Biliary Atresia at Red Cross War Memorial Children's Hospital: A retrospective descriptive study reviewing the age of presentation, clinical course and outcome of infants presenting to RCWMCH with biliary atresia.

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1. DECLARATION

I, Dr. Lindsey Nicola Levin, hereby declare that the work on which this mini dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part thereof has been, is being or is to be submitted for another degree in this or any other university.

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2. RESEARCH PROPOSAL: BILIARY ATRESIA AT RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL

Biliary Atresia at Red Cross War Memorial Children's Hospital: A retrospective descriptive study reviewing the age of presentation, clinical course and outcome of infants presenting to RCWMCH with biliary atresia.

INVESTIGATORS

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BACKGROUND

Biliary atresia is a progressive obstructive cholangiopathy of unknown aetiology, occurring during the perinatal period. If left untreated it progresses to liver fibrosis and cirrhosis in the first few months of life, with death occurring in the first few years (1). It is the leading cause of end-stage liver disease in the paediatric population and remains the most common indication for liver transplantation in children (2). The current surgical management of biliary atresia involves hepatic portoenterostomy (Kasai procedure) to re-establish bile drainage, thus delaying the progression of fibrosis, with subsequent liver transplantation still required in many cases (3).

The Kasai procedure was introduced in Japan in 1959 (3). The procedure aims to construct a new bile drainage system and if successful, increases survival and postpones liver transplantation. However, long term survivors are prone to develop complications, most commonly cholangitis, or liver cirrhosis leading to portal hypertension and oesophageal varices. Successful Kasai (re-establishment of bile flow) is much more likely if performed within the first 8 weeks of life, making early referral and prompt evaluation of suspected cases essential (4). A Canadian series found that patients receiving initial sub speciality care after 90 days of age had worse outcomes following Kasai operation (2).

The prevalence of biliary atresia is estimated as 1 in 20 000 live births (3). Since the global acceptance and utilisation of the Kasai procedure, many international studies have looked at the

outcomes and long term survival of post Kasai patients. Most of these studies have been conducted across Europe and North America, with very little research available in developing countries.

A French national series reported the outcomes of Western countries to show short-term clearance of jaundice in 50-60% of cases, with 30-40% of patients reaching 10 years with their native livers and a third of patients reaching 20 years of age (1).

A Canadian series conducted from January 1, 1985 to December 31, 2002 found 4- and 10-year survival rates of 77% and 75% respectively (2). The French national survey from 2003 to 2009 reported a 5-year overall survival of 89% (1). The Japanese registry for 1989 to 1994 documented 73.5% patient survival at 5 years (5). A study conducted across the United Kingdom and Ireland from 1993 to 1995 revealed an overall survival rate of 85% at 5 years (6).

Delayed presentation and late referral remain a major problem in the management of biliary atresia globally. Efforts aimed at promoting earlier referral through the creation of biliary atresia awareness at the primary health care level may play an important role in improving outcomes and survival post Kasai surgery. The United Kingdom has instituted a “yellow alert” educational programme aimed at expediting the referral of jaundiced infants. In Asia, a stool colour card is given to all mothers on discharge from postnatal services (2).

A report of the experience in Southern Nigeria showed that late presentation and lack of resources pose major problems in the management of biliary atresia with only 62.5% of patients undergoing Kasai procedure with a mean age of death at 14.2 months (7).

A previous study conducted in the surgical department at the Red Cross War Memorial Children’s Hospital (RCWMCH) reviewed 39 children undergoing Kasai procedure between January 1975 and January 1985 (8). The mean age of operation was 12.8 weeks. In the first 4 years of the study no patients established successful bile drainage. Thereafter 50% of Kasai procedures were successful in establishing bile drainage, however only 20% of these patients remained alive at the time of analysis.

These results are far below those seen in international studies. More recent statistics are not available at the RCWMCH. We are thus unable to compare our outcomes with current international experience.

Biliary atresia places a significant burden on hepatobiliary services. A review of liver transplantation at The Wits Donald Gordon Medical Centre in Johannesburg, South Africa reported biliary atresia as the most common indication for liver transplantation in paediatrics (9) and it accounts of 57% of liver transplants performed at RCWMCH (10, 11).

The purpose of this research is to review the current experience of biliary atresia at RCWMCH. Focus will be placed on the age of presentation, age at intervention, course of progress and outcomes of patients following Kasai operation. It is hoped that we will see a reduction in the mean age of surgery and improvement in the success of Kasai procedures since the earlier study. An awareness of the current management practices and outcomes within our referral system may identify areas of need in order to facilitate earlier referral and investigation of vulnerable patients.

AIM

To review the age of presentation to hospital, course and outcome of children presenting to or referred to Red Cross War Memorial Children's Hospital with biliary atresia.

OBJECTIVES

1. To document the mean age of referral/presentation to RCWMCH in to order to ascertain whether patients accessing primary health care facilities or general practitioners are being referred to tertiary services timeously.
2. To ascertain the mean age of Kasai (portoenterostomy) once within the RCWMCH system.
3. To establish and document the outcomes following Kasai procedures at RCWMCH.
4. To establish and document whether the age at Kasai procedure influences outcome of patients at RCWMCH.
5. To identify prognostic factors associated with poor outcome after Kasai procedure.

Outcomes

Outcomes will be assessed by looking at complications following Kasai and current survival rates. Complications looked at will include episodes of cholangitis, varices, ascites and bacterial peritonitis. Successful Kasai will be defined as complete clearance of jaundice with serum bilirubin < 20μmol/L within 3 months of Kasai procedure, as well as the presence of pigmented stools. Survival will be expressed as 2 year and 5 year survival rates, including overall survival, survival with native liver and survival post-transplant.

METHODOLOGY

1. STUDY DESIGN

Retrospective folder review

2. STUDY POPULATION

Patients managed for biliary atresia at RCWMCH during the period 2003-2013. This is estimated to be approximately 75-100 cases.

Inclusion Criteria:

1. All patients in whom a diagnosis of biliary atresia has been made by clinical, biochemical and radiological grounds with surgical findings and liver histology consistent with biliary atresia
2. Children with confirmed biliary atresia who did not undergo Kasai operation will be included in the study

Exclusion Criteria:

1. No records available
2. Other causes of neonatal cholestasis

3. DATA

Cases will be identified by a Clinicom search using the ICD 10 code for biliary atresia (Q44.2) within the defined study period. The Gastro-Intestinal Unit departmental database of liver biopsies and histology, as well as the RCWMCH transplantation register will be consulted. Medical records of identified patients will be retrieved for data collection.

Data will be collected from patient files by the primary investigator only. A numerical identifier will be allocated to each case. Data collected will be captured on appropriate data collection sheets (Appendix 1), transcribed for digital storage and analysed using Microsoft excel.

Data will include the following:

Demographics: Date of birth, gender, race, age at first presentation to Red Cross, source of referral and number of visits to primary care before referral.

Presenting features: reason for presentation, presence or absence of jaundice, stool colour, presence or absence of associated congenital abnormalities

Diagnostic Studies: ultrasound, cholangiogram and biopsy

Clinical and laboratory information:

The following values will be collected at presentation, Kasai, 3 months post Kasai and annually thereafter.

1. Growth Parameters: weight, height (expressed as Z scores)
2. Laboratory values: Bilirubin (total and conjugated), AST/ALT, ALP/GGT, Albumin, FBC, INR.

Age at Kasai procedure

Complications post Kasai: Cholangitis, varices, ascites, growth failure and bacterial peritonitis

Outcomes: clearance of jaundice, progression to requiring transplantation, 2 and 5 year survival

4. ANALYSIS

This will largely take the form of a descriptive study. Data will be analysed by standard statistical methods using appropriate analytical software for statistical analysis. For descriptive statistics, continuous variables will be expressed as means ± SD (for normally distributed variables) or medians and interquartile ranges. Survival analysis will be by way of Kaplan-Meier curves. Where 2 groups are being compared using the log-rank test, a p-value of 0.05 will be regarded as significant. Statistical support will be obtained from the UCT School of Public Health prior to data analysis.

5. DISSEMINATION PLAN

Data will be presented at the SCAH Research Day, as well as at content relevant congresses.

ETHICAL CONSIDERATIONS

Potential risks and discomforts:

No risk is anticipated with this study, as it is a retrospective study and no physical contact with the patient is required. No adverse effects are foreseen in any subject as a result of this study.

Potential benefits:

Potential benefits include improving the delivery and outcomes of Kasai procedure by identifying strategies for earlier referral and diagnosis of infants with biliary atresia. The study further offers the opportunity for RCWMCH to benchmark its treatment outcomes against available international standards.

Confidentiality:

Confidentiality of individual patients will be assured since the data will only be analysed by the principal investigator. All patient data captured will be anonymised by assigning a numerical identifier.

Informed Consent:

As this is purely a retrospective study, informed consent from each patient is not required.

Ethical Approval:

Approval will be obtained from SCAH Ethics Committee at RCWMCH as well as the University of Cape Town Faculty of Health Sciences Ethics Committee.

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3. LITERATURE REVIEW

METHODS

Objectives of literature review

The objective of this study is to describe the current experience and outcomes of biliary atresia managed at Red Cross War Memorial Children's Hospital (RCWMCH).

The main objective of this literature review is to identify studies that would be useful in comparing our data to other cohorts, in both developed and developing countries.

There is little information available in an African context, so recent work from other developing countries is valuable in assessing South African data.

Literature Search Strategy

An internet search, using the PubMed and Africa-Wide Information databases was undertaken. Terms used in the search were Biliary Atresia AND Kasai AND Outcome.

The PubMed search revealed 178 articles with limits set as Humans, English, Full text, Child: birth-18years. Titles and abstracts of articles were reviewed. 9 articles did not relate to biliary atresia and were immediately excluded. Articles were allocated to 3 groups of interest, namely, those describing international cohorts of biliary atresia with outcomes, long-term survivor experience and the effect of age at Kasai portoenterostomy. Surgical research pertaining to anatomical patterns, surgical techniques, re-do Kasai procedures, post-operative protocols and transplantation techniques were not reviewed. Research into laboratory indices as predictors of outcomes was also excluded. 40 relevant abstracts where identified, however 2 of these could not be retrieved in full text. A further 4 articles were identified from the references of these texts.

A separate search was done for Biliary Atresia AND South Africa. This revealed 15 results, none of which discussed outcomes of Kasai. 3 of these papers were selected, which discussed the experience of liver transplantation services in South Africa. 1 article known to have been previously published from RCWMCH which was not identified via the PubMed search was retrieved individually.

The Africa-Wide Information database revealed 9 articles. Duplicated articles from the previous searches were excluded. 3 original articles were identified and retrieved for analysis.

In total 41 of these articles were used in this literature review.

LITERATURE

Biliary atresia is a progressive obstructive cholangiopathy of unknown aetiology, occurring during the perinatal period. If left untreated it progresses to liver fibrosis and cirrhosis in the first few months of life, with death occurring within 2 years (1, 2). There is a wide variation in incidence across the globe, ranging from the highest incidence of 1 in 5000 live births occurring in Taiwan to 1 in 20 000 live births in Northern Europe (1, 3-8). Despite these variations it is the leading cause of end-stage liver disease in the paediatric population and remains the most common indication for liver transplantation in children (1, 9). The current surgical management of biliary atresia involves hepatic portoenterostomy (Kasai procedure) to re-establish bile drainage, thus delaying the progression of fibrosis, with subsequent liver transplantation still required in many cases (10).

Biliary atresia can be described in 4 broad groups: Biliary atresia splenic malformation syndrome (BASM), Cystic biliary atresia (CBA), Cytomegalovirus-associated biliary atresia and isolated biliary atresia (4). BASM and CBA are considered “developmental biliary atresia” in which the onset of biliary occlusion is prenatal and evident by the time of birth. These carry a worse prognosis (4). Isolated biliary atresia accounts for 80-90% of cases (1) and may vary in time of presentation, level of obliteration of the biliary tree and degree of inflammation present (4).

Biliary obstruction is classified into 3 anatomical types according to the Japanese Association of Paediatric Surgeons, based on the most proximal level of occlusion in the extrahepatic biliary tree (4, 11). In type I obstruction is present at the level of the common bile duct, type II involves obstruction of the common hepatic duct and in type III there is obstruction of the entire biliary tree (11). Type III is the most common type (accounting for 90% of cases) and carries the worst outcome (4, 11, 12).

The Kasai portoenterostomy (KP) was first described by Morio Kasai and was introduced in Japan in 1959 (4, 10). However, it was only accepted in international practice in the 1970s (13). The procedure involves excision of the gallbladder and extrahepatic biliary tree to expose the porta hepatis. A loop of jejenum is then anastomosed to the cut surface to create a bilio-intestinal conduit (1, 4).

The procedure aims to construct a new bile drainage system and if successful, prolongs survival with native liver (SNL). Complete clearance of jaundice (defined at total bilirubin < 20μmol/L) post KP is achievable, with restoration of excretory and synthetic liver function (1, 4). However, long term survivors are prone to develop complications, most commonly cholangitis and portal hypertension. Even those with successful drainage post Kasai will continue to develop fibrosis or cirrhosis. Ascites and variceal haemorrhage can complicate portal hypertension and will require prompt treatment. Long-term survivors with native liver are at an increased risk of malignancy including hepatoblastoma, hepatocellular carcinoma and cholangiocarcinoma (1).

