Introduction

Biliary atresia (BA) is a rare disease of the newborn that causes persistent conjugated jaundice, together with pale stools and dark urine; and, if left untreated can cause fibrosis, cirrhosis and ultimately end-stage liver disease within the first year of life (1).

Its incidence varies across the world from 1 in 5,000–10,000 in Taiwan and Japan (2,3) to 1 in 15,000–20,000 in Europe and North America (4-6). The reason for the disparity is not known but, interestingly, other conditions such as congenital choledochal malformations also show the same trend.

Pathologically it is an obstructive cholangiopathy affecting both the intra- and extra-hepatic bile ducts to varying degrees. Sometimes there is a marked inflammatory component, while in others there is simply absence of parts of the extrahepatic bile duct system. Fibrosis is age-related and not present at the time of birth but becomes increasingly obvious beyond 3–4 months of life. This is invariably accompanied by portal hypertension and splenomegaly. The most proximal level of obstruction is used to characterise the type of BA with >90% having an obstruction at the level of the porta hepatis (type 3). Less commonly the obstruction is at the level of the common hepatic duct (type 2) and common bile duct (type 1). It is possible to have cystic change within an otherwise obliterated biliary tree (cystic BA) which may even contain bile.

The aetiology of BA is not fully understood however it is very unlikely that there is single underlying cause for all cases; rather a number of different mechanisms and factors are thought to lead to a final common pathway that is the recognizable as the BA phenotype, a phenomenon known as aetiological heterogeneity (7). Figure 1 summarizes some of these possibilities.

We have described from experience with our English
cohort of BA a number of distinct variants including a syndromic form biliary atresia splenic malformation (BASM) (8,9) cystic BA (10) and more recently CMV IgM +ve associated BA (11). The remaining cases, indeed the majority, do not have any other defining characteristics and are referred to as isolated BA (Table 1). The implication being that in BASM (and those with other non-syndromic anomalies) and in cystic BA the cause is abnormal first and second trimester respectively bile duct developmental impairment. In those with perinatal CMV exposure, and hence IgM antibodies, the implication is that the hepatotropic virus triggers an immune-mediated injury in a normally developed bile duct. This concept has replaced the previous dichotomy between “embryonic” and “perinatal” BA, which is simply naive.

The clinical presentation of BA is typically with progressive, conjugated jaundice, pale acholic stools and dark urine. Other early presenting features may include a vitamin K dependent coagulopathy; with later ones being, ascites, portal hypertension and splenomegaly.

Pre-operative diagnosis is possible but there are many protocol variations between different centres. Standard tests include liver biochemistry, ultrasonography and exclusion of medical causes such as α1-antitrypsin deficiency, cystic fibrosis, Alagille’s syndrome etc. Other more contentious tests include dynamic radioisotope biliary imaging, duodenal bile aspiration, ERCP and percutaneous liver biopsy (12). Our preference is still for liver biopsy and the histological features of note are portal tract oedema, bile duct plugging and proliferation together with a small cell infiltrate and occasionally giant cell formation.

Early diagnosis and prompt Kasai portoenterostomy (KPE) are essential for the best outcomes (13) with the aim of surgery being to restore bile flow, reduce clinical jaundice and (hopefully) reverse liver fibrosis, thus salvaging the native liver. The operation removes the entire obliterated extrahepatic biliary remnant leaving the denuded (often apparently solid) portal plate which is then anastomosed to a jejunal Roux loop. In a small number of usually late-presenting infants (>100 days of age) where this approach may be deemed futile, primary liver transplantation should be considered.

Still, the outcome following KPE is highly variable in the literature and the real world with effective drainage of bile achieved in around 50% of children (14,15). Liver transplantation is indicated (obviously if available) for those who do not achieve adequate resolution of jaundice or who are affected by the severity of complications (16).
The aim of this review is to discuss and focus on adjuvant therapy and treatments designed to improve outcome after KPE.

**Adjuvant therapy**

**Corticosteroids (Figure 2)**

Inflammation is thought to be a key factor in the pathogenesis of many cases of BA. There is abnormal expression of MHC class 2 antigens, with upregulation of pro-inflammatory cellular adhesion molecules ICAM and VCAM (17,18) in up to 40% of cases of BA. This is accompanied by a small cell infiltrate of activated CD4 lymphocytes and NK cells (17). Recent work from our laboratory showed that the T cell infiltrate is predominantly Th1 and Th17 cells with the former having a particular association in those with CMV IgM antibodies (19). This inflammatory response can also be seen with elevated plasma levels of sICAM, sVCAM together with cytokines such as IL-2, IL-4, IL-18, TNF, interferon (which are not only present at the time of KPE but for up to 6 months post-operatively) (20).

