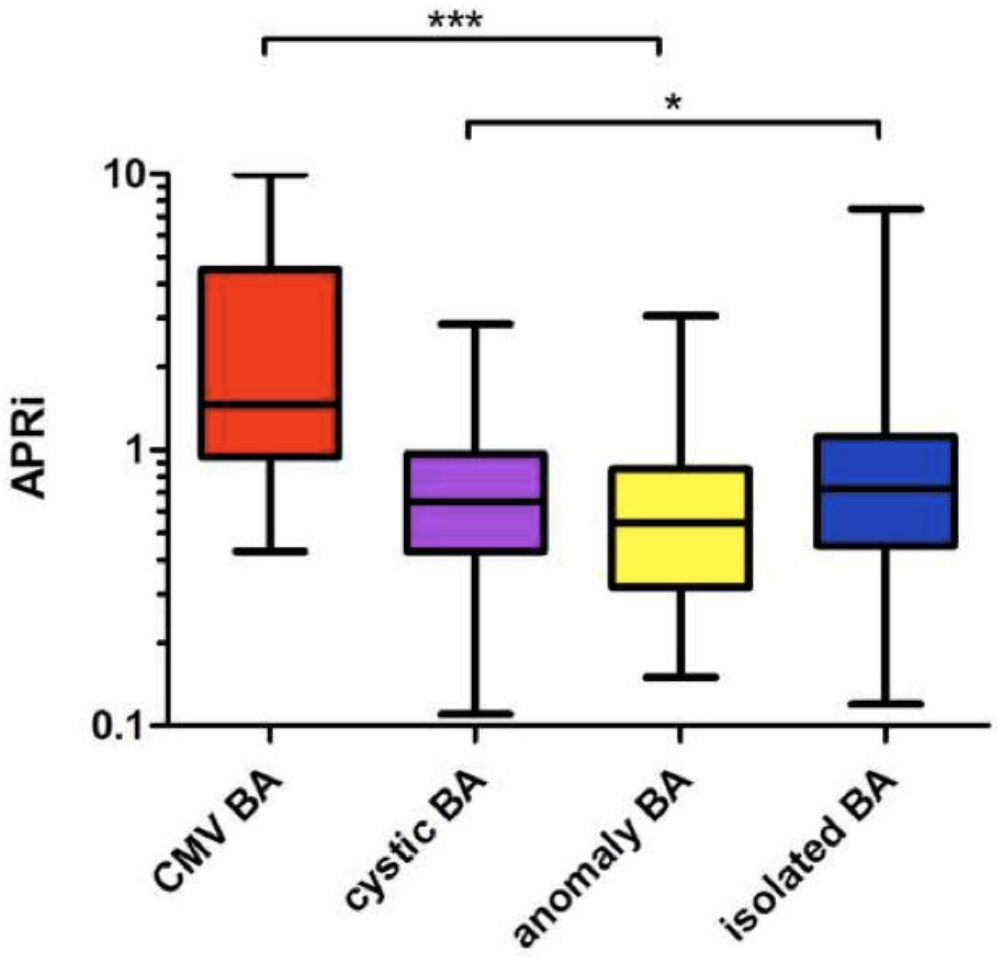


FIGURE 3 APRi and biliary atresia subtypes (N.B logarithmic axis)

Overall, APRi showed a moderate degree of correlation with age at surgery (r_s = 0.44; P < 0.0001), bilirubin (r_s = 0.45; p <0.0001) and spleen size (r_s = 0.48; P < 0.0001) (Table 3). However, when they were divided on the basis of sub-group the

correlational relationship changed. For example, although there was a moderate positive correlation of APRi with bilirubin overall, this only applied to those with isolated BA (r_s = 0.46 ; P < 0.0001) and even more so for those with CMV IgM+ve BA (r_s = 0.95; P < 0.0001) but not CBA (r_s = 0.04; P = 0.42) or Anomaly BA (r_s = 0.17; P = 0.13).

Variable	**Value Median (IQ range)**	**Correlation Coefficient (r_s)**	**P value**	**Normal values (range)**
Age at KP	58 (45-71) days	0.43	<0.001	
Liver Biochemistry				
Bilirubin (µmol/L)	157 (130-194)	0.44	<0.0001	0-20
AST	188 (130-277)	0.83	<0.0001	0-45
γGT (IU/L)	527 (295-963)	- 0.15	0.01	0-45
Alkaline phosphatase (IU/L)	527 (404-701)	0.34	<0.001	30-120
Haematology				
Platelet count (10^3)	536 (402-691)	- 0.63	<0.0001	150-450 laboratory 150-750 at 3 months of age
Clinical				
Spleen size (cm)	5.9 (5.2-6.6)	0.48	<0.0001	2.8-7.0

TABLE 3 Relationship of clinical and biochemical variables with APRi at time of Kasai portoenterostomy

Prediction of cirrhosis

Macroscopic liver cirrhosis was evident in 28 (10.8%) infants at time of laparotomy, 20 (71%) having isolated BA of which 3 were CMV IgM+ve. The predictive value of the pre-operative APRi was evaluated using ROC curve analysis and showed a best cut-off value of 1.22 [AUC 0.83 (95% CI 0.73 – 0.90), P < 0.0001]. Using this then APRi had a sensitivity of 75% and a specificity of 84% in the whole group, with a Likelihood ratio of 4.4 (Fig. 4). When narrowed down to evaluate only the isolated BA

group (n = 171) the AUC increased to 0.87 (95%CI 0.77 - 0.97; P < 0.0001) with sensitivity 88% and specificity of 85% and Likelihood ratio of 5.9.

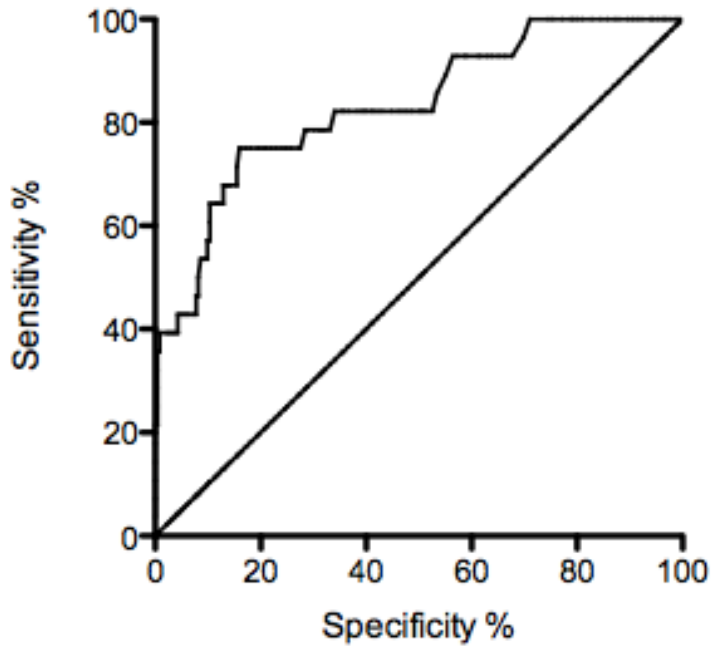


FIGURE 4 ROC of APRi and prediction of clinical cirrhosis (n = 28) in biliary atresia (n = 260). AUC = 0.83 for an APRi value of 1.22 with a sensitivity of 75% and a specificity of 84%.

Prediction of Outcome

Clearance of jaundice was achieved in 135 (51.9%) infants by six months following KP. These infants had a significantly lower median APRi at the time of KP [0.62 (0.40 – 0.95) vs. 0.81 (0.48 – 1.4); P = 0.0004]. No child cleared their jaundice when the APRi was > 3.0.

Figure 5 illustrates native liver survival when the cohort was divided into APRi quartiles. Only those in the lowest quartile (< 0.43) had a statistically higher native liver survival compared to the other three (X^2= 11.6; P = 0.009).

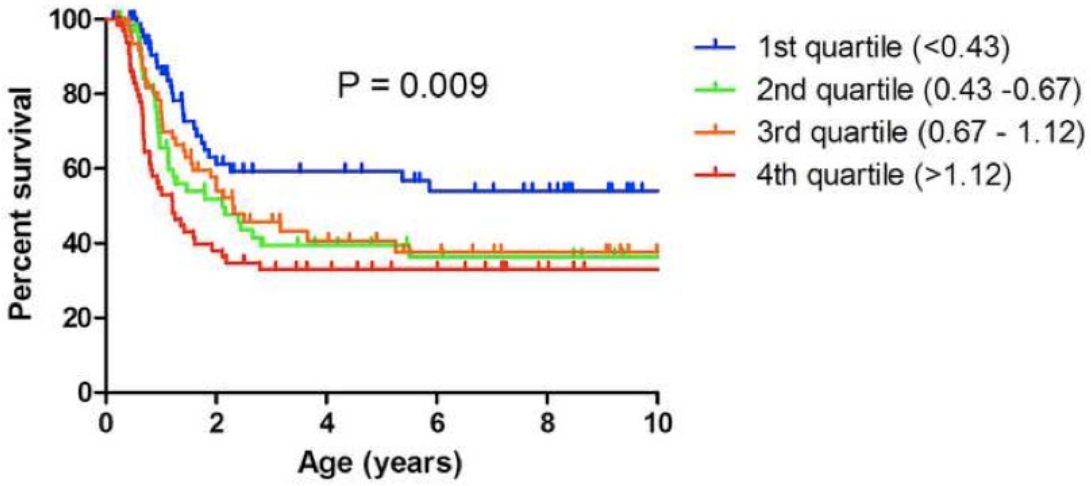


FIGURE 5 Native liver survival curves of infants with biliary atresia (n = 260): influence of APRi. Native liver survival of 1st quartile significantly better than remainder (P = 0.009).

Figure 6 illustrates the cohort by presence or absence of macroscopic cirrhosis showing a significant decreased native liver survival in the former (P = 0.03).