The Kasai procedure can be viewed as a palliative rather than a curative procedure, with the aim of delaying the development of cirrhosis and allowing time for growth prior to liver transplantation (1). Progression to chronic liver disease will still occur in 70% of patients in whom successful bile drainage is established, although the rate of progress varies. Time to transplant varies, but without establishment of biliary drainage is usually required within 6 months to 2 years (1, 4). Many studies have focused on trying to predict success of Kasai surgery and factors contributing to long-term survival with native liver.

Age at Kasai surgery

Emphasis is placed on early recognition, referral and intervention to optimise outcome. Liver fibrosis is a time-dependent process and it is thus only logical that the age at which Kasai is performed should affect its success rate (14). An age cut off of 60 days was previously suggested as a critical time, beyond which the success of Kasai rapidly declines (15), with primary liver transplantation recommended in patients presenting beyond this point (1, 16). Subsequent studies have failed to substantiate this notion and do not recommend a strict cut off in terms of offering Kasai. Unfortunately many patients still present beyond 60 days of life, with the best management in this scenario remaining controversial.

Davenport et al (2004) reported on 35 patients who underwent Kasai beyond 100 days of life (17). The 5-year and 10-year survival with native liver rates (SNL) were 45% and 40% respectively. He

suggests that no clinical test can be used to predict the success of Kasai without actually preforming the procedure.

Scheon et al (2001) in the United States concluded that there was no contraindication to performing Kasai beyond 75 days of life (16). This report looked at 31 children undergoing Kasai at the Children's Healthcare Centre of Atlanta. Those in whom Kasai was performed at or before 75 days had 52% rate of clearance (defined as bilirubin < 2mg/dL), whereas those undergoing Kasai beyond 75 days had a 83% success rate. This study only describes patients who underwent KP and does not offer any information on patients who may not have been offered KP. KP was only performed in 6 patients beyond 75 days of age, it is not known whether other patients beyond 75 days who may have presented with more advanced portal hypertension and cirrhosis were declined KP. The selection of patients with less severe clinical disease and complications may have contributed to the high success rate seen in this age group. Notably 4 patients underwent Kasai between 90 and 120 days, all of these were successful in clearing jaundice.

Wong et al (2010) reviewed 103 patients between 1980 and 2008 (15). Successful outcome was defined as clearance of jaundice (<20μmol/L) without the need for transplantation in the first year after surgery. Patients were stratified into 4 groups: Kasai performed < 60 days (success 53.7%), 61-80days (success 81.5%), 81-100 days (success 55.6%) and > 100 days (no successful cases). This study considered 100 days to be the point at which irreversible liver damage had already occurred.

Chen et al (2012) reviewed 452 infants with Type III biliary atresia and stratified age of Kasai in 3 age categories: < 60 days (success 39.7%), 60-90 days (success 41.8%) and > 90 days (success 36.1%) (18). (Success was defined as bilirubin < 20μmol/L.)

Nio et al (2010) reviewed 242 with Type III biliary atresia undergoing Kasai, at an average age of 79.7 days (19). Patients were allocated to 6 groups progressing in 30 day intervals, from group 1 being those who underwent Kasai less than 31 days and group 6 those in whom Kasai was performed beyond 150 days of life. None of the 8 procedures performed beyond 150 days were successful

(bilirubin < 2mg/dL). This study found that age at Kasai had a significant impact on clearance of jaundice, but not on SNL provided Kasai was done before 4 months of age (19).

The above research concluded that age is not the sole factor in determining prognosis and that Kasai surgery should not be denied on age criteria alone.

In contrast Serinet et al (2009), Tiao et al (2007) and Zhen et al (2015) considered age of Kasai to be an important determinant of success.[1]

Serinet et al assessed the impact of age at Kasai operation on its results in late childhood and adolescence (20). The study reported the best results if Kasai was performed before 30 days of life, however late Kasai operations (beyond 90 days) still carried a 15-year SNL of 13.4%.

Taio et al described 93 patients undergoing Kasai in a single-centre in Taiwan from 1986-2005 and compared those performed before and after 60 days of life (21). They found a significant difference in the jaundice-free survival rates (48% vs. 27%).

Zhen et al looked at designing an early scoring system to predict the early outcomes of Type III biliary atresia after Kasai operation (11). They showed the rate of early cholangitis, age at Kasai, time to clear jaundice, post-operative bilirubin and transaminases and surgical method used differed significantly between the survivor and non-survivor group at 2 years post Kasai. Early cholangitis was reported as the most powerful independent factor for prognosis. Tiao's study reported no significant difference in 3 and 5-year SNL between patients with and without cholangitis (21).

These figures suggest that an arbitrary age cut off should not be applied when offering Kasai procedure. There is still a significant chance of delaying liver transplantation even when performing Kasai beyond 100 days of life. Liver transplantation is more successful if performed later in life (16). Infant donor livers are in short supply and tend to have worse function than livers from older donors. Transplantation is technically more difficult in small children and carries higher complication rates. It is

[1] References provided below

therefore preferable to delay transplantation for as long as possible to allow for growth and development prior to transplantation, in order to achieve better success.

Nonetheless, the aim should still be towards prompt diagnosis and management of biliary atresia. The Canadian series (2007) found that patients receiving initial sub speciality care after 90 days of age had worse outcomes following Kasai operation (9). Data from the French National Series (2013) (2) and the Swiss National Study (2008) (6) both showed a strong correlation between age at Kasai and SNL.

Centralization of surgical care

Research in the United Kingdom showed that the outcome of children with biliary atresia is related to the caseload of the surgical centre performing KP, with centres performing more than 5 Kasai operations per year yielding better SNL and overall survival rates (7). In response, in 1999 3 designated centres were established where both Kasai operation and liver transplantation can be performed.

Davenport et al has subsequently reviewed the outcome of these policy changes, publishing their findings in 2004 and 2011 (14, 22). Both papers reported a median age at Kasai of 54 days with initial clearance rates (defined as bilirubin < 20μmol/L within 6 months of surgery) above 50%. In 2004 the 4-year SNL was estimated as 51%, with an overall survival of 89%. The 2011 article reports 5- and 10-year SNL as 46% and 40% respectively, with overall survival rates of 90% and 89%. These outcomes are a significant improvement on figures from the UK and Ireland reported for 1993-1994, with 5- and 10-year SNL of 30% and 44% respectively (7, 23).

The promising results of centralization of services were not reflected in Scotland, as reported by Tayler et al in 2013 (5). As none of the designated centres are located in Scotland, they attributed this to lengthy workups delaying referral for definitive management.

Serinet et al (2006) compares the decentralized policy of care in France with the centralized services in the United Kingdom (24). Overall 4-year survival between France and UK were comparable at

87.1% and 89% respectively. However, 4-year SNL was lower in the French data (42.7% compared to 57%). This was attributed to suboptimal results in the French centres performing 2 or less Kasai operations per year.

Schreiber et al (2010) reviewed the effect of centre caseload in Canada and reported no effect on biliary atresia outcomes between centres (25). However, this study only reported a 4-year SNL of 39%, which is significantly lower than the UK figures.

Anatomical malformations

Davenport et al (2008) compared the aetiology of biliary atresia and outcomes of KP in 225 cases (26). Patients were divided into 3 aetiological groups: isolated biliary atresia, BASM and CBA. The developmental biliary atresia's presented earlier at a median age 47 days, versus a median age a 58 days in the isolated biliary atresia group. Overall 56% achieved successful drainage post-Kasai with a 65% 2-year SNL. Infants with isolated biliary atresia showed no statistical difference in jaundice clearance or in SNL across the age cohorts. However, there was a marked detrimental effect of age at Kasai in the BASM and CBA groups.

In terms of isolated biliary atresia types I and II, carry a better prognosis than Type III (4).

International Cohorts/Outcomes

The success rates of Kasai operation, as well as long-term survival in biliary atresia vary within different centres worldwide, with many international studies looking at these outcomes.

A French national series reported the outcomes of Western countries to show short-term clearance of jaundice in 50-60% of cases, with 30-40% of patients reaching 10 years with their native livers and a third of patients reaching 20 years of age (2).

A Canadian series conducted from January 1, 1985 to December 31, 2002 found 4- and 10-year survival rates of 77% and 75% respectively (9). The French national survey from 2003 to 2009 reported a 5-year overall survival of 89% (2). The Japanese registry for 1989 to 1999 documented

75.3% patient survival at 5 years (27). A study conducted across the United Kingdom and Ireland from 1993 to 1995 revealed an overall survival rate of 85% at 5 years (7). A multi-centre study in the United States from 1997 to 2000 reported 56% SNL at 2 years, although this study had a shorter period of follow up than those conducted in other countries (28).

The following table summarises the age of Kasai surgery, jaundice clearance rates and survival amongst differing cohorts worldwide. (Table 1)

Table 1:

Country *Series*	**Period**	**n**	**Age at KP** [a]	**Jaundice clearance** [a/b]	**Survival native liver**		**Overall Survival**	
					4/5yr	10yr	4/5yr	10yr
Japan *Nio et al, 2003* (27)	1989-1999	1381		62% [b]	59.7%		75.3%	
Taiwan *Hsiao et al, 2008* (29)	2004-2005	75	55	59% [c]				
UK *Davenport et al, 2011 (14)*	1999-2009	443	54	55% [b]	46%	40%	90%	89%
France *Serinet et al, 2006 (24)*	1997-2002	271	57	39.5% [c]	42.7%		87%	
Switzerland *Wildhaber et al, 2008 (6)*	1994-2004	48	68		37.4%		91.7%	
Netherlands *De Vries et al, 2012 (8)*	1998-2008	104	59	38% [b]	42%		76%	
Canada *Schreiber et al, 2007 (9)*	1985-2002	349	65		33%		77%	
US *Shneider et al, 2006 (28)*	1997-2000	104	61		55.8% (2yr)		91.3% (2yr)	

[a] Reported as days in median/mean
[b] Defined as achieving a level of less than 20µmol/L (or 1.5mg/dL)
[c] Defined as achieving a level less than 2mg/dL

Long-term outcomes

Various authors have looked at the long-term outcomes of biliary atresia survivors. Bijl et al (2013) performed a systematic review of patients living beyond 20 years with their native livers (10). This review found 184 patients alive above the age of 20 years, 88% of these patients still had their native liver. Of those 60.5% suffered from liver related complications: 100% had experienced episodes of

cholangitis, 80% had portal hypertension with 45% of these having experienced an episode of gastrointestinal bleeding.

Another systematic review conducted by Jimenez-Rivera et al (2012) found that 10-year overall survival ranged from 66.75 to 89% (30).

Nio et al (1997) reviewed the outcomes of long-term survivors in Japan (31). Of 85 patients alive more than 10 years on from Kasai, 72 (84%) remained jaundice free.

Shinkai et al (2009) reviewed 80 patients undergoing Kasai at a single-centre in Japan (32). Survival rates with native liver were 63%, 54% and 44% at 5-, 10- and 20-years respectively. Of the 35 patients alive with native liver beyond 20 years, 71% had normal bilirubin levels, although 47% had already been diagnosed with cirrhosis and portal hypertension. This is not surprising as those with successful KP and clearance of bilirubin are more likely to survive. Two patients in this review died of liver failure and 5 underwent transplantation in their twenties.

Hadzic et al (2002) and McKiernan et al (2007) have reported on long-term experience in the UK. Hadzic reviewed 244 patient records and found 11% of patients with no features of chronic liver disease 10 years post Kasai (33). Despite normal liver function and good quality of life, 40% of these patients still had biopsy evidence of cirrhosis. The outcome in these patients was not influenced by isolated episodes of cholangitis.

McKiernan followed 91 patients receiving Kasai between 1993 and 1995 for 13 years (23). Of the 50 successful KP's, 80% of these patients were still alive with native liver, with a 13-year actuarial survival rate of 82.5%. KP was unsuccessful in 41 of the original patients. All of these had either died or received a liver transplant at the time of follow up. 13-year SNL was 43.8% and overall survival 83.8% for the entire group.

20 year SNL is possible, but the majority of these patients have complications of chronic liver disease, requiring close follow up and on-going management.

Experience in developing countries

Despite a wealth of information from developed countries, very little information is available in Africa and other developing nations.

Mshelbwala et al (2007) described the challenged in Nigeria (34). They reported 14 cases of biliary atresia, with a median age at presentation of 16 weeks. 7 of these patients had presented to primary health care services with neonatal jaundice, but were not referred for further investigation. 11 of the patients already had cirrhosis and coagulopathy at the time of presentation, the remaining 3 patients were fit for Kasai operation. 1 of these patients was well post Kasai, but died of gastroenteritis at 2 years; 1 died from cholangitis 8 weeks post Kasai and 1 did not recover from anaesthesia at the time of Kasai.

A subsequent report of the experience in Southern Nigeria by Okoro et al (2013), showed that late presentation and lack of resources still pose major problems in the management of biliary atresia, with only 62.5% of patients undergoing Kasai procedure and a mean age of death at 14.2 months (35). Liver transplants are not performed in Nigeria, which contributes to the poor overall survival.