This explains much of the rationale behind corticosteroid use in BA which is to try and limit this presumably detrimental response. However corticosteroids also have a less well studied choleretic effect which may also be beneficial.

**Clinical evidence**

Early clinical studies were reported during the 1980’s, describing a so-called ‘blast-regimen’. This was a short course of a high-dose corticosteroid to try and reverse the effect of cholangitis and restore bile flow (21,22). Following this a multitude of non-randomized small-scale studies were published (23-25) suggesting benefit in a variety of doses and duration.

The first randomized, double blind, placebo-controlled trial was published in 2007 from two UK centres (London and Leeds) and involved 71 infants (26). The regimen used in retrospect was low-dose and consisted of a starting dose of 2 mg/kg/day oral prednisone, reducing over a 4 weeks period. The primary outcome measures were defined as clearance of jaundice, early liver biochemistry and native liver survival. A significant difference in bilirubin levels at 1month post-KPE was found between the two groups. A further study was published in 2013 from the larger centre (27), restricted to those infants <70 days at KPE and adding a further group with a high-dose regimen (starting at 5 mg/kg/day). This showed a significant difference between placebo and steroid groups in terms of clearance of jaundice to normal levels (52% vs. 66%, P=0.037). Indeed a gradation of response could be seen between low and high

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**Table 1 Clinical variants of biliary atresia**

<table>
<thead>
<tr>
<th>Variant</th>
<th>Associated anomalies</th>
<th>Typical biliary appearance</th>
<th>Clinical outcome post-KPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASM (10% in Western series, &lt;2% in Eastern series)</td>
<td>Polysplenia, situs inversus, absence of IVC, preduodenal portal vein: cardiac defects</td>
<td>Atrophic with usually absence of common bile duct</td>
<td>Poor but maybe dependent on other anomalies</td>
</tr>
<tr>
<td>Cystic BA (5–10%)</td>
<td>–</td>
<td>Cystic change (± bile)</td>
<td>Excellent</td>
</tr>
<tr>
<td>CMV IgM +ve BA (10% prevalence in European series)</td>
<td>–</td>
<td>Inflammatory, hypertrophic bile duct remnant</td>
<td>Poor</td>
</tr>
<tr>
<td>Isolated BA (90–95%)</td>
<td>–</td>
<td>Wide spectrum from atrophy and missing parts to hypertrophic inflammatory appearance</td>
<td>About 50% will clear jaundice and 40–50% will have their own livers at 5 and 10 years</td>
</tr>
</tbody>
</table>

BASM, biliary atresia splenic malformation; BA, biliary atresia.

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**Figure 2** Chemical structure of prednisolone, a synthetic glucocorticoid: (A) ball and stick model (oxygen as red) and (B) steroid ring C21H28O5. [(A) reproduced under Creative Commons License, originator RingO].

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dose regimens. Other biochemical differences were also observed including reduced AST levels and AST-to-platelet ratio index (APRI) at 1 month post-operatively. It did not show any change in improved native liver survival or the need for transplantation.

The effects of a high-dose prednisone regimen was also tested in a placebo controlled trial in the North American multicenter (n=14) Steroids in biliary Atresia Randomised Trial (START) (28). It compared placebo (n=70) against a regimen of IV methylprednisolone (4 mg/kg/day) for 2 weeks tapering down to oral prednisolone (2 mg/kg/day) for a further 9 weeks (n=70). The primary endpoint was serum bilirubin <1.5 mg/dL at 6 months post KPE. The secondary outcome measure was native liver survival at 6 months. They reported an overall non-significant increase in jaundice clearance at 6 months (49% vs. 59%) in the steroid group.

Both of the placebo-controlled studies (27,28) identified a negative effect of increasing age on outcome and subset analysis in the START trial confirmed an increased proportion of those to clear their jaundice (71.8%), but again not to statistical significance. On review of their study design it appears that it was powered to require a difference of 25% in the primary outcome measure. This estimation was based upon a previously published American retrospective study (23) with very poor outcomes for its control group.