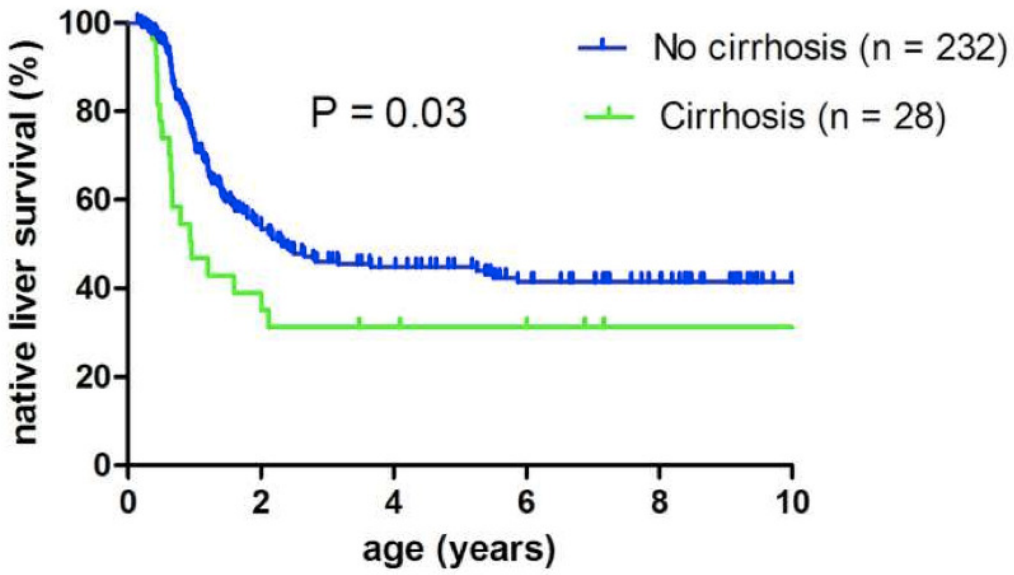


FIGURE 6 Native liver survival curves of biliary atresia (n = 260): influence of clinically evident "cirrhosis" at time of laparotomy.

There were 11 (4%) infants with an APRi of ≥3 [median age = 94 (range 60 -140) days]. All except one has required a liver transplant or died (n = 3) at a median age of 0.53 (range 0.37 – 2.1) years. Four were CMV IgM+ve.

In this cohort, 83 (32%) children underwent upper gastrointestinal endoscopy at a median time following KP of 352 (198-611) days. Indications were either bleeding or clinical suspicion of portal hypertension (e.g. splenomegaly). Of these, 42 (16% of original cohort) underwent endoscopic intervention (sclerotherapy or banding) for "significant" varices. Initial APRi values were analysed to try and predict "significant" varices using this group. Later APRi values noted at the time of endoscopy were also used to predict this. However, there was no significant difference in APRi values either at time of KP [0.84 (0.43-1.13) vs. 0.68 (0.43-1.20); P = 0.6] or at time of endoscopy ([2.30 (1.20-3.35) vs. 3.04 (1.37-3.90); P = 0.86) between intervention and no intervention.

Of the CMV + patients 3 (20%) were seen to have macroscopic cirrhosis at the time of KP. Only one of this CMV + cohort underwent endoscopy and required intervention for varices prior to transplant or death. Median Native liver survival for patients with positive CMV serology 0.93 years (0.41-1.61) was significantly lower (p=0.0047) that the remaining patients 1.55 (0.74-5.40) and has the poorest overall survival when broken down into various subgroups (Figure 7).

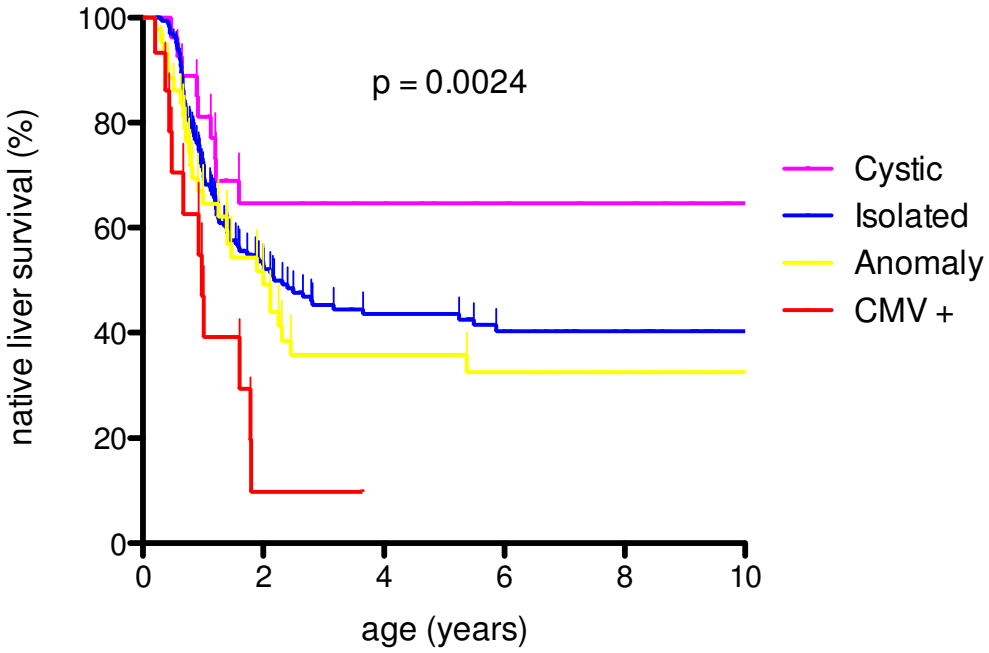


FIGURE 7 Native liver survival curves of biliary atresia per subtype.

CHAPTER 5 – Discussion

There are many potential factors involved in the prognostic outcome in patients with BA, including chronological factors, biochemical variables, histological factors, technical surgical issues, and infective factors. Many of these factors are beyond our control, however timing of surgery, surgical technique and having a dedicated and regular team helps with optimising outcomes for these patients (Davenport & Grieve 2012). Variable outcomes are seen post KP with approximately 66% of cases showing pigmented stools in the early post operative time period indicating some bile drainage into the intestine. However, only approximately 50% will end up being anicteric at six months (Cowles 2012). All BA patients may progress to end stage liver failure over time despite initial drainage and regular follow up is thus mandatory and consideration for liver transplantation with liver failure (Hartley, Davenport & Kelly 2009). There are various surgical and medical options available to improve outcomes in patients with BA and yet, regardless of maximal therapy, there will still be a small cohort of patients that require early transplantation to ensure survival (Hadzic 2012). The aim would be to identify this cohort of patients that, despite optimal surgical and medical management, still progress to early end stage liver failure. It would be prudent to rather optimise these patients and opt for primary liver transplantation, thus improving on the high levels of morbidity and potential mortality, as well as enabling better utilisation of medical resources. Unfortunately at present no single variable has proven to differentiate this group from others. Age at surgery and degree of liver cirrhosis at the time of KP are potential predictors of outcome, but there will continue to be outliers that do not adhere to these general principles.

The King's college unit had previously noted that a high platelet count and low AST levels at presentation were associated with a good outcome in a series of 194 infants with BA from the 1990s (Davenport, Betalli & Hadzic 2002). Furthermore multiple regression analysis suggested both variables were independent of each other. Subsequently, the composite index APRi was developed using both variables but validated largely in chronic liver disease in adults (Wai et al 2003).

Of the two original variables, the platelet count needs most discussion as to what it is reflecting in BA. Thus infants with BA appear to have a relative thrombocytosis compared to normal infants, however the upper limit of normal is defined (Christensen et al 2009, McPherson & Juul 2005, Weidmeier et al 2009). The reasons for this are not known, although most authors allude to thrombocytosis in infants as reactive to bacterial or viral infection, and a little more specifically, respiratory infectionand sometimes it can be secondary to maternal drug ingestion (e.g. anti-psychotics, opiates) (Denton & Davis 2007, Matsubara et al 2004, Nako et al 2001, Vora & Lilleyman 1993). Thrombopoietin is a glycoprotein produced in the liver from hepatocytes and sinusoidal epithelial cells that primarily regulates megakaryocyte development and platelet production and tends to be high during secondary thrombocytosis (Kaushansky 1998). In adults, it can be low in association with end-stage liver disease due to cirrhosis (Goulis et al 1999). Other than thrombopoietin, cytokines such as IL1, IL-3, IL6, and IL11 also stimulate platelet production in vivo (Hsu et al 1999). In our study there was a difference in platelet count according to sub-group and perhaps surprisingly given the potential infective causes listed above and the observed high APRi values in this group, CMV-associated BA infants had the lowest values. Anomaly BA infants had the highest values overall, and as most of these did have polysplenia perhaps this had an effect

by reducing platelet clearance. A previous study from King's college in older children with BASM, however, did not show any immunological deficit compared to children with BA and normal spleens (Taylor et al 2003).

Biochemical markers are being utilised with more frequency to establish the extent and severity of liver disease in place of regular and routine invasive biopsies. Numerous markers are available utilising standard biochemical parameters, specific biochemical parameters, physical parameters or a combination of these. As discussed in Chapter 1, the APRi is a simple marker that makes use of regular biochemical markers being surveyed routinely in patients with BA and liver disease eliminating the need for further invasive or non-invasive tests. It has also shown to correlate respectably with the standard tests looking at the level of fibrosis and cirrhosis in patients with liver disease.

There is very little data available on the use of biochemical markers as surrogates for fibrosis and cirrhosis in patients with BA apart from two studies in which the APRi is evaluated (dos Santos de Oliveira et al 2010, Kim et al 2010).