Aydogdu et al (2004) reviewed 27 patients with biliary atresia in Turkey, the median age at diagnosis was 63.5 days with median age at Kasai 67.5 days (36). Only 2 out of 25 Kasai operations performed were successful (bilirubin < 20μmol/L). Kasai operations were carried out at various hospitals and only referred to specialist liver unit post-operatively; this is likely to contribute to the low success rate. Overall 5 year survival was 10%, far lower than in other European cohorts.

Lee et al (2009) described the outcomes of 57 patients with biliary atresia at a single-centre in Malaysia (37). The median age at presentation was 62 days. 48 patients underwent Kasai at a median age of 70 days, with a success rate of 44% (bilirubin < 20μnol/L within 6 months). 2-year SNL and overall survival were 37% and 40% respectively. Liver transplantation is not available in the Malaysia, accounting for the low overall survival.

Reasons for late diagnosis in all of these reports were attributed to lack of parental insight and poor health seeking behaviours, geographical and financial accessibility of health care, repeated reassurance by medical staff without appropriate referral or investigation of neonatal jaundice and limited access to diagnostic procedures (35-37).

South African Experience:

A previous study conducted in the surgical department at the Red Cross War Memorial Children's Hospital (RCWMCH) reviewed 39 children undergoing Kasai procedure between January 1975 and January 1985 (12). The mean age of operation was 12.8 weeks. In the first 4 years of the study no patients established successful bile drainage. Thereafter 50% of Kasai procedures were successful in establishing drainage, however only 20% of these patients remained alive at the time of analysis.

These results are far below those seen in international studies. More recent statistics are not available at the RCWMCH. We are thus currently unable to compare our outcomes with those of international cohorts.

Biliary atresia places a significant burden on hepatobiliary services. A review of liver transplantation at The Wits Donald Gordon Medical Centre in Johannesburg, South Africa reported biliary atresia as the most common indication for liver transplantation in paediatrics and it accounts of 57% of liver transplants performed at RCWMCH (38-40).

The Role of Screening:

Delayed presentation and late referral remain a major problem in the management of biliary atresia globally. Efforts aimed at promoting earlier referral through the creation of biliary atresia awareness at the primary health care level may play an important role in improving outcomes and survival post Kasai surgery. The United Kingdom has instituted a "yellow alert" educational programme aimed at expediting the referral of jaundiced infants. In Asia, a stool colour card is given to all mothers on discharge from postnatal services (9, 29, 41).

The stool colour card was established in Taiwan in 2004 and comprises 6 photographs of infant stool. 3 colours of stool are labelled as abnormal, namely clay coloured, pale yellow and light yellow. Contact details are available on the card for a stool card registry centre which responds to all reports within 24 hours with advice and appointment date if indicated.

Since the introduction of this screening method biliary atresia has been diagnosed earlier with the percentage of patients undergoing Kasai before 60 days improving from 49.4% to 65.7%. Jaundice clearance post Kasai has improved from 34.8% to 60.8%, with 5-year jaundice free SNL improving from 27.3% to 64.3% (41).

What this research will offer:

Davenport suggests that a jaundice clearance rate of 50% should be the benchmark for institutions offering Kasai, with survival rates of 90% attainable in countries with access to liver transplantation (4).

We are currently unable to measure our experience at RCWMCH against the international standards described above. The purpose of this research is to review the current experience of biliary atresia at RCWMCH. It is hoped that we will see a reduction in the mean age of surgery and improvement in the success of Kasai procedures since the earlier RCWMCH study. An awareness of the current management practices and outcomes within our referral system may identify areas of need in order to facilitate earlier referral and investigation of vulnerable patients.

Focus will be placed on 3 important aspects which will assess the efficacy of different stages in our management pathway.

The median age at Kasai reflects effectiveness of primary health care services in identifying and referring suspected cases of biliary atresia. The diagnostic process in secondary and tertiary institutes also plays a role in improving the age at which Kasai is performed.

The percentage of jaundice clearance is a reflection of surgical expertise within the centre. Long-term survival with native liver reflects both surgical practice, as well as the level of on-going medical care post Kasai. Accessibility of services, which is known to be a problem in our context, will also impact on long-term survival.

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4. MANUSCRIPT IN PUBLICATION READY FORMAT

TITLE PAGE

TITLE: BILIARY ATRESIA EXPERIENCE IN SOUTH AFRICA: CLINICAL COURSE AND OUTCOME OF PATIENTS AT RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL

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ABSTRACT

Background

Biliary atresia (BA) is the leading cause of end-stage liver disease in the paediatric population and remains the most common indication for paediatric liver transplantation in South Africa.

Objectives

This study aimed to describe the age of presentation, clinical course and outcome of infants presenting to Red Cross War Memorial Children's Hospital (RCWMCH) with BA.

Methods

A retrospective folder review was conducted on all patients with BA presenting to RCWMCH between January 2003 and December 2013. The main outcomes assessed were median time to presentation to tertiary services, clearance of jaundice post Kasai procedure (bilirubin <20μmol/L) and 2- and 5-year overall survival (OS) and survival with native liver (SNL).

Results

The median age at presentation in the 80 cases reviewed was 70 days. Kasai procedure (KP) was performed in 62 (77.5%) patients at a median age of 68 days. 18 patients who presented late did not undergo KP. Clearance of jaundice was achieved in 39% of KPs. 13 patients underwent KP beyond 90 days with a success rate of 38%. 2- and 5-year SNL rates were 41% and 37.5% respectively with OS of 59% at 2-years and 56% at 5-years. Liver transplant was only performed in 12 of the 54 patients who showed progression to require transplantation.

Conclusions

Jaundice clearance post KP and SNL compared favourably with international figures, however, lower overall survival rates reflected lack of access to transplantation. Age at KP was not a predictor of poor outcome.

Key Words

Kasai portoenterostomy, developing nation, cholestasis, age at operation

What is known	What is New
• Short-term clearance of jaundice has been achieved in 50-60% of cases in developed countries. • Emphasis has been placed on early recognition, referral and intervention to optimise outcome. • Previously 60 days was recommended as a cut off for performing KP, currently much debate exists around the benefits of performing late KP.	• Little information is available in the developing world. • This provides valuable insight into what is achievable in a resource limited setting. • A significant proportion (38%) of patients benefited from KP performed beyond 90 days.

INTRODUCTION

Biliary atresia (BA) is a progressive obstructive cholangiopathy of unknown aetiology, occurring during the perinatal period. If left untreated it rapidly progresses to hepatic fibrosis and cirrhosis, with death occurring within 2 years (1, 2). It is the leading cause of end-stage liver disease in the paediatric population and remains the most common indication for liver transplantation in children (1, 3). The current surgical management of biliary atresia involves Kasai portoenterostomy (KP) to re-establish bile drainage, thus delaying the progression of fibrosis, with subsequent liver transplantation (LT) still required in many cases (1, 3-5).

Although KP can improve long-term survival, progression to chronic liver disease will still occur in 70% of patients in whom successful bile drainage is established (6). The rate of progression and time to transplant varies.

Factors thought to predict success of KP and long-term survival with native liver (SNL) include age at surgery (2, 7, 8), aetiology and anatomical pattern of BA (1, 9), experience of the surgical centre (1, 10) and the degree of liver fibrosis present at the time of KP (9, 11). Data from French (2), Swiss (7) and Canadian (8) cohorts showed a strong correlation between age at Kasai and SNL. Emphasis is therefore placed on early recognition, referral and intervention to optimise outcome. Recently researchers have challenged the concept of an age cut off beyond which Kasai should no longer be

offered. Davenport et al reported a 5-year SNL of 40% in patients undergoing Kasai beyond 100 days (12).

Outcomes vary world-wide, with short-term clearance of jaundice achievable in 50-60% of cases (10, 13-16), and a 10-year SNL of 30-40% (10, 13, 17, 18) reported in literature from Western countries.

Despite a wealth of information from developed countries, very little information is available in Africa and other developing nations. The purpose of this research is to review the current experience of biliary atresia at Red Cross War Memorial Children's Hospital (RCWMCH) in South Africa with emphasis on age at presentation, age at KP and outcomes in patients following KP at our institution.

METHODS

This is a retrospective descriptive study conducted within the Department of Paediatrics, RCWMCH, Cape Town, South Africa. Patients managed within the department from January 2003 to December 2013 were included. The study was approved by the University of Cape Town Faculty of Health Sciences Ethics Committee (HREC REF: 137/2014).

Inclusion Criteria:

Patients in whom a diagnosis of BA was made on clinical, biochemical and radiological grounds with surgical findings and liver histology consistent with BA, including those who did not undergo KP were analysed. Only patients managed primarily at RCWMCH during the study period were included. Patients referred for transplant assessment after initial management at other tertiary institutions did not form part of this study.

Analysis of Records:

Cases were identified through the RCWMCH clinical data base using the International Statistical Classification of Diseases Code (ICD-10) allocated to BA. Records of attendance for the liver and transplant clinics, as well as current and old transplant waiting lists were reviewed to ensure no cases were missed. A review of all files was conducted from May to July 2014, by the primary author.

Data collected from patient charts included unique patient code, date of birth, sex, referral site, age of jaundice onset (in weeks), date of presentation, presence of congenital anomalies, presence of

jaundice and stool pigment, imaging results (ultrasound, hepatobiliary scintigraphy, intraoperative cholangiogram), whether KP was performed, date of KP, liver histology, success of KP, complications (ascites, cholangitis, varices, fractures), tuberculosis, progression to require transplantation, suitability for transplant listing, whether liver transplant (LT) was performed, date of LT, date of last visit and final outcome including cause of death. Laboratory indices (full liver function tests, albumin, haemoglobin and international normalised ratio) were recorded at time of presentation, time of KP, 3 months post KP and annually thereafter.

Liver biopsies were scored using Lee's 15 point histological scoring system for neonatal cholestasis (19), by a single paediatric histopathologist. Cirrhosis was classified as present if there was porto-portal bridging involving more than 50% of the portal tracts.

Outcome measures:

The latest outcome status was documented as alive, dead, lost to follow up or transferred to another institution. Successful KP was defined as clearance of jaundice with serum bilirubin < 20μmol/L within 3 months of surgery. Survival was expressed as 2- and 5-year survival rates, including overall survival (OS) and survival with native liver. OS was defined as beginning at birth and ending at death or last follow-up; SNL starting at birth and ending at death (based on how many patients could have reach 2 or 5 years of age by 31 July 2014).

Statistical Analysis:

All analyses were done using Stata Statistical Software Release 13 (StataCorp LP, College Stat). Ages are quoted as median and interquartile range (IQR). Fisher's exact tests were used to compare categorical data. The distribution of numerical data was assessed and the Student t-test or Mann-Whitney U test applied as appropriate. A P-value of 0.05 was regarded as significant. Survival rates were estimated using the Kaplan-Meier method.

Full data was not available in all medical records, for these variables number (n) was declared. For patients lost to follow up (LTFU) the date of their last visit was utilised when calculating survival. Cases LTFU immediately post-KP were not included when calculating success of KP.

Limitations: Anatomical classification of BA was not recorded. Data was limited by that available in medical records and short follow-up periods of the cohort.

RESULTS

152 cases were identified, 6 files were not traceable and 3 were excluded due to insufficient clinical records. Of the remaining cases, 80 were managed primarily by RCWMCH (30 male, 50 female). Sixty-three patients were referred to RCWMCH at the time of transplant assessment, after initial management at other centres and were therefore not included in the cohort.

16 (20%) had associated congenital anomalies. 4 of these involved biliary atresia splenic malformation (BASM) syndrome (2 with significant cardiac lesions: 1 with septal defects and an interrupted inferior vena cava, 1 with transposition of the great vessels and asplenia). Situs inversus was not present in any of the BASM cases. The remaining anomalies included: 4 patients with haemodynamically insignificant cardiac lesions; 3 with major congenital cardiac lesions; 3 choledochal cysts; 1 case of renal tract pathology and 1 chromosomal disorder (47XXX) with cardiac lesions.

Age at presentation and surgery:

Jaundice was noted during the first week of life in 81% of cases (57/70). The median age at presentation was 70 days, IQR 44-105.5. Sixty two patients underwent KP at a median age of 68 days, IQR 54-86. The median age at presentation of the Kasai group was 61 days, IQR 42-79. Median interval between initial presentation and KP was 8 days, IQR 5-13. Demographic data as well as initial findings and investigations are summarised in Table 1.

Patients with no Kasai Procedure: (n = 18, see outcome summary in Figure 1)

18 (22.5%) patients who were referred late did not undergo KP (median age at presentation 166.5 days, IQR 142-208). None of these patients were alive with their native livers at the time of data collection. 3 patients were LTFU, 7 died without liver transplantation and 8 patients received cadaveric liver grafts, 2 of whom have died. 1 patient died 1 month post-transplant from overwhelming sepsis, the second patient died 6 months post-transplant secondary to hepatic vein thrombosis with fulminant liver failure. The median age of death in this group was 15.1 months, IQR 13.4-17.8.

Patients undergoing Kasai Procedure: (n = 62, see outcome summary in Figure 1)

KP was performed in 62 (77.5%) of patients with outcomes depicted in Figure 1. Three patients defaulted immediately post KP and were excluded from further analysis of KP success.