There is also a large non-randomized cohort study from Shanghai, China (29), which compared the outcome of low and high dose steroids in two consecutive periods 2004–2006 and 2007–2009. In total, 380 (n=253 in high dose group) infants underwent KPE. Although there was a significant difference in mean age at KPE (74 vs. 66 days; P=0.03) there was a significant difference in the proportion to clear their jaundice (39% vs. 53%) in favour of steroids.

Several systematic reviews have been published (30,31). The most recent meta-analysis was published by Chen et al. in 2015 which looked at 259 patients undergoing steroid therapy post-KPE (32). Of the studies meeting the inclusion criteria two were RCTs and five were observational studies, published from 1968 to 2014. They identified from their analysis that there was a significant difference in jaundice clearance in those where moderate to high-dose steroid versus placebo at 6 months post-KPE. They also suggested that longer regimens failed to elicit any further significant benefit and therefore a shorter course may be more prudent to avoid drug-related complications.

A more recent study from London (33) looked specifically at the “age effect” in a prospective, single-centre, single-surgeon review. One hundred and four infants with BA who underwent KPE at <70 days old and received high dose steroids were included. This group was subdivided into serial age cohorts and jaundice clearance at 6 months was assessed. This showed a significant trend over time favouring early KPE. Additionally, significant improvement in overall native liver survival those operated on before 45 days (the median age in the sample). This study for the first time shows that high-dose steroid not only augment jaundice clearance but can also translate to improved native liver survival.

Prednisolone is the most frequently prescribed steroid in most studies (21,26,28) with a usual starting dose of 4 or 5 mg/kg/day. Some protocols begin this with intravenous methyl prednisolone (23,28) although there is little evidence to suggest this has any extra effect. Dexamethasone has also been recommended by one English centre beginning oral dexamethasone (0.3 mg/kg twice daily for 5 days, 0.2 mg/kg twice daily for 5 days, and 0.1 mg/kg twice daily for 5 days), beginning on postoperative day 5 (25).

There are many potential side effects of steroids though none has actually been reported in the papers presented so far. Possible side-effects include increased risk of infection, poor wound healing, hyperglycemia, hypertension, gastrointestinal bleeding, poor growth, and an inadequate response to routine immunizations (27). The START trial suggested an increased but non-specific incidence of side effects but were unable to identify anything more tangible (28).

In practice, high-dose steroid use is ubiquitous in the UK, Japan and much of Europe. However its use is probably declining in the USA because of the negative influence of the START trial (28).

**Ursodeoxycholic acid (UDCA) (Figure 3)**

UDCA is a hydrophilic secondary bile acid that is an established part of treatment for cholestatic conditions in adults (34) (Figure 2). Its medicinal benefits were first identified during the Tang Dynasty in China as the traditional drug Shorea, and used to treat liver disease. Shorea is derived from the bile of adult black bears (Figure 4), which contains a high concentration of UDCA.

There is wealth of evidence supporting its use in primary biliary cirrhosis and primary sclerosing cholangitis with studies reporting improved clinical and biological parameters to reduced need for transplant (34,35). Other conditions suggested to benefit from UDCA are cystic
fibrosis and TPN associated cholestasis (36,37).

Alongside improving choleresis, UDCA is known to have an immunomodulatory effect. It has been shown to decrease cytokine production in PBC, alter HLA-class I antigens on hepatocytes (38,39) and suppress immunoglobulin production. There is a documented inverse relationship between increasing serum concentrations of UDCA and decreasing “toxic” endogenous bile salts. This is thought to be protective to hepatocytes and cholangiocytes adding a third rationale for potential benefit in BA. Although there are documented theoretical benefits for UDCA in BA, qualifying evidence is lacking.

A French group performed a prospective uncontrolled crossover trial assessing the effect of UDCA in stable patients post-KPE (40). Sixteen children met the inclusion criteria, having cleared their jaundice post-KPE and had been on UDCA (25 mg/kg/day TDS) for at least 1 year. The outcome measures assessed were clinical state (pruritus, ascites, organomegaly, bacterial cholangitis) and biochemical markers (bilirubin, liver enzymes). A discontinuation/reintroduction methodology was used with the hope that as patients were not taking any other medication (such as steroids), any change in outcome measure could be attributed to UDCA cessation/reintroduction. Thirteen of the 16 children were restarted on UDCA within 6 months of stopping. The main reason for resumption being worsening of liver enzyme profile; with one child having recurrence of jaundice. All of these improved on reintroduction of the drug. Although UDCA showed beneficial effects on liver biochemistry this study did not observe a pronounced effect on clinical status. None of the children experienced increased pruritus, bacterial cholangitis or hepatomegaly on discontinuation of UDCA.