We did not attempt to review liver histology in this cohort in order to prove the assumption that APRi was indeed a surrogate for liver fibrosis. This has been done in adult studies of hepatitis C (Adams 2011, Bedossa et al 1994, Boursier et al 2009, Shaheen & Myers 2007, Wai et al 2003). Previous studies from King's college institution have had limited success in histological assessment of the BA liver to predict outcome specific outcomes and there are other obvious problems related to sampling error in core biopsies and the heterogeneity of the disease process (Bedossa et al 1994, de Le'dinghen et al 2007, Kang et al 1993). Other institutions have also reported little correlation of conventional liver histology with outcome

(Azarow et al 1997, Weerasooriya, White & Sheperd 2004). Interestingly, one recent study also failed to establish a relationship of conventional histological grading (i.e. Ishak score) with outcome in BA but showed a relationship with newer techniques involving computerised quantification of a specialised stain – picrosirius red (Kang et al 1993).

This study has shown general trends of an increasing APRi to mirror, with other factors such as age at surgery, spleen size, and macroscopic liver cirrhosis, a worsening condition in patients with BA. We have also demonstrated that the APRi at the time of surgery could help predict long term native liver survival; none of the patients with an APRi > 3 achieved native liver survival of more than 2.1 years, with a median of 0.53 years of native liver survival in this cohort. Furthermore, our study demonstrated a significantly higher native liver survival in the quartile group with an APRi < 0.43. This information is important as it enables us too more accurately council parents on the possible outcomes in children. Moreover, we may consider listing patients for primary liver transplant if their ARPi is > 3, thereby avoiding unnecessary surgery as well as increased perioperative KP morbidity. In addition in such cases, this would eliminate the complications of postoperative ascending cholangitis and the potential difficulty of post KP ex-plantation of the liver at the time of transplantation.

In our population we showed that an APRi level of > 1.2 was predictive of macroscopic liver cirrhosis at presentation, and indeed in that group (11% overall) native liver survival was significantly worse. However, these only formed a relatively small part of our BA population and when the outcome of the whole group was

divided into quartiles and their native liver survival looked at, only those with the low APRi's (< 0.43) had demonstrably improved outcome.

Infants with CMV-associated BA were different and had the highest APRi values also proving to have the worse outcome. Although this cohort of patients came to surgery the latest of the subgroups with a median of 70 days (61-81days); it still fell well under the generally accepted 100 day cut off before a delay in surgery is noted to have a detrimental outcome (Caponcelli, Knisely & Davenport 2008). Since these patients have such a rapid deterioration in their clinical state they often do not come to endoscopy but rather have early transplantation or demise.

We know that even in those infants who achieve successful bile drainage and clearance of jaundice, the fibrotic process is progressive leading to splenomegaly and variceal formation (Hartley, Davenport & Kelly 2009). Previous experience has shown that liver histology at the time of KP fails to predict who will go on and develop significant varices later in life and a more recent study involving measurement of portal venous pressure at KP has also shown a lack of correlation with future variceal formation (Kang et al 1993, Shalaby, Makin & Davenport 2012). In this respect initial APRi also had little predictive value. What was less predictable was the failure of APRi measured at the time of endoscopy to distinguish those who had significant varices (defined by the need for treatment). This is in contrast to two studies, albeit in much older children. Chongsrisawat *et al.* used APRI and the ultrasound technique of transient elastography (FibroScan®) in 73 children with BA (mean age 9 years) (Chongsrisawat et al 2011). Both tests were similar in their ability to predict varices and using an APRi of ≥ 1.2 they achieved a sensitivity and specificity of 84 and 83% respectively. Colechhia *et al.* in older children with a median age of 12 years, found

that an APRi ≥ 0.96 showed a sensitivity of 86% and a specificity of 81% in predicting oesophageal varices at the time of endoscopy in their cohort of 31 patients with BA (Colecchia et al 2011).

In the resource limited environment of South Africa where access to liver transplantation is poor and often prolonged, the best chance of survival is still the KP procedure despite prognostic indicators that may suggest primary liver transplantation. The Red Cross Hospital in Cape Town is currently the only paediatric liver transplant unit in the state sector and transplants on average 5.5 patients a year but has additional referrals for transplantation of in excess of 20 paediatric patients per annum (Millar, Spearman & Khan 2009).

In conclusion, the APRi value confirms the heterogeneity of subgroups of BA patients. It is seen to be a useful adjunct in evaluating liver disease in patients with BA. Patients with an APRi > 3 are unlikely to benefit from a KP and should be considered for primary liver transplant workup. CMV IgM postitive patients are seen to have a poor prognosis in this UK cohort. APRi does not show any indication of which patients will require endoscopic intervention for oesophageal varices.

Benefits

- The APRi reduces the need for liver biopsy in patients with BA when used to determine the extent of fibrosis or cirrhosis.
- An APRi > 3 helps predict in which cases of BA KP surgery may be futile. Thus decreasing the need for unnecessary surgery and prompting early referral for liver transplantation.
- The APRi is not a good predictor of potential bleeding oesophageal varices when measured at the time of KP or during follow up of BA patients.
- Study has highlighted the specific group of CMV IgM + BA who have an extremely poor prognosis overall.

Limitations

- APRi not compared to histological grading of liver disease at the time of KP.
- Macroscopic assessment at the time of KP prone to bias as single operator and subjective in nature.
- Retrospective review and thus missing data cannot be recaptured.
- Difficult to assess extent of portal hypertension in all patients as only small numbers underwent endoscopy and no other measures of portal hypertension documented.

Possibilities for further research

- Local study of outcomes in BA in South Africa together with assessment of APRi.
- Implications of CMV IgM + serology and BA in local population with higher incidence of exposure.
- APRi and histology of BA in local population of KP patients.

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Appendices

Appendix A: Letter of Permission to use Data from Kings College Hospital

Mark Davenport ChM FRCS *(Paeds)* FRCS *(Glas & Lon)*

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To Whom It May Concern:

17th November 2011

RE: Andrew Grieve – use of data for APRI study in 2011.

This letter is to confirm permission to utilise clinical data from the 1st January 1999 to the 31st December 2010 for a retrospective study performed this year at Kings College Hospital.
This research is done as a retrospective audit of patients treated in the Paediatric Surgical Hepatobiliary Unit of King's College and thus does not require formal ethics approval in the United Kingdom, thus no ethics approval was applied for in the United Kingdom.

Yours truly,



Mark Davenport

Appendix B: Data collection table template

Patient Study number	M=1, F=2	Date Of Surgery	Age at Surgery (days)	BA Type I,II,III	Macro cirrhosis	Spleen size (cm) at KP	Other congenital abnormalities	Cystic	BASM	CMV IgM +	Bilirubin	AST	ALP
1													
2													
3													
Etc.													

Patients continued...	GGT	Platlets	APRI	Clear jaundice @ 6/12	Plt at OGD	AST at OGD	APRi at OGD	OGD intervention=1, Scope no intervention=2	Time to OGD from KP (days)	OGD Varices grade	RIP/Liver Tx	Date Last Seen	Native Liver Survival
1													
2													
3													
Etc.													

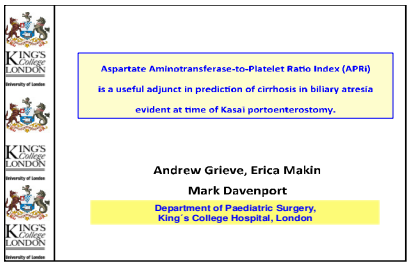
OGD: oesophagogastroduodenoscopy .
BA: Biliary atresia .
BASM: Biliary atresia splenic malformation
CMV: cytomegalovirus

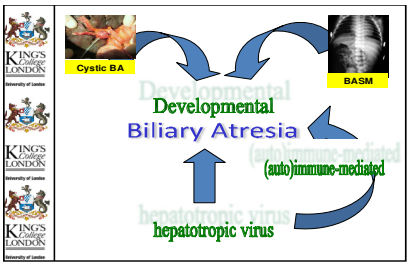
Appendix C: Slides from Presentations

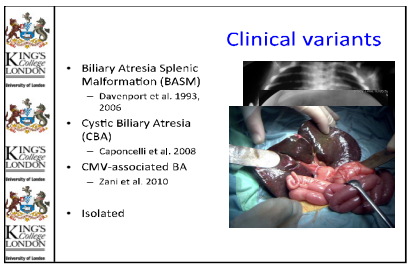
1. British Association of Paediatric Surgeons congress July 2011 (Belfast)

   APRi is a useful adjunct in prediction of cirrhosis in biliary atresia evident at the time of Kasai portoenterostomy.

29/11/2012









1

AASLD

Davenport et al. (2002)
Improved prognosis in BA (1992 – 2001) with
- High Platelet count
- Low AST level

AST-to-Platelet Ratio Index

$$\text{APRi} = \frac{\text{AST/Upper Limit of Normal Range}}{\text{Platelet count } (10^9/\text{L})} \times 100$$

1st used in adult Hepatitis C patients
Wai et al. 2003

Methods

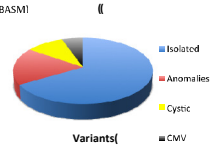
- Prospective
- Single surgeon series.
- January 1999 – December 2010.
- Kasai portoenterostomy or laparotomy alone
  - AST & platelet count
  - Clinical assessment of cirrhosis
- Statistical analysis
  - Non-parametric
    - Correlation (Spearman)
    - Comparison (Kruskal-Wallace ANOVA)
  - ROC curve
  - $P < 0.05$

Hypothesis

- If APRi is a surrogate marker of liver fibrosis
  - Variation with clinical type?
  - Variation with age?
  - Predictor of cirrhosis at time of surgery?