Thirty six (61%) of the 59 patients with known outcomes were ultimately unsuccessful (median age at operation 76.5 days, IQR 55.5-86.5). Three patients died of complications related to the KP (2 breakdown of anastomosis progressing to sepsis, bowel/liver necrosis and multi-organ failure (MOF) and 1 bowel obstruction with sepsis and MOF). Of the remaining 33 patients with persistent jaundice post KP only 9 were alive with native liver at data collection, 10 patients had been LTFU, 3 underwent LT (1 of whom suffered from chronic rejection and died 4 years post LT), 11 died without LT.

Sixteen of the 59 patients (27.1%) established successful drainage 3 months post KP, however a further 7 (11.9%) patients established clearance beyond this point (6 by 6 months, age of clearance was not documented in 1 case). Including the cases which cleared beyond 3 months increases the overall success rate to 39% (23/59). The median age at surgery of successful KP was 57 days, IQR 51-88. Twenty of these patients were still alive with their native liver, 19 of whom remained jaundice free. 1 patient underwent successful LT at the age of 2 years 6 months. Follow up data was not available on 2 patients.

Age at Kasai

Of the 59 patients where outcome of KP was known, 25 patients (42.4%) underwent KP before 60 days of life with a success rate of 28%. Twenty-one patients (35.6%) were operated between 61 and 90 days with a success rate of 19%. An additional 13 patients (22%) underwent KP beyond 90 days with a success rate of 38%. The differences in success rates across these age categories was not statistically significant (P = 0.9). The oldest age at KP was 148 days. This procedure was successful in establishing bile drainage and the patient remained jaundice free 4 years post KP.

Cirrhosis was evident in 58.8% of patients at the time of Kasai (30/51). The median age at Kasai in those with no evidence of cirrhosis was 56 days compared to a median of 82 days in those where cirrhosis was present (P = 0.001). There was no statistical significance between the presence of cirrhosis and success of KP (P = 0.576). Of those with evidence of cirrhosis on histology 26% (7/27) still had successful outcomes of KP.

Liver Transplantation:

Of 73 patients for whom follow up data was available 54 (74%) progressed to require transplantation. Only 20 of the 54 (37%) were placed on the transplant list (16 active, 4 inactive listing), with the

remaining 34 (63%) being excluded from potential transplantation. Unfavourable social circumstances such as poor compliance with medical treatment and follow up, inadequate sanitation, parental substance abuse and distance from the transplant centre formed the main grounds for exclusion (n=16, 47% of those excluded from LT; 30% of the 54 patients requiring LT). Two patients were medically unfit to undergo LT (1 due to active tuberculosis). One patient was not from South Africa, 8 defaulted prior to completion of the transplant assessment and 4 died before they could be listed

A total of 12 patients underwent LT (15% of the total cohort, 22.2% of those requiring LT) at a median age of 31.5 months, IQR 18.9-34.2. The outcomes of these patients were described above. (Figure 1)

Clinical course and complications:

Of the 29 patients who were alive with their native liver at the end of data collection, only 3 (10.3%) were alive without complications. Cholangitis was the commonest complication occurring in 21 of these patients (72.4%), of these 13 patients suffered recurrent episodes of cholangitis. Ascites occurred in 6 of the 29 patients (20.7%). Routine endoscopy was not performed and thus varices were only diagnosed after an episode of melaena or hematemesis. Five of the 29 patients (17.2%) experienced variceal bleeds. One patient suffered 2 consecutive femur fractures. Pulmonary tuberculosis occurred in 6 patients (20.7%).

The frequency of complications for the full cohort is expressed in Table 2.

Sixty two patients underwent KP and information regarding cholangitis was not available for 2 of these patients. Of the remaining 60 patients, 44 experienced episodes of cholangitis (73.3%). Fifteen of these patients were lost to follow up and hence outcome data was only available for 45 patients. Of the patients that died during the study period, 84.6% had experienced at least 1 episode of cholangitis (11/13). Cholangitis was also seen in 71.9% of patients who ultimately survived (23/32). There was no statistical significance in the occurrence of cholangitis and the outcome of survival (P=0.467).

Reliable growth parameters were not available in many records and growth failure was thus unable to be assessed. Bacterial peritonitis was not reported in any patients.

Outcome and Survival at 2 and 5 years:

Figure 1 highlights the outcomes of the 80 patients managed in this cohort. The overall 2-and 5 year survival rates were 58.8% (30/51) and 56.3% (18/32) respectively. Native liver survival was 41.2% (21/51) and 37.5% (12/32) at 2 and 5 years respectively. See Figure 2 for Kaplan Meier survival curves.

DISCUSSION

The RCWMCH is currently the only dedicated tertiary paediatric hospital in South Africa. The hospital receives referrals from all 9 provinces within the country as well as from across African borders. Many of these patients come from poor socioeconomic backgrounds with limited access to primary health care and follow a prolonged route to tertiary referral.

All KPs performed at RCWMCH follow a standard post-operative protocol including steroids and antibiotic prophylaxis, with vitamin K and ursodeoxycholic acid commenced from day 5 post procedure. As this is the standard of care for all patients, the benefits were not assessed in this study. (Appendix attached for shared surgical/medical care package)

Liver transplantation remains a scarce resource. Transplants have been performed at RCWMCH since 1991 (20). In 2005 the Donald Gordon Medical Centre (DGMC), a privately-owned medical facility began its own transplant programme (20, 21). Currently the RCWMCH is only able to offer cadaveric LT, with the shortage of donor livers contributing to long waiting lists. Socio-economic factors play a pivotal role in the evaluation of transplant candidates. Many patients live in rural areas situated far from the transplant centre with limited access to medical facilities, especially specialised services; immunosuppressive therapy and intensive monitoring that would be required post transplantation. These families often live in extreme poverty with no electricity, inadequate sanitation, as well as high rates of malnutrition and infectious diseases (22). In this series 30% of patients requiring LT were excluded from the transplant list due to the above mentioned socioeconomic factors preventing safe transplant.

Late presentation to tertiary services remains a major problem in South Africa. The median age at presentation was 70 days in this study, much later than that reported in International Cohorts. McKiernan reported a median age at referral to surgical centre of 40 days in the United Kingdom and

Ireland (17). The United States (16) and Canada (8) have reported 53 and 55 days at referral to tertiary centres respectively. Our figures are closer to those reported in other developing countries, namely Malaysia (62 days) (23) and Turkey (63.5 days) (24). Reasons for late presentation in our setting included poor parental knowledge resulting in delayed health seeking behaviours, distance from health care facilities and financial constraints limiting access to medical services, high work-loads and lack of awareness regarding causes of prolonged neonatal jaundice amongst primary health care staff resulting in inappropriate advice being given to parents and delayed referral.

Recent research has challenged the concept of a critical time point of 60 days beyond which patients should be offered primary liver transplantation instead of KP. This series showed a median age at KP of 68 days, with an overall success rate of 39%. There was no statistical significance comparing age at KP with success of the procedure. When stratified by age the highest success rate (38%) was achieved in the group who underwent KP beyond 90 days. This figure is comparable to that reported by Chen et al which showed 39% clearance when performed beyond 90 days (25). Similar results were published by Schoen et al in the United States who showed 83% beyond 75 days (26) and by Wong et al in Hong Kong who reported 55.6% success between 81 and 100 days (11). Davenport et al reported 5- and 10-year survival rates of 45% and 40% respectively in patients undergoing KP beyond 100 days (12).

Figure 2b depicts the Kaplan Meier survival curves stratified by age at KP. In this graph the 61-90 day subgroup appears to have worse outcomes than those who underwent KP beyond 90 days. It should be considered that the Kaplan Meier method provides a crude estimation of survival without adjusting for any confounding factors. Cox-regression analysis was not possible due to the small subgroup sizes. This difference did not appear to be due to the degree of fibrosis present at the time of KP or the presence of other congenital anomalies. The apparent better performance of the > 90 days group could possibly be explained by sample bias as KP was not offered to all patients beyond 90 days. Those with clinical cirrhosis and portal hypertension did not undergo KP. These patients would have had a poor outcome if shown in this graph.

These figures suggest that an arbitrary age cut off should not be applied when offering Kasai procedure. There is still a significant chance of delaying liver transplantation even when performing Kasai beyond 100 days of life (12). KP should be considered on an individual basis in cases of late

presentation taking into account the clinical severity of disease, presence of established cirrhosis and portal hypertension. In the South African context where access to LT is limited, life expectancy and quality of life can be improved by offering KP even in those presenting beyond 90 days. In patients who fulfil the social criteria for transplantation, delaying LT benefits patients by allowing time for growth and immunisations prior to transplantation.

Definitions of successful KP vary amongst authors. Bilirubin values of <20µmol/L are the most commonly quoted (2, 9, 11, 18, 25, 27), but <2mg/dL (34µmol/L) has also been used (26, 28). Grizelj et al used a cut off of 3 months post KP to define successful clearance of jaundice (27), although other authors have been more liberal in their cut off with Davenport (9), Lee (23) and Hung (29) using 6 months and McKiernan (18) and Tiao (28) accepting the target bilirubin levels at any time post KP. When applying a cut off of 3 months in this study successful clearance of jaundice was only achieved in 27% of cases. However, if this is expanded to include the 7 cases which cleared beyond 3 months, the success rate increased to 39% which is comparable with jaundice clearance rates achieved in France (40%) (15), Netherlands (38%) (30) and US (40%) (16). This is still below those achieved in Japan (62%) (13), Taiwan (59%) (14) and the UK (55%) (10).

Cirrhosis was evident in almost 60% of patients undergoing KP. Although the presence of cirrhosis did not have a statistical impact on the success of procedure, cirrhosis was associated with increasing age at KP. Davenport et al reported that histological degree of fibrosis had no value in predicting success and furthermore that the presence of cirrhosis at the time of KP had no significant survival disadvantage, in a series reviewing the outcomes of KP beyond 100 days (12).

This cohort showed a high rate of cholangitis and complications of portal hypertension including ascites and variceal bleeding. Cholangitis was common, occurring in 73% of those who underwent KP (Table 2). This is comparable to figures seen in other literature (28, 29). Zhen et al (31) reported early cholangitis to be the single most powerful predictor for prognosis, although other authors have found no association between cholangitis and survival (16, 28). In this study cholangitis was not associated with a worse survival outcome, although the limited numbers need to be taken into account when interpreting the statistical significance of this data. It is essential that cholangitis is treated promptly

and aggressively to preserve liver function. 20% of patients in this cohort were lost from follow up. Parents should be counselled about the potential for complications and the development of chronic liver disease post KP, as life-long follow up is essential in order to delay progression of liver disease and begin appropriate transplant assessment when required.

Twenty six per cent of patients were treated for pulmonary tuberculosis (PTB) in this cohort. PTB is highly prevalent in the Western Cape and the presence of active disease is an exclusion to transplantation until fully treated. The majority of these cases were not culture confirmed, but were considered as probable PTB and therefore treated empirically. The higher number of TB cases in the non-Kasai group may be due to the effects of chronic illness and poor nutritional status on immune function.

The 37.5% 5-year SNL in this cohort is comparable with figures from Canada (33%) (8), France (40%) (2), Switzerland (37% 4-year SNL) (7), Netherlands (42% 4-year SNL) (30) and United Kingdom (46%) (10). However, the overall 5-year survival of 56% falls far below those seen internationally, which range from 77% in Canada (8) to 90% in the UK (10) and 92% in Switzerland (7). The low overall survival rates can be explained by poor access to LT with only 12% of our initial cohort undergoing transplantation, as opposed to 40% seen in the BARC (Biliary Atresia Research Consortium) cohort reported by Shneider et al (16) and 44% reported by Davenport et al in the UK (10).

A previous study conducted in the surgical department at RCWMCH reviewed 39 children undergoing Kasai procedure between January 1975 and January 1985 (32). The mean age of operation was 12.8 weeks. In the first 4 years of the study no patients established successful bile drainage. Thereafter 50% of KPs were successful in establishing drainage, however only 20% of these patients remained alive at the time of analysis. The current cohort (2003-2013) shows a dramatic improvement in age at KP which has decreased from 12.8 weeks to 68 days as well as in overall survival.

Delayed presentation and late referral remain a major challenge in the management of BA. Efforts aimed at promoting earlier referral through the creation of biliary atresia awareness at the primary health care level play an important role in improving outcomes and survival post Kasai surgery. The

most successful strategies have been the stool colour cards established in Taiwan in 2004 and the "yellow alert" educational programme in the UK aimed at expediting the referral of jaundiced infants (8, 14, 33).

Since the introduction of stool colour cards in Taiwan, BA has been diagnosed earlier with the percentage of patients undergoing Kasai before 60 days improving from 49.4% to 65.7%. Jaundice clearance post Kasai has improved from 34.8% to 60.8%, with 5-year jaundice free SNL improving from 27.3% to 64.3% (33). As Taiwan has the highest incidence of BA, screening in this population is bound to yield positive results. A US study on the cost-effectiveness of a screening programme concluded that the use of stool colour cards resulted in lower costs and better outcomes for patients with BA (34).