In contrast, there is a larger retrospective study from Egypt which looked at a cohort of 141 infants of which 108 had received UDCA and they suggested that this group had had a worse outcome (41). However, this may just reflect poor results—only 19 (13%) infants overall cleared their jaundice.

Antibiotics

Cholangitis remains a very serious post-operative complication following KPE, reportedly affecting over 50% of patients (42). The common causative organisms have been identified as Klebsiella spp., Escherichia coli, Pseudomonas aeruginosa, Escherichibia cloacae, A. baumann, Streptococcus mitis and Salmonella typhi (43-45). The mechanism is most likely to be an ascending cholangitis via the Roux loop into the intrahepatic duct system, primitive and distorted though it is. Other contributory mechanisms may also be involved including bacterial overgrowth of bacteria in the gut, translocation from lymphatics and haematogenous spread via the portal vein. The incidence ranges in surgical series between 40–93% (46-48).

The practice of prescribing prophylactic antibiotics to try and reduce the incidence of cholangitis is extremely variable. There is no consensus as to what drug to give, for what duration and indeed if there is even any clinical benefit. A recent systematic review by Decharun et al. (49) sought to systematically review current evidence on prophylactic
antibiotics and their prevention of cholangitis post-KPE. Four studies met the eligibility criteria; one randomized control trial (50) and three retrospective cohort studies (51-53). A lack of high quality evidence for antibiotic prophylaxis is evident when from 87 abstracts reviewed, only one RCT was selected. The primary outcome measures were the incidence of cholangitis, recurrent cholangitis and transplant-free survival and a total of 329 patients were included in total (196 receiving antibiotics, 133 control).

There was wide variation in results from the smaller studies; with the largest (53) recruiting from a Dutch national cohort (n=214). This latter study did not identify any reduction in cholangitis rates, however interestingly it was associated with a higher 4 years transplant free survival rate (54% in antibiotic group, 34% in the control group).

The randomized control trial by Bu et al. (50) concentrated on antibiotic prevention of recurrent cholangitis. Nineteen children, all of who had had one episode of cholangitis were randomized to receive either trimethoprim-sulfamethoxazole (Septrin\textsuperscript{TM}) (n=9) or oral neomycin (n=10). A historical control group (n=18) of patients who did not receive antibiotics was also used. The authors found that rates of recurrent cholangitis were significantly lower in both antibiotic groups, with no significant difference between the two. Neomycin delayed the first episode of recurrence and was associated with a higher survival rate.

This review does reinforce the high incidence of cholangitis in children post-KPE, and the expectation that most episodes will occur in the first year post-operatively. A sensible approach to duration of antibiotic therapy may be to give them concomitantly with steroids due to immunosuppression risks or if a longer course is desired to stop at 1 year post-KPE, when the cholangitis risk significantly decreases.

Native liver survival decreases markedly if there are repeated episodes of cholangitis especially within the first 2 years of surgery. In one recent study of 76 infants, Koga et al. showed that liver transplantation was ultimately required by all jaundice-free children who had had cholangitis within 3 months of the KPE (54).

Cholangitis occurring after some years of infection-free life in children who have cleared their jaundice should make one suspicious of an actual mechanical problem with the Roux loop (43). These children should be fully investigated with radionuclide imaging, ultrasound and MRCP. Balloon enteroscopy may also have a diagnostic role enabling visualization of the Roux limb. Dilatation proximal to the retrocolic tunnel is the key diagnostic feature and if there is sufficient evidence of this then laparotomy should be embarked upon.

**Ganciclovir**

Cytomegalovirus IgM +ve associated BA has been proposed as a distinct group of infants with a different aetiology and a worse prognosis (11). Perinatal viral exposure may lead to destruction of fully formed bile ducts. This may either be via deleterious effects of the hepatotropic virus itself or a secondary autoimmune reaction (55-57).