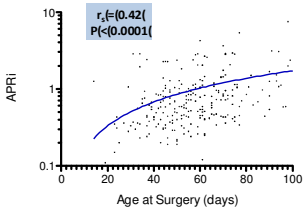
Demographic Data

- N = 260 (147 female)
- Median age at surgery 59 (IQ 45-71) days
- Type 3 (98.5%)
- Variants
  - Anomalies (e.g. BASM)
    - n = 50 (19%)
  - CBA
    - n = 27 (10%)
  - CMV IgM+ve BA
    - n = 15 (6%)
  - Isolated
    - n = 183 (70%)

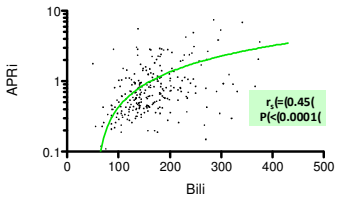


Variants(

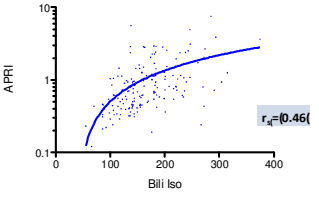
APRi and Age at KP

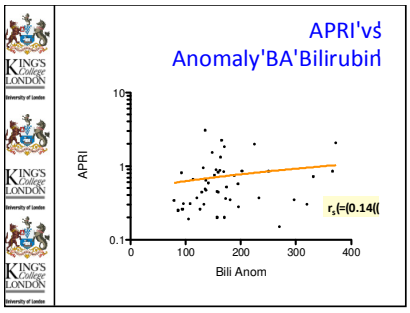


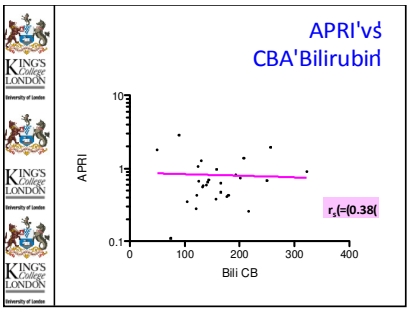
APRi vs Bilirubin

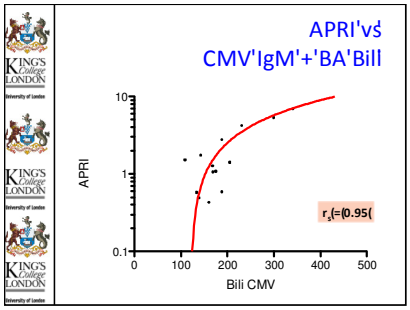


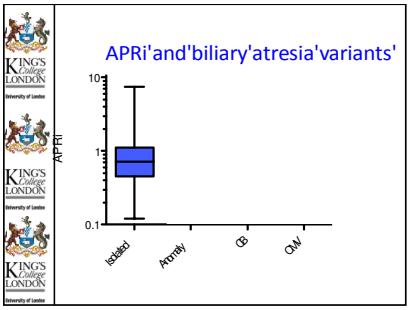
APRI vs Isolated BA Bilirubin



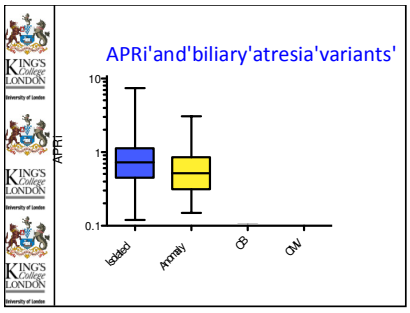

APRI'vs'
Anomaly'BA'Bilirubin'
APRI
Bili Anom
r_s=(0.14((

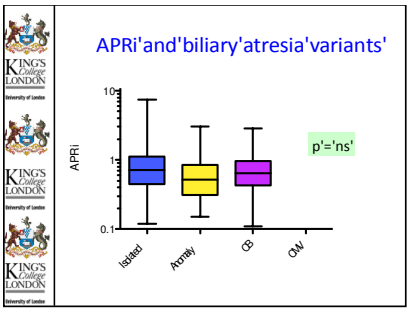

APRI'vs'
CBA'Bilirubin'
APRI
Bili CB
r_s=(0.38(

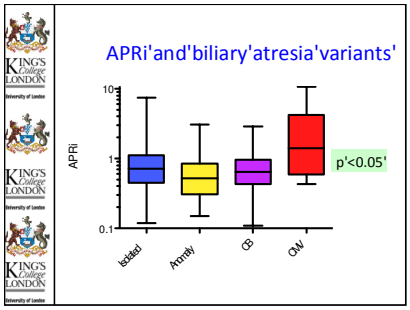

APRI'vs'
CMV'IgM'+'BA'Bill
APRI
Bili CMV
r_s=(0.95(

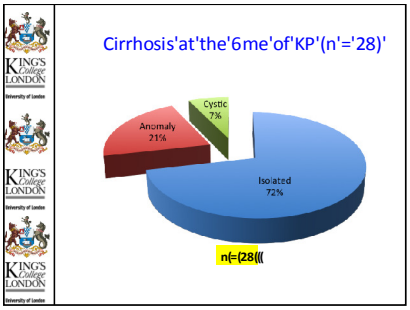

APRi'and'biliary'atresia'variants'
APRI
Isolated
Anomaly
CB
CMV

4


APRi'and'biliary'atresia'variants'
APRI
10
1
0.1
Isolated
Anomaly
CB
CMV

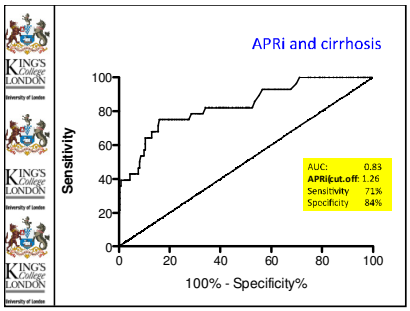

APRi'and'biliary'atresia'variants'
APRi
10
1
0.1
p'='ns'
Isolated
Anomaly
CB
CMV

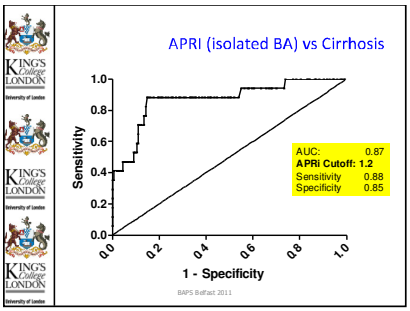

APRi'and'biliary'atresia'variants'
APRi
10
1
0.1
p'<0.05'
Isolated
Anomaly
CB
CMV


Cirrhosis'at'the'6me'of'KP'(n'='28)'
Cystic
7%
Anomaly
21%
Isolated
72%
n(=(28(((

5

.





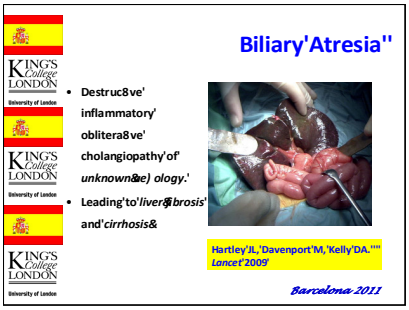
Conclusion

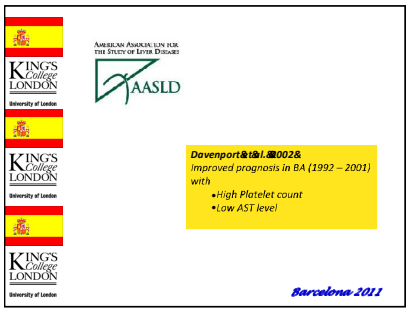
- APRi supports
  - *ae#ological)heterogeneity* in biliary atresia.
- APRi is
  - useful predictor of cirrhosis in biliary atresia.
  - may be used to support option of primary transplant.

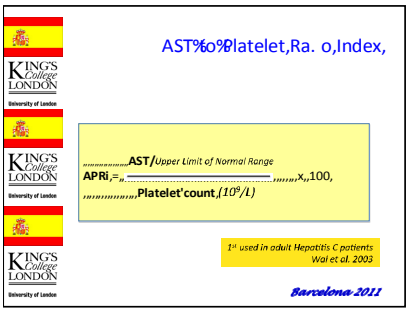
Thank you.

2. European Paediatric Surgical association congress June 2011 (Barcelona) *Prognostic value of APRi in Biliary atresia*









Aim

- To explore its value in relation to outcome

Surrogate marker of liver fibrosis

Barcelona 2011

Patients & Methods

- Jan. 1999 – Dec. 2010
- Single-centre prospective cohort
- n = 260
  - 98% Type 3 BA
- APRi calculated at time of Kasai portoenterostomy

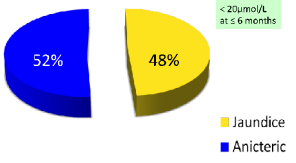
Barcelona 2011

Outcome measures

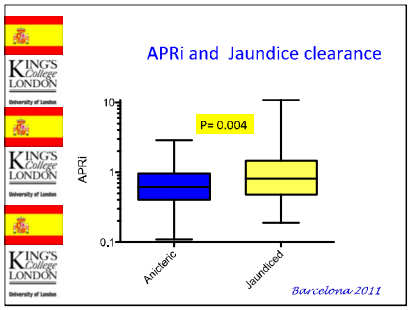
- Clearance of Jaundice
  - (< 20 µmol/L within 6 mths)
- Development of oesophageal varices
  - requiring intervention.
- Actuarial native liver survival
  - Kaplan Meier curves with log-rank test