Differentiating physiological jaundice in the neonate from pathological jaundice is essential to facilitate early referral. Pale stools, dark urine and jaundice persisting beyond 14 days of life warrants urgent referral for further investigation (including conjugated bilirubin). In the RCWMCH cohort initial stool colour was not documented in 20% of patients, a figure which is likely to be higher at primary health care facilities. This highlights the need to improve awareness amongst both parents and all health care professionals. Although it is unlikely to be financially feasible to include stool colour cards in the current Road to Health Card Booklets, posters should be made available at antenatal services and baby clinics as part of an educational campaign. Nursing staff administering 6 week immunisations should be encouraged to enquire about stool colour. Although referral at 6 weeks would already be considered late, it would still allow for KP to be performed within 60 days and would be an improvement on our current age at presentation. Employing an algorithm for the appropriate management of conjugated hyperbilirubinaemia at peripheral services would aid in facilitating earlier referral.

This study has confirmed that patients can still benefit from undergoing KP beyond 90 days. In a country with limited access to transplantation, KP should still be offered to patients presenting late. This will allow a significant proportion (38%) of cases the opportunity for successful KP with improved

quality of life, as well as delaying the need for transplantation and immunosuppression and alleviating strain on the organ donor system.

In this study age at KP, degree of fibrosis and complications were not associated with poor outcome of KP.

In conclusion, although overall survival figures still lag behind international data, we have made enormous progress over the last 30 years within a limited resource setting. Improved awareness and earlier referral of infantile cholestasis will improve these figures further.

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Table 1: Cohort characteristics

	n* (%)	
Overall cohort	80	
Sex (n=80)		
Male	30 (37.5%)	
Female	50 (62.5%)	
Median age at presentation	70	
Median age at KP	68	
Presence of jaundice		
Yes	79 (99%)	
Not recorded	1	
Pale Stools		
Yes	61 (76.2%)	
No	3 (3.8%)	
Not recorded	16 (20%)	
Ultrasound findings (n=54)		
Consistent with BA	41 (76%)	
Not suggestive of BA	13 (24%)	
Cholangiogram (n=53)		
Consistent with BA	52 (98%)	
Not consistent with BA	1**	
Biopsy (n=60)		
Biliary atresia	56 (93.3%)	
Neonatal hepatitis	2 (3.3%)	
Cystic biliary atresia	2 (3.3%)	
Age at KP (days)***	Successful	Unsuccessful
< 31 (n=5)	1 (20%)	4 (80%)
31-60 (n=20)	6 (30%)	14 (70%)
61-90 (n=21)	4 (19%)	17 (81%)
> 90 (n=13)	5 (38.5%)	8 (61.5%)
Cirrhosis at KP (n=51)		
No	21 (41.2%)	
Yes	30 (58.8%)	

* Number of cases with information available
** Biopsy revealed biliary atresia, with subsequent cholangiogram and KP being performed
*** 3 cases LTFU immediately post KP, were not included in this analysis

Table 2: Frequency of complications (n depicts number of cohort for which information was available)

Complication	**Total incidence**	**Kasai Group**		**No Kasai Group**	
Ascites	47.3% (27% recurrent)	39.2%	n* = 56	72.2%	n = 18
Cholangitis	70.1% (40.3% recurrent)	73.3%	n = 60	58.8%	n = 17
Varices with bleed	35.1%	28.1%	n = 57	58.8%	n=17
Fractures	9.1% (4.55% recurrent)	9.8%	n = 51	6.7%	n =15
TB	26.7%	22.8%	n = 57	38.9%	n = 18

* Number of cases with information available

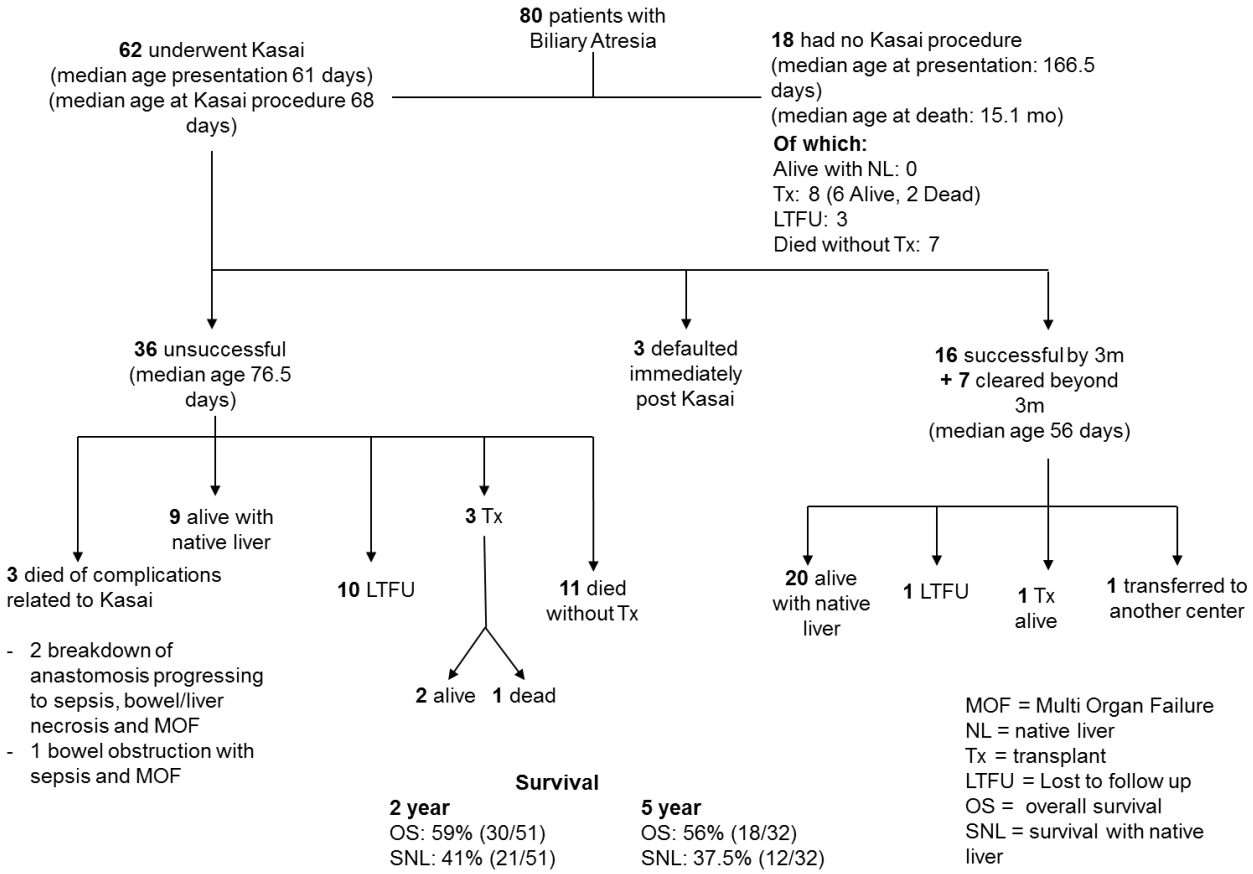
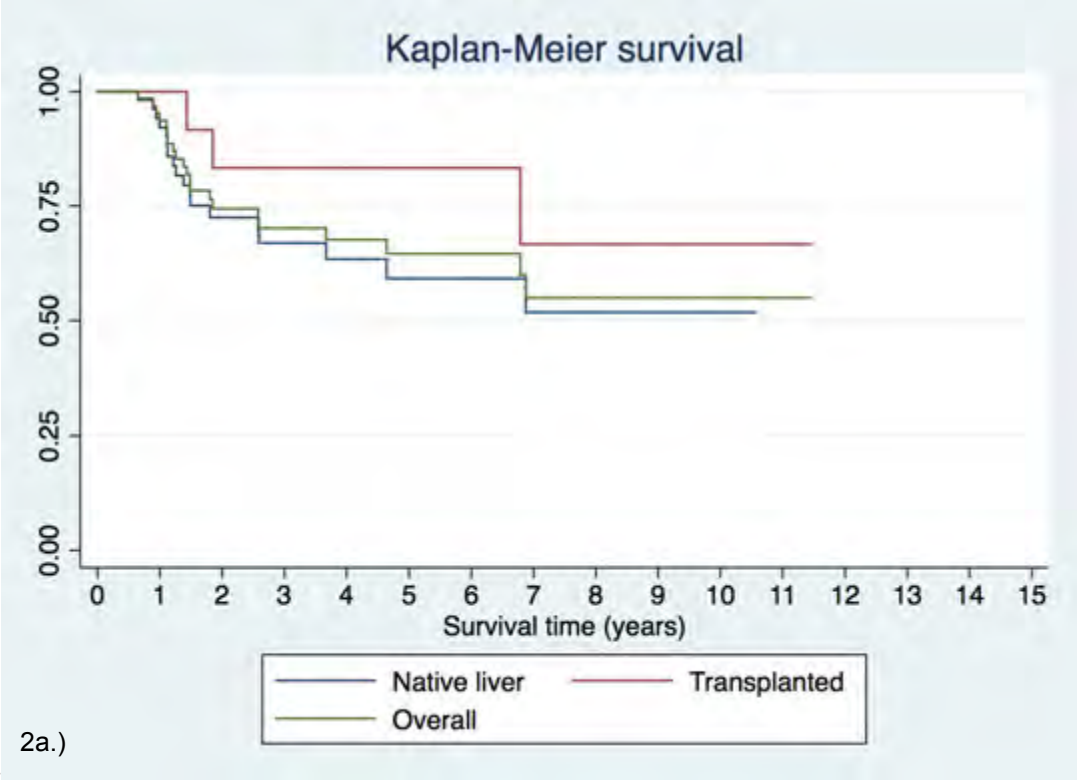
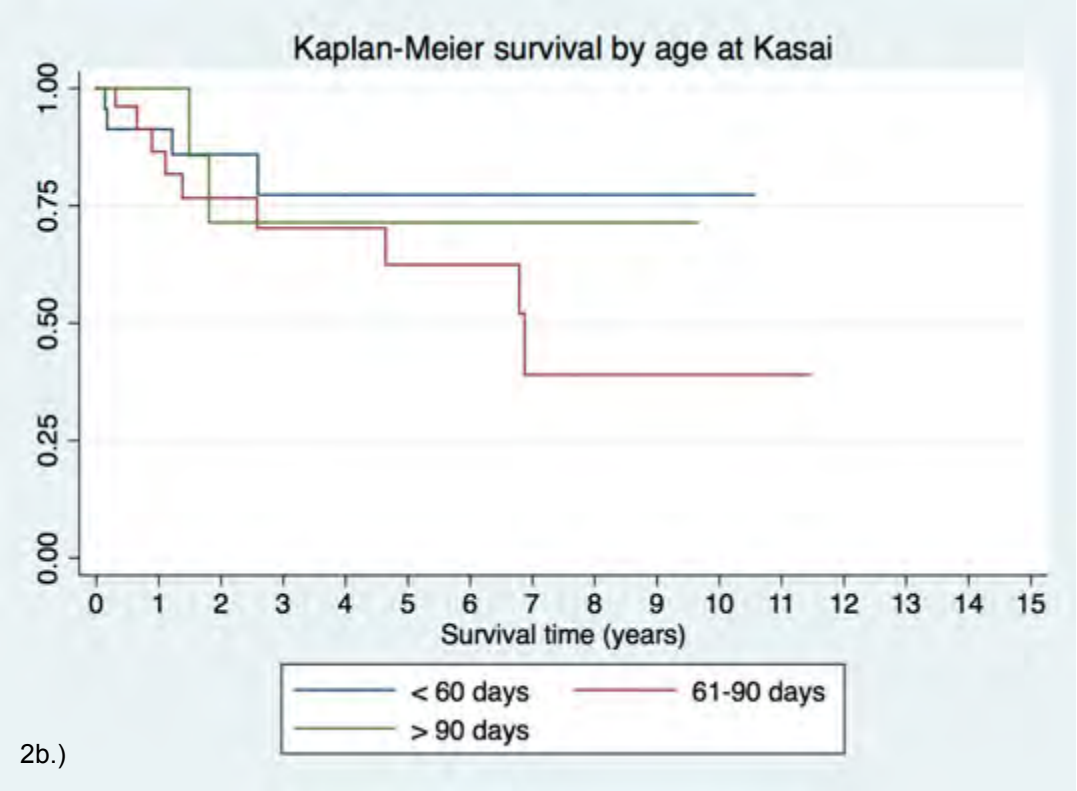


Figure 1: Outcomes of 80 patients with biliary atresia managed at RCWMCH 2003-2013



2a.)



2b.)

Figure 2 Kaplan Meier survival curves: 2a.) Overall, native liver and transplanted liver survival in patients with biliary atresia, 2b.) effect of age at Kasai on overall survival

5. APPENDICES

ETHICAL APPROVAL

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: lameez.emeds@uct.ac.za
Website: www.health.uct.ac.za/research/humanethics/forms

16 April 2014

HREC REF: 137/2014

Dr E Goddard
Paediatrics GIT
Red Cross

Dear Dr Goddard

PROJECT TITLE: BILIARY ATRESIA AT RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL: A RETROSPECTIVE DESCRIPTIVE STUDY REVIEWING THE AGE OF PRESENTATION, CLINICAL COURSE AND OUTCOME ON INFANTS PRESENTING TO RCWMCH WITH BILIARY ATRESIA.