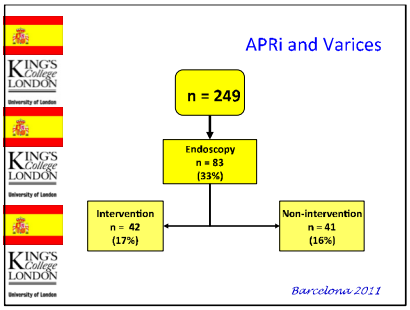
Barcelona 2011

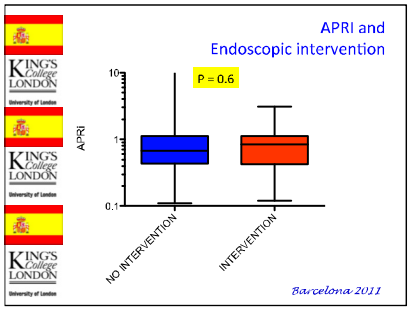
Clearance of Jaundice

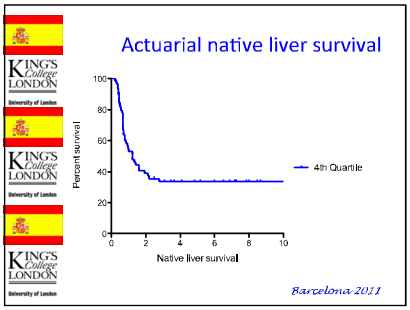


Barcelona 2011

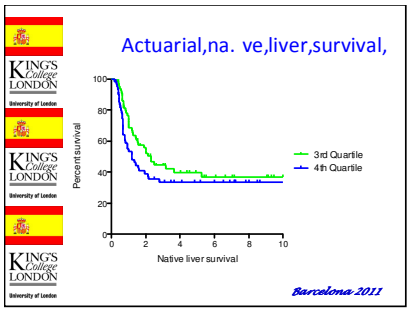

APRi and Jaundice clearance
P= 0.004
APRi
10
1
0.1
Anicteric
Jaundiced
Barcelona 2011

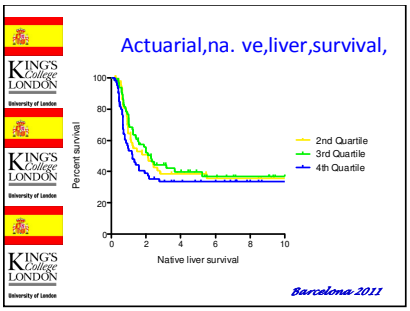

APRi and Varices
n = 249
Endoscopy
n = 83
(33%)
Intervention
n = 42
(17%)
Non-intervention
n = 41
(16%)
Barcelona 2011

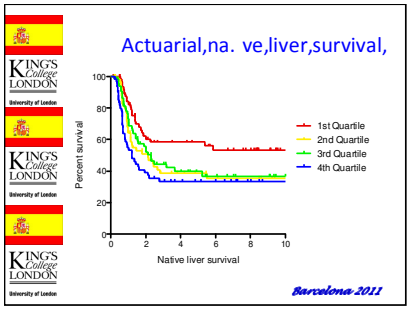

APRI and
Endoscopic intervention
P = 0.6
APRi
10
1
0.1
NO INTERVENTION
INTERVENTION
Barcelona 2011

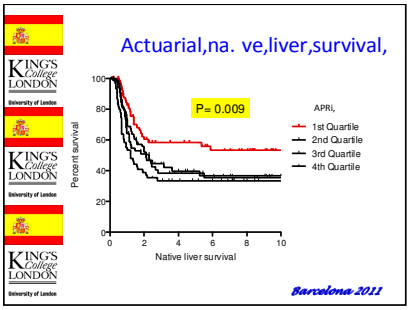

Actuarial native liver survival
Percent survival
100
80
60
40
20
0
0
2
4
6
8
10
Native liver survival
4th Quartile
Barcelona 2011

3


Actuarial,na. ve,liver,survival,
Percent survival
Native liver survival
3rd Quartile
4th Quartile
Barcelona 2011


Actuarial,na. ve,liver,survival,
Percent survival
Native liver survival
2nd Quartile
3rd Quartile
4th Quartile
Barcelona 2011


Actuarial,na. ve,liver,survival,
Percent survival
Native liver survival
1st Quartile
2nd Quartile
3rd Quartile
4th Quartile
Barcelona 2011


Actuarial,na. ve,liver,survival,
P= 0.009
APRI,
Percent survival
Native liver survival
1st Quartile
2nd Quartile
3rd Quartile
4th Quartile
Barcelona 2011

Conclusion

- APRi:
  - Easy to calculate
  - Uses routine investigations
- Poor predictor of need for endoscopic intervention

But

- Prognostic value
  - Clearance of jaundice
  - Native liver survival

Barcelona 2011



Barcelona 2011

5

Appendix D: Ethics approval.

Deirdre Kruger

From: Peter Cleaton-Jones
Sent: 10 December 2012 04:57 PM
To: Deirdre Kruger
Subject: RE: DR ANDREW GRIEVE

To whom it may concern at the Faculty of Health Sciences.

I confirm that the following MMed project by Dr Andrew Grieve:

"*Does the Aminotransferase Aspartate to Platelet Ratio Index (APRi) value at the time of Kasai portoenterostomy (KP) show any relationship to long-term outcome in patients with Biliary Atresia*"

has ethics approval from the Human Research Ethics Committee (Medical). At present Mrs Anisa Keshav of the Wits Research Office secretariat for the Committee is in the USA undergoing training. It will not be possible to provide a standard clearance form before January 2013.

I request that the MMed research report be accepted for examination on the strength of this email. A signed copy of the clearance will be provided in the new year.

Prof P Cleaton-Jones
Chair, HREC(Medical)

From: Deirdre Kruger
Sent: Friday, December 07, 2012 12:36 PM
To: Peter Cleaton-Jones
Subject: DR ANDREW GRIEVE

Dear Prof

Many thanks for your help in issuing us with the clearance certificate for Dr Andrew Grieve.

The title of his project at the time of original ethics application submission in September 2011 was:
"*Does the Aminotransferase Aspartate to Platelet Ratio Index (APRi) value at the time of Kasai portoenterostomy (KP) show any relationship to long-term outcome*".

This title was changed in November 2011 to:
"*Does the Aminotransferase Aspartate to Platelet Ratio Index (APRi) value at the time of Kasai portoenterostomy (KP) show any relationship to long-term outcome in patients with Biliary Atresia*".

Thank you again.

Kind regards,

Deirdré

Dr. Deirdré Kruger, PhD
Research Coordinator (Surgery) and Specialist Scientist
University of the Witwatersrand
Wits Medical School - Department of Surgery
Rm 9M09, 7 York Road, Parktown, Johannesburg, 2193.
Tel: +27 (0) 11 717 2376

1

Appendix E: Letter of acceptance from the Journal of Pediatric Surgery.

-----Original Message-----
From: ees.jpedsurg.f.1c4bea.8e059271@eesmail.elsevier.com
[mailto:ees.jpedsurg.f.1c4bea.8e059271@eesmail.elsevier.com] On Behalf Of
Juan Tovar
Sent: 03 October 2012 09:07
To: markdav2@ntlworld.com
Subject: Your Submission JPEDSURG-D-12-00460R2

Ms. Ref. No.: JPEDSURG-D-12-00460R2
Journal of Pediatric Surgery

Dear Professor Davenport,

I am pleased to inform you that your manuscript JPEDSURG-D-12-00460R2 CR, titled, "Aspartate Aminotransferase-to-Platelet Ratio index (APRi) in infants with biliary atresia: prognostic value at presentation", has been accepted for publication in the Journal of Pediatric Surgery. The manuscript will be published as an original paper. The editors wish to thank you and your co-authors for submitting this manuscript to the Journal.

If you have any questions regarding publication of this manuscript, please refer to your manuscript identification number when contacting us.

You will receive further information regarding galley proofs and the exact issue in which your manuscript will appear as that determination is made. Thank you for your support of the Journal of Pediatric Surgery.

Very sincerely yours,

Juan A. Tovar, MD, PhD
Regional Editor
Journal of Pediatric Surgery

****
For further assistance, please visit our customer support site at http://support.elsevier.com. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer
On 10 Oct 2012, at 10:36 AM, Deirdre Kruger wrote:

Appendix F: Journal article as accepted by the Journal of Pediatric Surgery.

Aspartate Aminotransferase-to-Platelet Ratio index (APRi) in infants with biliary atresia: prognostic value at presentation

*Andrew Grieve MD	Clinical Fellow
**Erica Makin FRCS MSc	Specialist Registrar
**Mark Davenport ChM FRCS (Paeds)	Professor of Paediatric Surgery

* Travelling Clinical Fellow: Department of Paediatric Surgery, University of the Witwatersrand.

** Department of Paediatric Surgery, King's College Hospital, London, UK. SE5 9RS

Corresponding author

Prof Mark Davenport

King's College Hospital,

Denmark Hill, London, SE5 9RH

0044 203 299 3350

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Markdav2@ntlworld.com

Key Words

Biliary atresia; prognosis, liver fibrosis, aspartate aminotransferase-to-platelet ratio index,

Abstract

Background: Biliary atresia (BA) is a progressive obliterative cholangiopathy leading to liver fibrosis and cirrhosis. The aspartate aminotransferase-to-platelet ratio index (APRi) has been used in other liver diseases and in older children with BA as a surrogate marker of liver fibrosis. The aim of this study was to calculate APRi at time of presentation and relate this to operative findings and early outcome.

Methods: Prospective single surgeon cohort study of infants with BA (January 1999 - December 2010). Initial APRi values were related to other biochemical indices and liver appearance at the time of Kasai portoenterostomy. Data are expressed as median (interquartile range). Non-parametric comparison was performed and a P-value of ≤ 0.05 was regarded as significant.

Results: Overall 260 infants were included in the study. Median APRi was 0.67 (0.43-1.12) at a median age of surgery of 58 (range 14 - 209) days. APRi correlated with age (r_s=0.44; P<0.0001), spleen size (r_s=0.48; P<0.0001) and bilirubin (r_s=0.45; P<0.0001). Liver assessment at operation was divided into cirrhosis [n=28 (10.8%)] or non-cirrhosis. Using a cut-off value of 1.22 [AUC 0.83 (95% CI 0.73-0.90)] showed a sensitivity of 75% and a specificity of 84% for macroscopic cirrhosis.

Native liver survival was significantly different but improved only for those in the lowest APRi quartile (<0.43; P<0.009). APRi values at presentation had no significant association with later development of significant oesophageal varices.

Conclusion: APRi at the time of KP is a useful adjunct in evaluating severity of liver disease in BA at presentation.

Introduction

Biliary atresia is characterised as an obliterative cholangiopathy with early progression to liver fibrosis and cirrhosis (1). The aetiology is not known with any precision though it should probably be viewed as a consequence of diverse causes (developmental, infective, immune-mediated etc.). Certainly, various clinical sub-types can be recognised – the biliary atresia splenic malformation syndrome (BASM) (2,3) and cystic biliary atresia (4) are examples where there are distinct clinicopathological characteristics and different responses to surgery (4,5).

In most infants an attempt is made to restore bile flow and preserve the native liver by excision of solid extrahepatic biliary remnants and reconstruction using a Roux loop [Kasai portoenterostomy (KP)] with primary liver transplantation reserved for those where this is considered futile largely on the grounds of age and established end-stage liver disease and cirrhosis (6).

A number of factors evident at the time of initial surgery have been studied previously to try and determine outcome post-KP. These can be categorised as the effect of biliary remnant histology [e.g. portal plate remnant ductal size (7,8)], the effect of macroscopic sub-type of BA (3,5,9), the effect of age at surgery (5,10,11) and the effect of circulating mediators of inflammation (12). Progressive cholestasis within the liver substance itself together with the development of fibrosis and cirrhosis are time-dependent consequences of BA and the prognostic value of liver histology itself has been also evaluated in a number of studies (6, 13, 14).

In 2002, we reported a study on the value of various biochemical and haematological values at presentation in predicting outcome in BA and showed that infants with high platelet counts and low aspartate aminotransferase (AST) levels had a significantly improved prognosis (15). Subsequently, and presumably independently, Wai *et al*. (16) developed the Aspartate Aminotransferase-to-Platelet Ratio index (APRi) (Fig. 1.) for use in hepatitis C adults to establish the need for anti-retroviral therapy without the requirement for repeated liver biopsies.

The aim of this study was to look at the APRi and identify its relationship with various facets of BA at the time of presentation together with a detailed study of its use in prognosis.

Methods

Kings College Hospital is the largest tertiary institution for the treatment of pediatric hepatobiliary disease in the UK and derives most of its patients from the South-East of England. This is a prospective study of data collected at the time of initial laparotomy, with only the APRi calculated in retrospect. We included all those infants, born in the UK with histologically-proven BA coming to laparotomy at King's College Hospital, London in the period Jan 1999 - December 2010. All surgical procedures were performed by a single surgeon (MD).

Data collected at the time of KP included: biochemical and haematology values [platelet count, AST, γ-glutamyl transpeptidase (GGT), total bilirubin; cytomegalovirus (CMV) serology (where available); age at surgery and spleen size (maximum diameter as measured at pre-operative ultrasound). Infants were also subdivided into four sub-groups depending on putative aetiological features. These sub-groups therefore included Isolated BA, Anomaly BA (typically BASM), Cystic BA (as noted at laparotomy) and CMV IgM+ve BA (at time of presentation). During the laparotomy a macroscopic assessment of the degree of fibrosis and specifically cirrhosis was made and recorded prospectively.

Recently published studies of the normal range of platelet counts in infants were used to compare with observed values (17,18,19).

Routine follow-up data was collected which included liver biochemisty and clinical signs of portal hypertension. Upper gastrointestinal endoscopy was performed following clinically evident bleeding or for evaluation of splenomegaly etc. Clinical outcome was defined by clearance of jaundice (to bilirubin $\leq 20\mu mol/L$) and actuarial native liver survival.

The study was approved following institutional board review although it was regarded primarily as an audit of clinical outcome using widely available routine tests.

Data are quoted as median (interquartile range) unless otherwise indicated. Statistical comparison of groups was done using non-parametric tests (e.g. Kruskal-Wallace ANOVA and Mann-Whitney test) because of predominantly skewed nature of the data (as assessed by the D'Agostino-Pearson normality test). Actuarial native liver survival was compared using log-rank tests (Mantel-Cox). Principle statistical analysis was performed using

GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). A P value of ≤ 0.05 was regarded as significant.

Results

During the period Jan. 1999 – Dec. 2010, 260 (147 female) infants underwent a KP for biliary atresia. The median age at surgery was 58 (45-71) days with a range of 14 - 209 days. Table 1 illustrates the type and variant of BA in the cohort, and as expected the majority were isolated type 3 BA (n = 255). CMV serological results were available for 111 (43%) infants and 15 (13.5%) proved to be CMV IgM +ve. US measured spleen size was available in 198 (76.1 %) infants with a median value 5.9 (5.2 - 6.6) cms and a range of 4 - 10.2 cms.

Median platelet count and serum AST levels were 536 (402 - 691) x 10^9 /L and 188 (130 -277) IU/L respectively. There was a low, but significant degree of intercorrelation (r_S = -0.14; P=0.02) and neither set of values passed a test of normality (P < 0.0001). Thrombocytosis is difficult to define however, if the traditional upper limit of >450 x 10^9/L were used then 176 (67.7%) could be so defined. However, a recent American study looked at platelet counts obtained from a population of over 47,000 neonates up to 90 days of age and noted two peaks; one at 2 to 3 weeks and a second at 6 to 7 weeks. The 95th percentile during these peaks were as high as 750 x 10^9 /L. Using this limit, then 47 (18%) infants could be defined as thrombocytosis. The platelet count also varied significantly according to underlying sub-group (Fig. 2) with CMV BA having the lowest counts and Anomaly BA the highest (P = 0.0002).

Median APRi was 0.67 (0.43-1.12) with a range of 0.11 - 10.81 but was not uniform across the various sub-groups (KW = 17; P = 0.007). Specifically, higher values were found in the CMV IgM+ve BA sub-group compared to the two developmental sub-groups Anomaly BA (P = 0.01) and Cystic BA (P< 0.01) (Fig. 3).

Overall, APRi showed a moderate degree of correlation with age at surgery (r_s = 0.44 ; P < 0.0001), bilirubin (r_s = 0.45; p <0.0001) and spleen size (r_s = 0.48; P < 0.0001) (Table 2). However, when they were divided on the basis of sub-group the correlational relationship changed. For example, although there was a moderate positive correlation of APRi with bilirubin overall, this only applied to those with isolated BA (r_s = 0.46 ; P < 0.0001) and even more so for those with CMV IgM+ve BA (r_s = 0.95; P < 0.0001) but not CBA (r_s = 0.04; P = 0.42) or Anomaly BA (r_s = 0.17; P = 0.13).

Prediction of cirrhosis

Macroscopic liver cirrhosis was evident in 28 (10.8%) infants at time of laparotomy, 20 (71%) having isolated BA of which 3 were CMV IgM+ve. The predictive value of the pre-operative APRi was evaluated using ROC curve analysis and showed a best cut-off value of 1.22 [AUC 0.83 (95% CI 0.73 – 0.90), P < 0.0001]. Using this then APRi had a sensitivity of 75% and a specificity of 84% in the whole group, with a Likelihood ratio of 4.4 (Fig. 4). When narrowed down to evaluate only the isolated BA group (n = 171) the AUC increased to 0.87 (95%CI 0.77 - 0.97; P < 0.0001) with sensitivity 88% and specificity of 85% and Likelihood ratio of 5.9.

Prediction of Outcome

Clearance of jaundice was achieved in 135 (51.9%) infants by six months following KP. These infants had a significantly lower median APRi at the time of KP [0.62 (0.40 – 0.95) vs. 0.81 (0.48 – 1.4); P = 0.0004]. No child cleared their jaundice when the APRi was > 3.0.

Figure 5 illustrates native liver survival when the cohort was divided into APRi quartiles. Only those in the lowest quartile (< 0.43) had a statistically higher native liver survival compared to the other three (X^2= 11.6; P = 0.009). Figure 6 illustrates the cohort by presence or absence of macroscopic cirrhosis showing a significant decreased native liver survival in the former (P = 0.03).

There were 11 (4%) infants with an APRi of ≥3 [median age = 94 (range 60 -140) days]. All except one has required a liver transplant or died (n = 3) at a median age of 0.53 (range 0.37 – 2.1) years. Four were CMV IgM+ve.

In this cohort, 83 (32%) children underwent upper gastrointestinal endoscopy at a median time following KP of 352 (198-611) days. Indications were either bleeding or clinical suspicion of portal hypertension (e.g. splenomegaly). Of these, 42 (16% of original cohort) underwent endoscopic intervention (sclerotherapy or banding) for "significant" varices. Initial APRi values were analysed to try and predict "significant" varices using this group. Later APRi values noted at the time of endoscopy were also used to predict this. However, there was no significant difference in APRi values either at time of KP [0.84 (0.43-1.13) vs. 0.68 (0.43-1.20); P = 0.6] or at time of endoscopy ([2.30 (1.20-3.35) vs. 3.04 (1.37-3.90); P = 0.86) between intervention and no intervention.

Discussion

We had previously noted that a high platelet count and low AST levels at presentation were associated with a good outcome in a series of 194 infants with BA from the 1990s (15). Furthermore multiple regression analysis suggested both variables were independent of each other. Subsequently, the composite index APRi was developed using both variables but validated largely in chronic liver disease in adults (16).