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th April 2015.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/research/humanethics/forms)

We acknowledge that the MMed student Dr Lindsey Levin will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC reference in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN I ,ICS
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

137/2014

DATA CAPTURE SHEETS

Index Case Number				
Folder Number				
Age at presentation (days)				
Race 1 African; 2 Mixed; 3 White; 4 Other				
Sex 1 Male; 2 Female				
Source of referral 1 GP; 2 clinic/day hosp; 3 secondary; 4 tertiary; 5 other				
Number of visits prior to referral				
Date of birth				
Date of presentation				
Date of Kasai				
Date entered transplant list				
Date transplanted				
Date of death				
Presenting features **Jaundice** (0 no; 1 yes) **Pale stools** (0 no, 1 yes) **Other**				
Associated Congenital Abnormalities 0 = nil; 1 = yes (specify)				
LABORATORY VALUES				
Bili (TB/CB) P = Presentation; K = Kasai; K3 = 3/12 post Kasai; A1/2/3/4… = annually				
AST P = Presentation; K = Kasai; K3 = 3/12 post Kasai; A1/2/3/4… = annually				
ALT P = Presentation; K = Kasai; K3 = 3/12 post Kasai; A1/2/3/4… = annually				
ALP P = Presentation; K = Kasai; K3 = 3/12 post Kasai; A1/2/3/4… = annually				

Laboratory continued				
GGT P = Presentation; K = Kasai; K3 = 3/12 post Kasai; A1/2/3/4… = annually				
Albumin P = Presentation; K = Kasai; K3 = 3/12 post Kasai; A1/2/3/4… = annually				
INR P = Presentation; K = Kasai; K3 = 3/12 post Kasai; A1/2/3/4… = annually				
Hb P = Presentation; K = Kasai; K3 = 3/12 post Kasai; A1/2/3/4… = annually				
GROWTH PARAMETERS				
Weight (Z scores) P = Presentation; K = Kasai; K3 = 3/12 post Kasai; A1/2/3/4… = annually				
Height (z scores) P = Presentation; K = Kasai; K3 = 3/12 post Kasai; A1/2/3/4… = annually				

DIAGNOSTIC STUDIES	
Ultrasound	
Cholangiogram	
Biopsy	

KASAI OPERATION	
Age in days	
Surgeon performing Kasai 0 = consultant; 1 = registrar	
Pigmented stools 3/12 post Kasai 0 = no; 1 = yes	
Clearance of jaundice 3/12 Post Kasai 0 = no; 1 = yes	
COMPLICATIONS POST KASAI	
Ascites 0 = no; 1 = yes (number of episodes)	
Cholangitis 0 = no; 1 = yes (number of episodes)	
Varices 0 = no; 1 = yes (number of episodes)	
Bacterial peritonitis 0 = no; 1 = yes (number of episodes)	
Growth failure 0 = no; 1 = yes	
Other (e.g. TB, rickets, specify)	

OUTCOMES	
Successful Kasai 0 = no, 1 = yes	
Progression to require transplant 0 = no; 1 = yes	
Transplanted 0 = no; 1 = yes	
Alive at data collection 0= no (capture cause of death); 1 = yes; 2 = LTFU; 3 = transferred to another centre	

Addendum:

Race was not captured as it is not recorded in hospital records.

HISTOLOGICAL SCORING SYSTEM FOR NEONATAL CHOLESTASIS

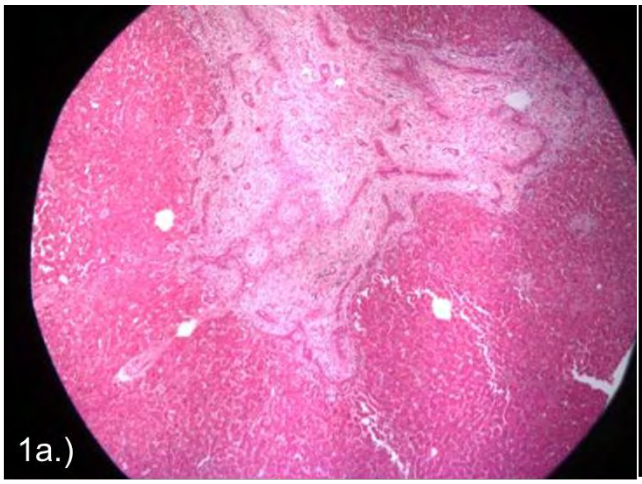
Seven-feature, 15-point histological scoring system devised for the interpretation of liver biopsy materials in neonatal cholestasis

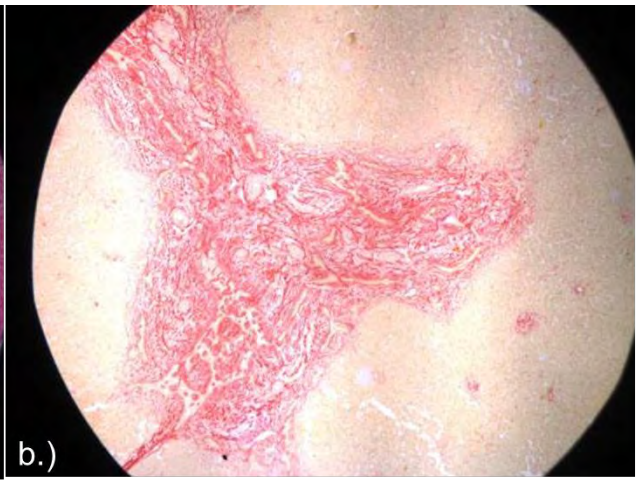
Parameter	Histological characterization	Histological grade
Portal ductal proliferation	None	0
	Mild	1
	Moderate	2
	Marked	3
Bile plug in portal ductules	Absent	0
	Present	2
Porto-portal bridging	None	0
	< 50% of portal tracts	1
	> 50% of portal tracts	2
Lymphocytic infiltrate in portal region	None	2
	Mild	1
	Moderate/severe	0
Multinucleated hepatocytes	None	2
	Only around central vein	1
	Diffuse	0
Neutrophils in the infiltrate	Absent or mild	1
	Moderate or marked	0
Hepatocellular swelling	None	2
	Mild/focal	1
	Periportal/diffuse	0

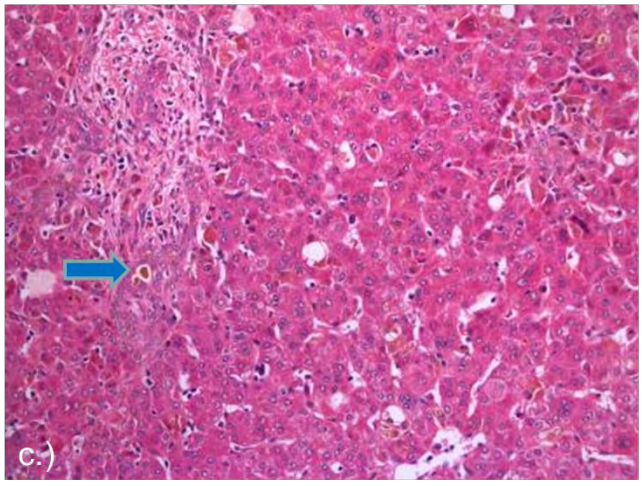
[1]The higher the score, the more likely to be biliary atresia.

Citation: Lee WS, Looi LM. Usefulness of a scoring system in the interpretation of histology in neonatal cholestasis. *World J Gastroenterol* 2009; 15(42): 5326-5333

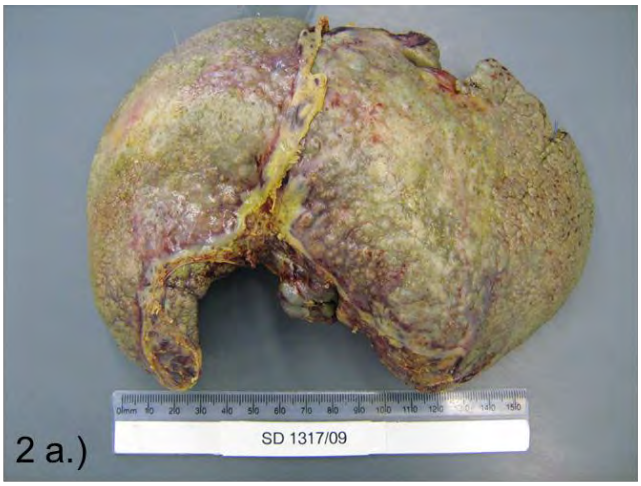
BILIARY ATRESIA PATHOLOGICAL IMAGES







Histological slides depicting the classical features of biliary atresia. Images 1a) & b) demonstrate the expanded portal tract with bile ductular proliferation. Image c) arrow highlights a bile plug. Giant cell transformation and hepatocyte ballooning are inconspicuous.



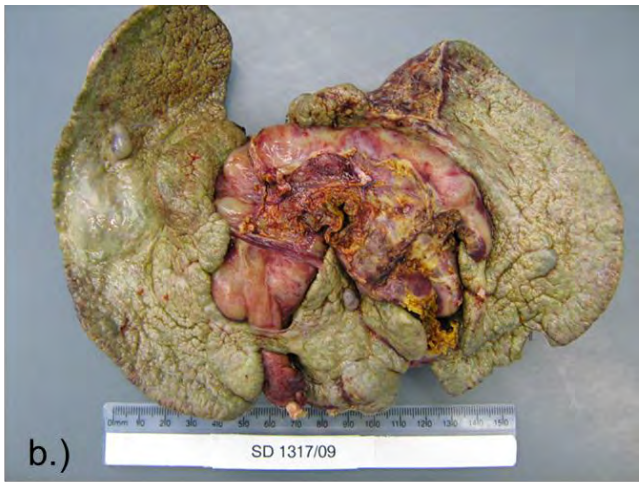


Image 2: explanted liver specimen; site of Kasai portoenterostomy visible on undersurface of liver show in b).

SHARED CARE GUIDANCE: FOLLOW UP OF CHILDREN POST SURGERY FOR BILIARY ATRESIA

SHARED CARE GUIDANCE

Follow up of children post surgery for Biliary Atresia

CONTENTS

INTRODUCTION

We look forward to sharing care of this infant who has Biliary Atresia, and underwent a Kasai Portoenterostomy. Biliary drainage is achieved in up to 80% of patients. Subsequently some of these children will develop complications of cirrhosis (portal hypertension, liver dysfunction etc.) and will be considered for liver transplant electively. Children who do not achieve bile drainage (i.e. remain jaundiced) have an unsuccessful Kasai and should be referred for liver transplant.

CONTACT NUMBERS

Ward D2, GIT Unit on Ward E2

Please feel free to discuss any problems at any time.

FOLLOW UP

2^{nd}, 3^{rd} & 4^{th} week	SOPD
5^{th} week	GIT follow-up in Liver OPD Monday am.
2 - 3 month	GIT Liver clinic
6 month	GIT Liver clinic
1 year and then yearly	GIT Liver clinic

MEDICATION

The following regimen is followed:

Steroids (two week course)

Initially IV in hospital Methylprednisolone 20mg commencing on POD 1, decreasing 2.5mg daily until 5mg/day. This is then completed at a dose of 5mg orally until POD 14.

Further need for steroids to be assessed in clinic but may be reintroduced as a short course if an episode of cholangitis results in sudden cholestasis after good bile drainage.

Antibiotic prophylaxis for 5 days

From induction IVI Ampicillin 25mg/kg/dose 6hrly, Gentamycin 6mg/kg/dose daily (check levels) and Metronidazole 7.5mg/kg/dose 8hrly

Long term oral antibiotic prophylaxis

From Day 6 Ciprofloxacin 12.5mg/kg/dose bd for one month then daily for 3 months

Then a cycle of: Amoxycillin 25mg/kg once a day for 12 weeks (max of 1 gram)

Cephalexin 12.5mg/kg once a day for 12 weeks (max of 500 mg)

Then cycle indefinitely

Vitamin supplements

The infant will be receiving the following vitamins

Vitamin A (retinol) (started in out patients at first surgical review)

Trade Name = Arovit®

Presentation = 150,000 iu/ml (equivalent to 5,000iu/drop)

<1 year 5000 units/day oral

Vitamin D (alfacalcidol) (started in BCH out patients at first surgical review)

Trade Name = One Alpha®

Presentation = 2mcg/ml (equivalent to 100ng/drop)

Neonate + premature 50-100 nanograms/kg/daily oral

Vitamin E (alpha tocopheryl) (started in BCH out patients at first surgical review)

Trade Name = Ephynal®

Presentation = 500mg/5ml suspension

<1 year 50mg/day oral

Vitamin K (phytomenadione) (From Day 5 post surgery) give as either

Trade Name = Menadiol® liquid

Presentation = 5mg in 5mls

Neonate + premature 300 micrograms/kg/day oral

or

Trade Name = Konakion® MM Paediatric

Presentation = 2mg in 0.2ml

Neonate + premature 300 micrograms/kg/day oral

These should stop at 1 year unless failing to thrive or having significant cholangitis

See Appendix for details of suppliers and dosages for older children

(iv) Other medication (from day 5 post surgery)

Ursodeoxycholic acid

5 – 7mg/kg 2-3 times a day Max 45mg/kg/day

Suspension 250mg in 5ml

Phenobarbitone (alcohol, sugar and colour free)

3-5mg/kg daily - all ages

oral liquid 50mg in 5mls

Ranitidine

2mg/kg tds oral

Oral liquid 75mg in 5mls

Omeprazole

1mg/kg/day oral

Fish oil (empirical dose of 100mg/day for Essential Fatty Acid support)

These will be reviewed periodically and duration of use will depend on the success of the operation, but are usually given for at least 3 months after the operation.

VACCINATIONS

Vaccination may start after finishing Prednisolone. No live vaccines should be given until steroid free for 3 months. These infants should receive Prevenar® (Pneumoccal Conjugate Vaccine) This is a 3 dose course that can be given alongside the normal immunisations of DtaP/IPV/HIB+Men C. Practically to avoid 3 injections given at once time, the Prevenar® can be given 2 weeks after each dose of DtaP/IPV/HIB+Men C to reduce the number of injections given at one time. There is a 2 dose schedule between 7-11 months with at least 1 month between doses. A third dose is recommended in the second year of life for whatever scheme is used. They should have Pneumovax II® when they reach 2 years of age. We recommend a course of Hepatitis B once the primary vaccination course is finished. Infants who start their course of Prevenar® after 1 year of age should have 2 injections separated by 2 months followed by Pneumovax II® when they get to 2 years of age. We also recommended Influenza vaccination annually when 6 months of age.

COMPLICATIONS/PROBLEMS

Cholangitis

Suspicion if

Fever > 37.3 for 24 hours or if child is unwell and no other obvious cause (check urine, ENT and chest)

Jaundice/pale stools/abnormal transaminases, rise in ALP/GGT/increased neutrophils

Management

Septic Screen (including two blood cultures at 1 hour interval, chest x-ray, urine m + c & s) no lumber puncture unless indicated. Please discuss with the on call liver unit registrar at this point.

Start IV antibiotics

Ceftazidime 30mg/kg/dose tds for 10 days

Ampicillin 50mg/kg/dose tds for 10 days

(change antibiotic if needed according to culture)

If temperature does not settle after 2 days or returns on discontinuation of antibiotics, start CIPROFLOXACIN (10Mg/kg/8hrs) at full dose. Please discuss with on call liver unit registrar at Red Cross Hospital. The child may need an ascitic tap and liver biopsy for bacteriological culture and further therapy based on the c & s.

The second line treatment is usually

Meropenem	10-20mg/kg tds
Vancomycin	10mg/kg tds
	15/kg loading dose (levels 4th dose)

Lab tests

Monitor full blood count and liver function test at least twice a week

Pruritus

This can be present in unsuccessful Kasai. If the baseline treatment of Ursodeoxycholic acid dose and Phenobarbitone dose is not adequate at their maximum doses the following can be tried up to maximum dose sequentially.

In general a single drug should be used with a stepwise dose increase till effective or maximum dose reached. Combination treatment should be avoided

Rifampicin

3-10mg/kg once a day liquid 100mg in 5ml Rifadin® available

Cholestryramine

Under 6 years 2g (½ sachet) day Over 6 4g (1sachet) day

Questran Light® 4g/sachet Increase dose according to response

Give as single dose or in up to 4 divided doses Other drugs especially vitamins should be given 1 hour before or 6 hours after

Odansetron

2-4mg bd up to 12 years of age then 4-8mg 12-18 year. Solution Zofran® 4mg in 5ml available

Professor Alastair J.W. Millar

APPENDIX

VITAMIN REGIMEN

VITAMIN A (RETINOL)

Trade name	=	Arovit® (unlicensed)
Formulation	=	Solution
Presentation	=	150,000 iu/ml (equivalent to 5,000iu/drop)
Supplier	=	Idis Ltd (Tel: 0208 4100710)

<1 year 5000 units/day

>1 year up to 10,000 units/day

VITAMIN D (ALFACALCIDOL)

Trade name	=	Dependent on presentation
Formulation	=	Solution and capsules
Presentation	=	1mcg capsules – One Alpha®

0.25mcg capsules – Alfa D®

2mcg/ml drops – One Alpha®(equivalent to 100ng/drop)

Supplier = Wholesalers (e.g. AAH)

Neonate + premature 50-100 nanograms/kg/daily

Up to 20kg	25 – 50 nanograms/kg daily
Over 20kg	1 microgram/day

Up to 2 micrograms in severe cases

VITAMIN E (ALPHA TOCOPHERYL)

Trade Name = Ephynal®

Formulation = Suspension and tablets

Presentation = 500mg/5ml suspension

50mg and 200mg tablets

Supplier = Wholesalers (e.g. AHH)

<1 year 50mg/day

>1 year 100 – 200mg/day

12- 18 months 200mg/day

VITAMIN K (PHYTOMENADIONE)

Trade Name = Konakion®MM Paediatric

Formulation = Injection (for IV and oral use) (Mixed micelles)

(Menadiol is the preferred formulation for oral use)

Presentation = 2mg in 0.2ml

Supplier = Wholesalers (e.g. AHH)

Trade Name = Menadiol®

Formulation = Tablets Liquid (unlicensed)

Presentation = 10mg and 5mg/5ml

Supplier = Wholesalers Boots contract manufacturing

(e.g. AAH) (ex-specials)

1 – 5 mg/day

Maximum 10mg/day

Based on 300 micrograms/kg/day

Vitamins A and E levels are monitored in Red Cross Hospital.

Journal of Pediatric Gastroenterology and Nutrition

Online Submission and Review System

SCOPE

The *Journal of Pediatric Gastroenterology and Nutrition* publishes original articles, special reports, review articles, rapid communications, case reports, letters to the editor, short communications, and commentaries on all aspects of pediatric gastroenterology, hepatology, pancreatology, and nutrition.

The journal follows the International Committee of Medical Journal Editors' Uniform Requirements for Manuscripts Submitted to Biomedical Journals (URM). Manuscripts must be prepared in accordance with the URM (N Engl J Med 1997;336:309-15 and updated at http://www.icmje.org/). Manuscripts not prepared according to the Instructions to Authors will be returned to the author(s) without review.

ETHICAL AND LEGAL CONSIDERATIONS

A submitted manuscript must be an original contribution not previously published (except as an abstract), must not be under consideration for publication elsewhere, and, if accepted, it must not be published elsewhere in similar form, in any language, without the consent of Wolters Kluwer. Each person listed as an author is expected to have participated in the study to a significant extent. Although the editors and reviewers make every effort to ensure the validity of published manuscripts, the final responsibility rests with the authors, not with the *Journal*, its editors, or the publisher.

Documented review and approval from a formally constituted review board (Institutional Review Board or Ethics committee) is required for all studies involving people, medical records, and human tissues, and for all animal studies. For authors/investigators that do not have access to formal ethics review committees, the principles outlined in the Declaration of Helsinki should be

followed. If the study is judged exempt from review, a statement from the committee should be provided. Informed consent by participants should always be sought. If not possible, an institutional review board must decide if this is ethically acceptable.

Plagiarism detection

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Declaration of Funding Source

ACKNOWLEDGMENT OF GRANTS OR FINANCIAL SUPPORT MUST BE DECLARED FOR ALL MANUSCRIPTS. In addition, authors of all articles in which the effect of a drug, appliance, or treatment is evaluated must also acknowledge all support from the manufacturer of such drug, appliance, or treatment or its competitor. Authors of review articles, editorials, letters to the editor, and other commentaries must disclose any financial interests that might have an impact on the views expressed in the article, editorial, letter, or commentary. The Declaration of Funding Source statement will be included in the published article or commentary.

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Authors should remove patients' names and other identifying information from figures. If any identifying details appear in text, tables, and/or figures, the author must provide proof of informed consent obtained from the patient (i.e., a signed permissions form). Photographs with bars placed over eyes of patients should NOT be used in publication. If they are used, permission from the patient is required.

The corresponding author of a Case Report must provide the editorial office at the time of submission of the manuscript a written guarantee indicating that the subject(s) of the case report or their parents (or guardians) are aware of the intent to publish and agree to it.

If the parents or guardian were unable to be located for their consent, a signed statement from

the Chair of the Department may be accepted. The statement must read: all attempts have been exhausted in trying to contact the parents or guardian for the purpose of attaining their consent to publish the Case Report.

Conflicts of Interest

Authors must state all possible conflicts of interest in the manuscript, including financial, consultant, institutional and other relationships that might lead to bias or a conflict of interest. If there is no conflict of interest, this should also be explicitly stated as none declared. All sources of funding should be acknowledged in the manuscript. All relevant conflicts of interest and sources of funding should be included on the title page of the manuscript with the heading "Conflicts of Interest and Source of Funding:" For example:

Conflicts of Interest and Source of Funding - A has received honoraria from Company Z. B is currently receiving a grant (#12345) from Organization Y, and is on the speaker's bureau for Organization X – the CME organizers for Company A. For the remaining authors, none are declared.

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Use nonproprietary names of drugs, devices, and other products, unless the specific trade name is essential to the discussion. The trade name may appear once in the Abstract and once in the Introduction or Methods section, followed by the nonproprietary name, manufacturer, and manufacturer location in parentheses; all other mention of the product must use the generic name. Trade names of drugs and other products must not appear in the article title.

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ARTICLE TYPES

Rapid Communication: This article type allows for rapid review (within 10 days) and publication of original studies. Manuscripts considered for rapid review will be limited to reports judged to be of general scientific or public health importance. Authors submitting Rapid Communications must provide a detailed cover letter outlining the rationale for fast tracking their work. Authors must state whether the findings could alter current standards of patient care (e.g., finding efficacy or lack of efficacy of treatment), and/or if the findings suggest a novel mechanism or understanding of disease process (e.g., new susceptibility gene identification in *H pylori* organism).

Rapid Communications should contain no more than 3000 words, structured abstract with no more than 250 words and no more than four figures and tables combined (for example, a submission may include 4 figures, 1 figure and 3 tables, 4 tables, etc., but not 2 figures and 3 tables) and no more than 50 references. Submissions exceeding these parameters without justification or without a detailed cover letter explaining the rationale for a Rapid Communication will be returned to the author for correction prior to review.

Original Articles: Original articles are full-length reports of original research. Original articles are accepted based on their scientific relevance, the originality of the work, and the priority of the work for *JPGN* and its readership. Authors should aim for accuracy, clarity, and brevity. Long introductions, repetition of data among tables, figures, and the text, and unfocused discussions should be avoided.

Original research articles should be approximately 18 double-spaced, numbered pages, including the title page, references, figures, and tables. Failure to comply with length restrictions may result in a delay in the processing of your paper. The following length targets are recommended for Original Articles:

- Structured Abstract: maximum of 250 words
- Introduction: 1 page
- Methods: 2-3 pages
- Results: 2-3 pages
- Discussion: 3-5 pages
- References: limited to those critical and relevant to the manuscript (not more than 50)
- Tables and Figures: 4 total
- Additional/supplemental content may be submitted as "Supplemental Digital Content (SDC)"

Clinical Trials: Clinical trials are Original Articles of studies that prospectively assign human subjects to specific intervention or comparison groups and determine the relationship between an intervention and outcome. To ensure consistency with the guidelines of the Clinical Trial

Registration Statement from the International Committee of Medical Journal Editors (http://content.nejm.org/cgi/content/full/NEJMe078110), all trials submitted to the Journal with patient enrollment commencing after January 1, 2009 must be registered in a public trials registry prior to enrollment of the first subject. The registry must incorporate free public access, and must be searchable, open to prospective registrants, and have not-for-profit management. The following information must be included in the registry: (1) unique identifying number, (2) statement of intervention(s), (3) hypothesis, definition of primary and secondary outcome measurements, eligibility criteria, target number of subjects, funding source, contact information for principal investigator, and dates of registration, start and completion. Authors should provide the URL (website address) and trial identification number on the title page of the manuscript. This information will be published with the article. Structured Abstract (no more than 250 words). No more than 50 references permitted. Clinical trial reports should comply with the Consolidated Standards of Reporting Trials (CONSORT) (www.consort-statement.org).

Review Articles: Review articles are usually solicited by the Editorial Board. However, unsolicited reviews of exceptional interest will also be considered. Authors should contact the Editors before submitting a review to determine whether the topic and contents are appropriate for JPGN. All proposed reviews will be approved based on a submitted list of author(s) and a brief outline for the proposed review. Reviews should be balanced and unbiased. Review articles undergo peer review. Please include an unstructured abstract (no more than 250 words). For Systematic reviews/Meta-analyses structured reviews (no more than 250 words).

Short Communications: This category comprises brief reports on topics relevant to the JPGN reader and preliminary reports of original studies of relevant scientific importance. Short Communications must not exceed 8 manuscript pages including tables and references (calculated at 250 words per page, and including figures, where two figures equal one typed page). Include an unstructured abstract of 150 words or less. No more than 50 references permitted (included in the page count).

Invited Commentary: These submissions are typically no more than 500-1000 words in length and are usually considered by invitation only. Requests to submit a commentary regarding a 'hot topic' should be made to one of the editors. Generally no more than 5-10 references, and 1 table and/or 1 figure are permitted. No abstract needed.

Topic of the Month: These submissions are typically no more than 1000-1500 words in length and are usually considered by invitation only. Unstructured abstract no more than 150 words. References should be limited to those critical and relevant to the manuscript. No more than 30 references and a total of 2 figures and/or tables.