Of the two original variables, the platelet count needs most discussion as to what it is reflecting in BA. Thus infants with BA appear to have a relative thrombocytosis compared to normal infants, however the upper limit of normal is defined (17,18,19). The reasons for this are not known, although most authors allude to thrombocytosis in infants as reactive to bacterial or viral infection, and a little more specifically, respiratory infection (20, 21,22) and sometimes it can be secondary to maternal drug ingestion (e.g. anti-psychotics, opiates) (23). Thrombopoietin is a glycoprotein produced in the liver from hepatocytes and sinusoidal epithelial cells that primarily regulates megakaryocyte development and platelet production and tends to be high during secondary thrombocytosis (24). In adults, it can be low in association with end-stage liver disease due to cirrhosis (25). Other than thrombopoietin, cytokines such as IL1, IL-3, IL6, and IL11 also stimulate platelet production in vivo (26). In our study there was a difference in platelet count according to sub-group and perhaps surprisingly given the potential infective causes listed above and the observed high APRi values in this group, CMV-associated BA infants had the lowest values. Anomaly BA infants had the highest values overall, and as most of these did have polysplenia perhaps this had an effect by reducing platelet clearance. A previous study from our group in older children with BASM, however, did not show any immunological deficit compared to children with BA and normal spleens (27).

The APRi is an example of a non-invasive biochemical marker presumed to reflect the degree of intrahepatic fibrosis. It was introduced into adult practice to monitor and evaluate liver disease progression (typically hepatitis C (16, 28), but also non-alcoholic steatohepatitis (NASH) (29)) to try and reduce dependence on serial liver biopsies but has also been used in other scenarios in children (e.g. short gut, 30). Such markers can be divided into Class I and II, with examples of the former include hyaluronic acid, metalloproteinases, Tissue Inhibitors of Matrix Metalloproteinases (TIMPS), Transforming Growth Factor (TGF-β1), all of which are specific in some way to, and reflect the development of, fibrosis in the liver (31,

32, 33). These are not usually routine laboratory investigations and tend to be expensive. Alternatively, Class II markers make use of a combination of factors algorithmically manipulated to help indicate the current state of hepatic damage. These factors often include common serological tests in combination with various types of biochemical data e.g. Hepatoscore, King's score, APRi, Fibrotest (16, 34, 35, 36, 37, 38, 39).

There is very little data available on the use of the APRi in BA (32, 40). Dos Santos de Oliveira *et al.*, from Brazil (32), studied the relationship of APRi and serum TGF-β1 to liver collagen density liver histology in 17 patients (10 at time of KP and 7 at time of liver transplant). Although not in the results, it is possible to calculate a correlation with collagen density (reflecting fibrosis) in the groups. As such, no correlation can be found either *ab initio* or using the entire BA group, but neither was there correlation with the class I marker TGF-β1 either. Kim *et al.* (40), looked at APRi and liver fibrosis (assessed as Metavir index (41) on wedge liver biopsy) in 35 infants. APRi values were calculated at the time of initial KP and compared to liver biopsies taken during KP. Eleven patients were classified as having "no - moderate fibrosis" and 24 patients "severe fibrosis – cirrhosis". A cutoff value of 1.42 separated these two groups with an AUC of 0.91. They also allude to worse long term outcomes in the group with the higher APRi values, but without specific details.

One of our recent themes has been to emphasis the heterogenous nature of BA with definition of a number of clinically distinct groupings (2, 4). There were no statistical differences between the APRi of the two larger sub-groups though (Isolated and Anomaly BA). We had also failed to show a difference in the histological degree of fibrosis in a previous study comparing BASM with isolated BA so perhaps this was not unexpected (2). Infants with CMV-associated BA were different and had the highest APRi values. This sub-group clearly have significantly higher AST values and a more "hepatitic" appearance to their liver histology (unpublished observation) and also proved to have the worse outcome.

We did not attempt to review liver histology in this cohort in order to prove the assumption that APRi was indeed a surrogate for liver fibrosis. This has been done in adult studies of hepatitis C (16, 33, 34, 41, 42). Previous studies from our institution have had limited success in histological assessment of the BA liver to predict outcome specific outcomes (43) and there are other obvious problems related to sampling error in core biopsies (42) and the heterogeneity of the disease process (36). Other institutions have also reported little

correlation of conventional liver histology with outcome (6, 13). Interestingly, one recent study also failed to establish a relationship of conventional histological grading (i.e. Ishak score) with outcome in BA but showed a relationship with newer techniques involving computerised quantification of a specialised stain – picrosirius red. (43).

In our population we showed that an APRi level of > 1.2 was predictive of macroscopic liver cirrhosis at presentation, and indeed in that group (11% overall) native liver survival was significantly worse. However, these only formed a relatively small part of our BA population and when the outcome of the whole group was divided into quartiles and their native liver survival looked at, only those with the low APRi's (< 0.43) had demonstrably improved outcome.

BA is a progressive disease consistent with the detrimental effects of on-going cholestasis and fibrosis. The APRi does have a correlation with age at surgery and several other biochemical markers indicative of progressive liver disease such as bilirubin and splenomegaly. Can it be used as a biological marker for when KP might be a futile undertaking and consideration be given to primary liver transplantation? Age by itself is not a great guide and our previous study looking at those coming to surgery at >100 days did show relatively good results with a five-year native liver survival of >40% even in this beleaguered cohort (45). The current analysis has shown that jaundice clearance was not achieved in any child with an initial APRi of >3.0 and furthermore all required liver transplant to survive. This lends weight to our impression that this indeed is a better indicator of the status of the liver at the time of presentation than anything currently available and maybe the most important practical observation.

We know that even in those infants who achieve successful bile drainage and clearance of jaundice, the fibrotic process is progressive (1) leading to splenomegaly and variceal formation. Our previous experience has shown that liver histology at the time of KP fails to predict who will go on and develop significant varices later in life (43) and a more recent study involving measurement of portal venous pressure at KP has also shown a lack of correlation with future variceal formation (46). In this respect initial APRi also had little predictive value. What was less predictable was the failure of APRi measured at the time of endoscopy to distinguish those who had significant varices (defined by the need for treatment). This is in contrast to two studies, albeit in much older children. Thus,

Chongsrisawat *et al.* (47) used APRI and the ultrasound technique of transient elastography (FibroScan®) in 73 children with BA (mean age 9 years). Both tests were similar in their ability to predict varices and using an APRi of ≥ 1.2 they achieved a sensitivity and specificity of 84 and 83% respectively. Colechhia *et al.* (48), in older children with a median age of 12 years, found that an APRi ≥ 0.96 showed a sensitivity of 86% and a specificity of 81% in predicting oesophageal varices at the time of endoscopy in their cohort of 31 patients with BA.

This series illustrates the value of a simple composite index even in a complex heterogenous disease such as BA. We have shown its usefulness as a non-invasive biological tool to evaluate the liver at the time of presentation; even suggesting a point beyond which attempts at KP may be futile. Once again, it also seems to emphasize the differences that exist within the various clinical sub-groups of BA.

Acknowledgements

This series reflects the clinical input of many people including Profs G Mieli-Vergani and A Dhawan, and Drs N Hadzic and A Baker.

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Figures

FIGURE 1 Aspartate Aminotransferase-to-Platelet Ratio index (APRi) equation.

$$APRi = \left(\frac{AST / Upper\ value\ of\ normal\ AST}{Platelet\ count\ (10^{9}/L)} \right) x \quad 100$$

FIGURE 2 Platelet count in biliary atresia (n = 260), by sub-group.

[* P < 0.05, *** P < 0.0001]

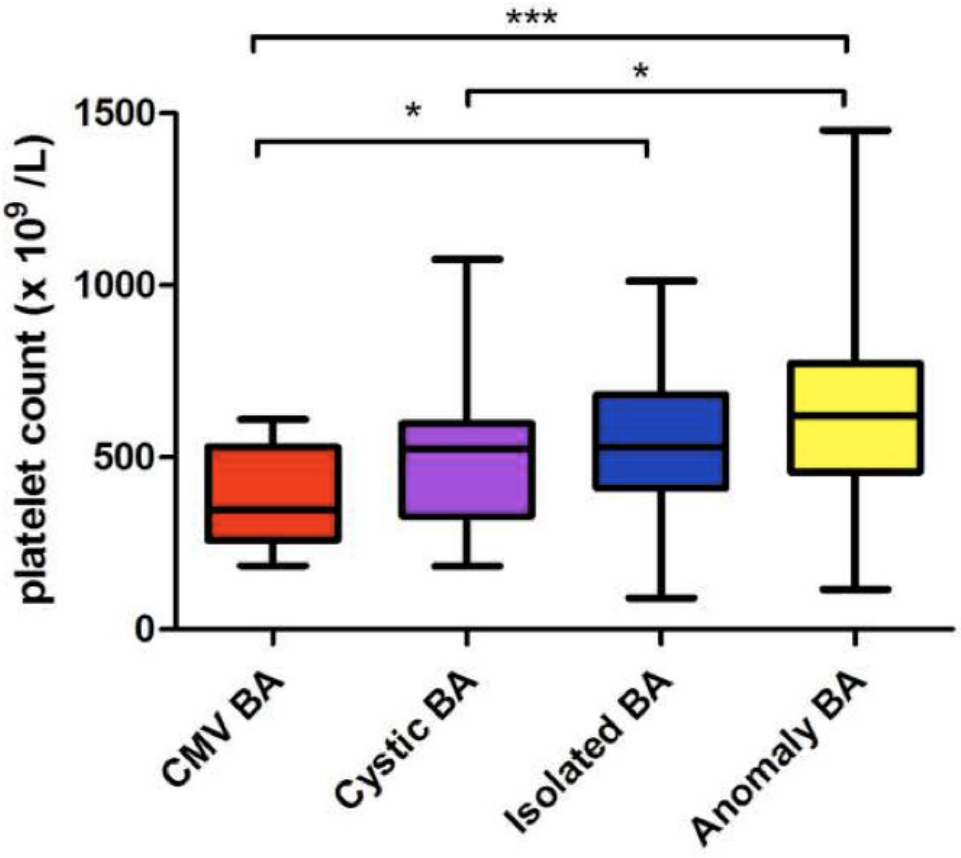


FIGURE 3 APRi and biliary atresia subtypes (N.B logarithmic axis)

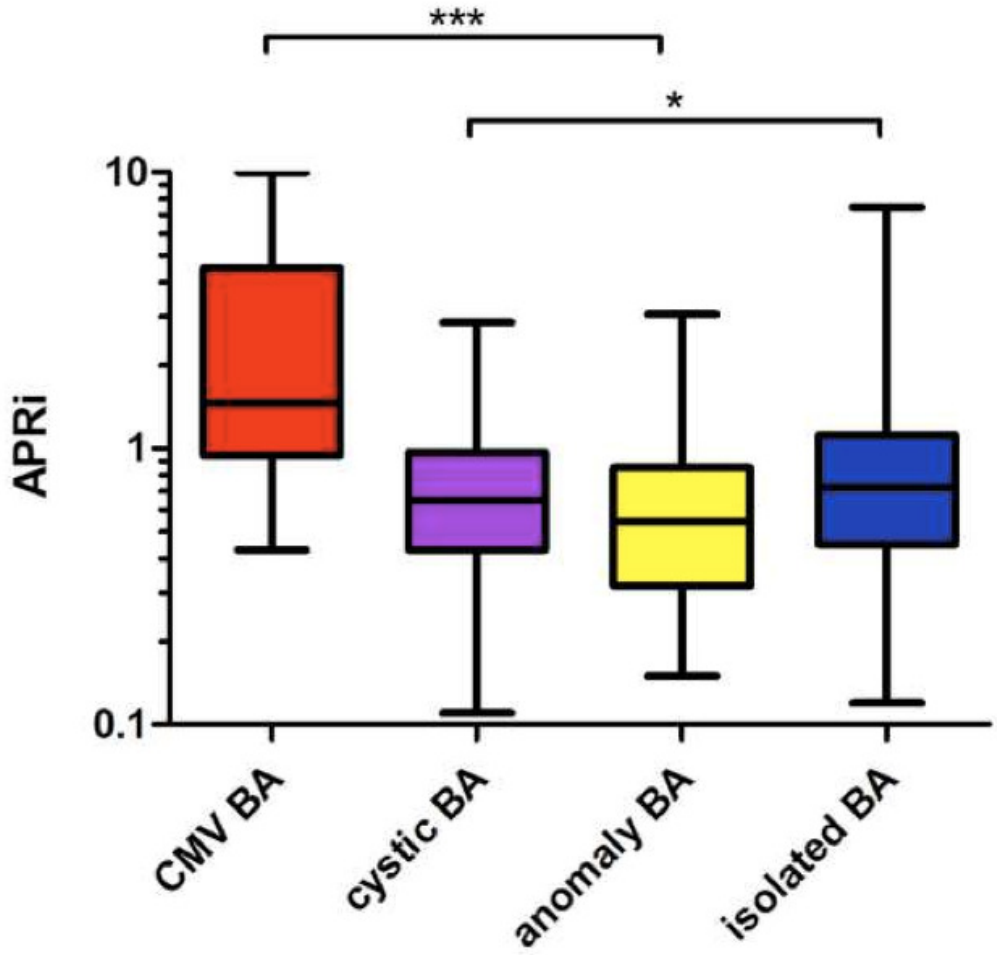


FIGURE 4 ROC of APRi and prediction of clinical cirrhosis (n = 28) in biliary atresia (n = 260). AUC = 0.83 for an APRi value of 1.22 with a sensitivity of 75% and a specificity of 84%.

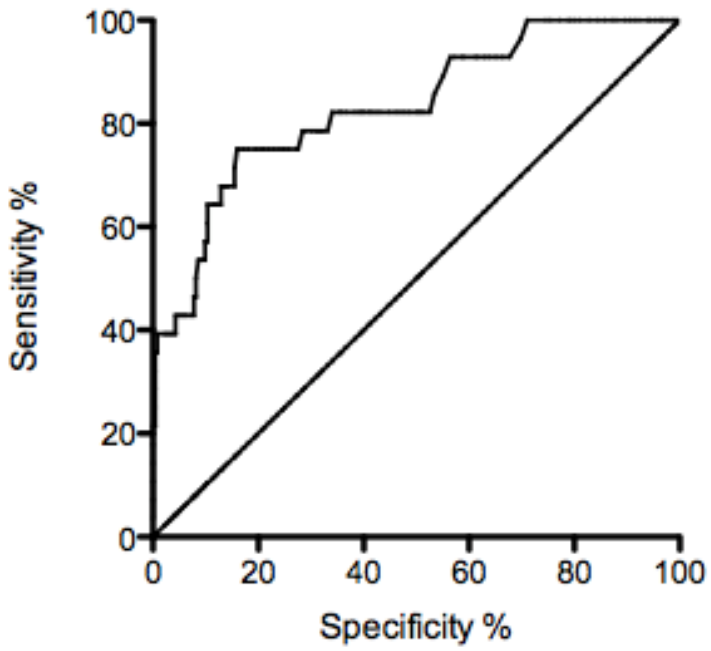


FIGURE 5 Native liver survival curves of infants with biliary atresia (n = 260): influence of APRi. Native liver survival of 1st quartile significantly better than remainder (P = 0.009).

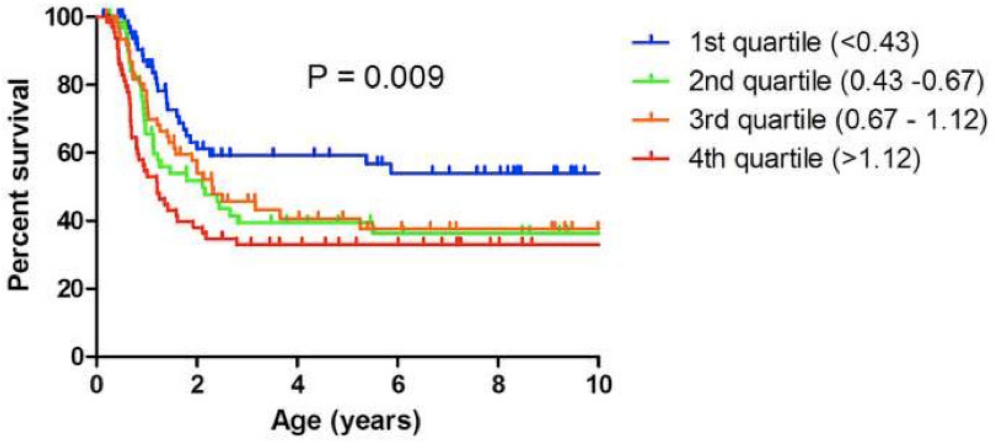
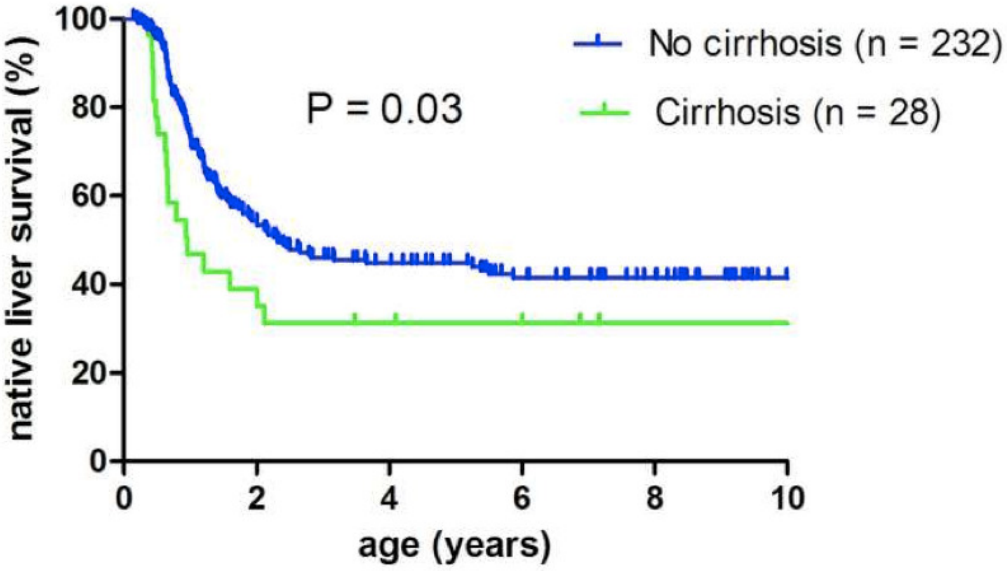


FIGURE 6 Native liver survival curves of biliary atresia (n = 260): influence of clinically evident "cirrhosis" at time of laparotomy.



Tables

TABLE 1 Characteristics of biliary atresia (n = 260)

Biliary atresia characteristic	N (%)	Age at surgery Median (IQ range)	Clinical cirrhosis
Macroscopic type			
1	2 (0.7%)	58 (45 – 71)	0
2	3 (1.2%)		1
3	255 (98.1%)		27
Clinical Variant			
Isolated	171 (65.7%)	60 (47 - 72)	17
CMV IgM +ve	15 (5.8%)	70 (61 - 81)	3
Cystic biliary atresia	27 (10.4%)	47 (35 – 54)	2
Anomaly associated (including BASM)	47 (18.1%)	53 (44 – 65)	6
Anomaly associated (excluding BASM)	10 (3.8%)		*1*

TABLE 2 Relationship of clinical and biochemical variables with APRi at time of Kasai portoenterostomy

Variable	Value Median (IQ range)	Correlation Coefficient (r_s)	P value
Age at KPE	58 (45 – 71) days	0.43	< 0.001
Liver Biochemistry			
Bilirubin (µmol/L)	157 (130 – 194)	0.44	< 0.0001
AST	188 (130-277)	0.83	<0.0001
γGT (IU/L)	527 (295 – 963)	- 0.15	0.01
Alkaline phosphatase (IU/L)	527 (404-701)	0.34	<0.001
Haematology			
Platelet count	536 (402-691)	-0.63	<0.0001
Clinical			
Spleen Size (cms)	5.9 (5.2-6.6)	0.48	< 0.0001