Societal Papers: These submissions are coordinated by ESPGHAN and/or NASPGHAN. For more information about submitting a societal paper, please contact the ESPGHAN or NASPGHAN Consulting Editor for Societal Papers. Abstract optional, no more than 250 words, can be structured or unstructured.

Online Only Article Types

Please Note: Case Reports, Image of the Month, Video of the Month, and Letters to the Editor will be reviewed and accepted as online only content. Papers in these sections will still be assigned to an issue and listed in the issue's Table of Contents, and will appear in full on the journal website and in all electronic versions of the journal.

Case Reports (online only): As of October 1, 2014, JPGN has temporarily suspended the submission of new Case Reports until further notice. Rare exception will be made for unique cases that are deemed important to the health of our patients or the advancement of the knowledge base in our field. If you wish to submit a Case Report under this condition, please e-mail a brief paragraph that explains why your case meets the above criteria to the applicable Editorial Office. Alternately, you may submit your report as a Letter to Editor. Please review submission parameters for Letters may include up to one figure or table.

Case Reports will be considered for publication only if they concern a hitherto unrecognized condition or offer new insight into the pathophysiology, diagnosis, or treatment of a disease. Patients should always be referred to as "the patient"; initials or other identification should not be used. Case Reports must not exceed 1,000 words, and may include up to three tables and figures, and no more than 8 references. An abstract is not required, and if present, it will be included in the word count.

Image of the Month (online only): Submissions for the "Image of the Month" should include one (at most two) high quality TIF endoscopic, histologic, radiologic, or photographic images of unusual or informative findings. A brief description of no more than 200 words should accompany the images. No more than 8 references permitted. No abstract.

Video of the Month (online only): Submissions for the "Video of the Month" should include high quality endoscopic video of unusual or informative findings. One or two additional associated photos, such as radiologic, pathologic, or photographic images, can also be submitted for online publication. A brief description of no more than 200 words should accompany the video. No more than 8 references permitted. No abstract. Videos should be uploaded as Supplemental Digital Content with one of the following file extensions: .wmv, .mov, .qt, .mpg, .mpeg, .mp4; and formatted with a 320 x 240 pixel minimum screen size. For more information, please review Wolters Kluwer's requirements for submitting supplemental digital content: http://links.lww.com/A142.

Letters to the Editor (online only): A Letter to the Editor may be in response to an article published in *JPGN* or may comment on a controversial issue. Letters should be brief (less than 250 words), and will be published at the discretion of the editor. No abstract.

Filler material (in print only): Material such as historical vignettes, photographs, or brief poems/stories/comments may be submitted as 'filler' material that are inserted by the publisher into extra space as available. "Filler" space is available when a paper does not fill at least 50% of the last page in the proofs of the paper. "Fillers" are accepted at the discretion of the Editors in Chief.

MANUSCRIPT PREPARATION

Manuscripts that do not adhere to the preceding guidelines and following instructions will be returned to the corresponding author for technical revision before undergoing peer review. Concise, clearly written articles are more likely to be accepted for publication in the *Journal of Pediatric Gastroenterology and Nutrition*. Authors whose first language is not English are STRONGLY encouraged to ask a native English-speaking colleague or a professional author's editor, preferably with knowledge in the subject matter contained in the manuscript, to edit their manuscript before submission. A list of editing services is available athttp://journals.lww.com/jpgn/_layouts/1033/oaks.journals/editservices.aspx.

Cover Letter: In the cover letter provide a statement as to whether the paper was previously published in any language, including the abstract and whether the paper is currently under consideration elsewhere for publication.

Title page: Include on the title page (a) complete manuscript title; (b) authors' full names, in order first name (given name) then last name (family name), highest academic degrees, and affiliations; (c) name and address for correspondence, including fax number, telephone number, and email address; (d) address for reprints if different from that of corresponding author; (e) all sources of support, including pharmaceutical and industry support, that require acknowledgment; (f) the URL (website address) and trial identification number; (g) disclosure of funding received for this work from any of the following organizations: National Institutes of Health (NIH); Wellcome Trust; Howard Hughes Medical Institute (HHMI); and other(s); and (h) the word count of the manuscript body (excluding abstract except in Case Reports, keywords, references and figure legends), number of figures and number of tables.

All relevant conflicts of interest and sources of funding must also be included on the title page of the manuscript with the heading "Conflicts of Interest and Source of Funding." If there is no conflict of interest, this should also be explicitly stated as none declared.

On a separate page, list each author and his/her respective roles in the submitted work, documenting appropriate input for authorship (http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html#two).

Title length: The manuscript title should have no more than 120 characters including spaces. Keywords for referencing should be included in the title. Please no abbreviations. Fancy or comical titles are inappropriate and will be asked to be revised. Trade names of drugs and other products must not appear in the article title.

Structured abstract and key words: Limit the abstract to 250 words. Do not cite references in the abstract. Limit the use of abbreviations and acronyms. At first mention, please write out the full term for abbreviations (e.g. Celiac Disease (CD)). Use the following subheads in your structured abstract: Objectives, Methods, Results, and Conclusions.

List three to five key words that are not included in the title.

Abstract Chart

Article Type	Abstract requirement (maximum length)	"What is known/What is new"
Rapid Communication	Structured (250 words)	Yes
Original Articles	Structured (250 words)	Yes
Clinical Trials	Structured (250 words)	Yes
Systematic reviews	Structured	Yes

/Meta-analyses	(250 words)	
Review Articles	Unstructured (250 words)	Yes
Short Communications	Unstructured (150 words)	Yes
Invited Commentary	-	No
Topic of the Month	Unstructured (150 words)	Yes (for social media only)
Societal Papers	Optional -No strict rules (250 words)	Yes (for social media only)
Online Only Article Types		
Case Reports*	Unstructured (150 words)	No
Image of the Month	-	No
Video of the Month	-	No
Letters to the Editor and Response	-	No

*PLEASE NOTE: *As of October 1, 2014, JPGN has temporarily suspended the submission of new Case Reports until further notice. Alternatively you may submit your report as a Letter to Editor. Please review submission parameters for Letters. You may include up to one figure or table.*

What is Known/What is New: Immediately following the abstract for all article types except where indicated in chart above, authors should include text for a summary box that will be published on the first page of all accepted articles. This text should highlight the significance of the article with the following guidelines in mind: What is known about this subject? What are the new findings and/or what is the impact on clinical practice? Use the format:

- What is known (2-4 bullet points listed beneath this heading)
- What is new (2-4 bullet points listed beneath this heading)

The total text should not exceed 100 words. At first mention of an abbreviation, please write out the full term.

Text: Organize the manuscript into four main headings: Introduction, Methods, Results, and Discussion. If a brand name is cited, supply the manufacturer's name and address (city and state/country). Under Methods, include ethical approval information, if applicable.

Data Analysis: Description of data analyses should provide the specific methods used, their rationale, their assumptions, whether data met those assumptions, and how any missing data were handled.

Abbreviations: For a list of standard abbreviations, consult the Council of Biology Editors Style Guide (available from the Council of Science Editors, 9650 Rockville Pike, Bethesda, MD 20814) or other standard sources. Write out the full term for each abbreviation at its first use in abstract, what is known, manuscript body and in each table and figure unless it is a standard unit of measure.

References: Please adhere to the reference limits noted for each article type above. The authors are responsible for the accuracy of the references. Key the references (double-spaced) at the end of the manuscript. Cite the references in text in the order of appearance. Cite unpublished data—such as papers submitted but not yet accepted for publication and personal communications, including email communications—in parentheses in the text. If there are more than three authors, name only the first three authors and then use et al. Refer to the List of Journals Indexed in Index Medicus for abbreviations of journal names, or access the list at http://www.nlm.nih.gov/tsd/serials/lji.html.

Sample references:

Journal article

I. Rautava S, Lu L, Nanthakumar NN, et al. TGF-β2 induces maturation of immature human intestinal epithelial cells and inhibits inflammatory cytokine responses induced via the NF-κB pathway. J Pediatr Gastroenterol Nutr 2012;54:630-8.

Book chapter

2. Todd VR. Visual information analysis: frame of reference for visual perception. In: Kramer P, Hinojosa J, eds. Frames of Reference for Pediatric Occupational Therapy. Philadelphia: Lippincott Williams & Wilkins; 1999:205–56.

Entire Book

3. Ming S-C, Goldman H. Pathology of the Gastrointestinal Tract. Philadelphia: Lippincott Williams & Wilkins; 1998.

Software

4. Epi Info [computer program]. Version 6. Atlanta: Centers for Disease Control and Prevention; 1994.

Online journals

5. Friedman SA. Preeclampsia: a review of the role of prostaglandins. Obstet Gynecol [serial online] January 1988;71: 22-37. Available from: BRS Information Technologies, McLean, VA. Accessed December 15, 1990.

Database

6. CANCERNET-PDQ [database online]. Bethesda, MD: National Cancer Institute; 1996. Updated March 29, 1996.

World Wide Web

7. Sullivan D. Major search engines and directories. SearchEngineWatch Web site. http://www.searchenginewatch.com/links/article.php/2156221. Published May 8, 2011. Accessed July 13, 2012.

Figure legends: Each figure must have a legend. Legends should be brief and should be typed on a separate manuscript page, directly following the reference list. Use scale markers in the image for electron micrographs, and indicate the type of stain used. Please let the editors and reviewers know if any of the figures (e.g., figures of study design) are appropriate for the on-line supplemental digital content (SDC) rather than needing to be in-print version.

Figures:

A) Creating Digital Artwork

- Learn about the publication requirements for Digital Artwork: http://links.lww.com/ES/A42
- Create, Scan and Save your artwork and compare your final figure to the Digital Artwork Guideline Checklist (below).
- Upload each figure to Editorial Manager in conjunction with your manuscript text and tables.

B) Digital Artwork Guideline Checklist

- Artwork should be saved in TIFF, Word Doc, PPT or EPS format (PDF is not recommended).
- Artwork is created as the actual size (or slightly larger) it will appear in the journal. (To get an idea of the size images should be when they print, study a copy of the journal to which you wish to submit. Measure the artwork typically shown and scale your image to match.)
- Crop out any white or black space surrounding the image.
- Diagrams, drawings, graphs, and other line art must be vector or saved at a resolution of at least 1200 dpi.
- Photographs, radiographs and other halftone images must be saved at a resolution of at least 300 dpi.
- Photographs and radiographs with text must be saved as postscript or at a resolution of at least 600 dpi.
- Each figure must be saved and submitted as a separate file. Figures should not be embedded in the manuscript text file.

C) Remember:

- Cite figures consecutively in your manuscript.
- Number figures in the figure legend in the order in which they are discussed.
- Upload figures consecutively to the Editorial Manager web site and number figures consecutively in the Description box during upload.

Tables: Cite tables consecutively in the text and number them in that order. Each table should be submitted as a separate Word document in text format. Each table must have a title. Use footnotes to define abbreviations and for other explanatory detail in a legend below the Tables. Tables should be self-explanatory and must supplement, rather than duplicate, the material in the text. Please let the editors and reviewers know if any of the tables (e.g., large data tables) are appropriate for the on-line supplemental digital content (SDC) rather than needing to be in-print version.

Supplemental Digital Content (SDC): Authors may submit supplemental digital content to enhance their article's text and to be considered for online-only posting. Supplemental digital content may include the following types of content: text documents, graphs, tables, figures, graphics, illustrations, audio, and video. Cite all supplemental digital content consecutively in the text. Citations should include the type of material submitted, should be clearly labeled as "Supplemental Digital Content," should include a sequential number, and should provide a brief description of the supplemental content. Provide a legend of supplemental digital content at the end of the text, listing captions in the order in which the material is cited in the text. The legends must be numbered to match the citations from the text. Include a title and a brief summary of the content. For audio and video files, also include the author name, videographer, participants, length (minutes), and size (MB). No patient-identifying information should be used in supplemental digital content unless written consent from the patient, the patient's parents or the patient's guardian has

been obtained. Documentation regarding this consent must be submitted with the manuscript. Copyright and Permission forms for article content including supplemental digital content must be provided at the time of submission.

Supplemental Digital Content Size & File Type Requirements: To ensure a quality experience for those viewing supplemental digital content, it is suggested that authors submit supplemental digital files no larger than 10 MB each. Documents, graphs, and tables may be presented in any format. Figures, graphics, and illustrations should be submitted with the following file extensions: .tif, .eps, .ppt, .jpg, .pdf, .gif. Audio files should be submitted with the following file extensions: .mp3, .wma. Video files should be submitted with the following file extensions: .wmv, .mov, .qt, .mpg, .mpeg, .mp4. Video files should also be formatted with a 320 X 240 pixel minimum screen size. For more information, please review Wolters Kluwer's requirements for submitting supplemental digital content: http://links.lww.com/A142.

Reviewers: The JPGN editors encourage authors to submit names, departments, institutions, and e-mail addresses of 3-7 potential reviewers with appropriate expertise to evaluate the manuscript. These potential reviewers should be outside all authors' institution(s) and have no known potential conflicts of interest. Names of persons who should not be asked to review the manuscript due to potential conflicts of interest should also be submitted. Final choice of reviewers, however, remains with the editors.

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