In a recent prospective cohort study, Zani et al. (11) clearly identified CMV IgM +ve status as a negative prognostic factor. This single center cohort study identified 20 CMV IgM +ve infants and compared a range of observations (biochemical, radiological, histological) to a control group of 111 CMV −ve infants over a 7 years period. In terms of demographics the CMV IgM +ve patients were older at time of KPE and a more ethnically diverse group with most patients from a non-Caucasian background. They were found have significantly worse preoperative liver function, a higher APRI, more splenomegaly, more inflammation and fibrosis on histology. Postoperatively the CMV IgM +ve infants had significantly reduced rates of jaundice clearance and higher mortality rate. Of note, immunohistochemistry from liver/biliary tissue at KPE was not able to confirm the presence of CMV in any patient. This difficulty in proving active replication of CMV has been noted in previous studies and Zani et al. hypothesized that it may imply clearance of infection. A question left unanswered is time of acquisition of the virus as maternal serology was not available.

Antivirals therapy has been suggested for CMV IgM +ve BA (11). It is known to be effective for congenital CMV infection. Thus ganciclovir was used in 100 infants with CMV disease affecting their central nervous system (58) and shown to reduced disease progression. Its use was however associated with myelosuppression (neutropenia) in two thirds. Its use in patients with BA, by contrast, is poorly reported.

Fischler et al. (59) aimed to describe the effect of ganciclovir on patients with CMV associated cholestasis. They identified six patients in their tertiary referral center with CMV infection (CMV IgM +ve/CMV in urine). Of these six, two had BA, one had septo-optic dysplasia and three had intrahepatic cholestasis attributed to CMV hepatitis. One of the BA patients was diagnosed at <3 weeks of age suggesting congenital infection. Ganciclovir was
given at 5 mg/kg BD for 2 weeks then reduced to 5 mg/kg daily thereafter. The first BA patient received ganciclovir pre-KPE for 3 weeks then post-KPE for a further 2 weeks. The second BA patient had an isolated 4 weeks course. The response was measured biochemically, virologically and by clinical outcome. The results in this very small group of CMV BA patients treated with ganciclovir are unclear. The first patient displayed improvement in markers of cholestasis. Unfortunately the second infant did not benefit from KPE and subsequently died from complications following a liver transplant. The authors noted that the concomitant use of UDCA may have influenced results but acknowledge that successful surgery is the biggest factor in improved cholestasis and outcome.

Shah et al. from Mumbai, India report success using valganciclovir for CMV-associated BA in one case (60). One patient who had not cleared their jaundice a month post KPE was given a 6 weeks course of valganciclovir. The patient’s bilirubin and CMV viral load subsequently improved to normal values.

The current lack of data makes it impossible to draw any conclusions regarding the efficacy of ganciclovir or its oral equivalent prodrug valganciclovir in CMV IgM +ve associated BA. Remaining challenges to be overcome will be determination of time of onset of infection and a safe method of delivery of the drug and isolation.

**Vitamin supplementation**

BA is associated with nutritional deficiencies due to reduced intraluminal bile acids. These acids are essential, and must be present at the critical micellar concentration for the adequate absorption of fat and fat soluble vitamins (A, D, E, K) (61). There are multiple potential consequences of fat soluble vitamin deficiency such as rickets, fractures and coagulopathy. An essential part of the care for patients with BA is recognizing nutritional deficiencies and providing adequate oral replacement. Venkat et al. (61) suggests that total bilirubin can be used as an effective surrogate for fat soluble vitamin insufficiency to guide treatment.

A recent paper from London by Ng et al. (62) focused specifically on vitamin D deficiency in BA. This single-centre retrospective review investigated the pre- and post-KPE levels of 25 hydroxyvitamin D (25 OHVD), liver and bone biochemistry in 129 infants. Each child received high-dose intramuscular vitamin D (30,000 IU or 60,000 in the presence of rickets) followed by oral supplementation post-operatively. Those who were still vitamin D deficient on monthly blood check received further IM dosing (10,000 IU/kg). Children were followed up at 1, 4, 6 and 12 months. The authors found that vitamin D deficiency was invariable in infants with BA on presentation and that despite supplementation deficiency continued to be a problem post-KPE. This was noted especially in those still jaundiced. The authors postulated several theories for this, including poor absorption secondary to cholestasis and inadequate metabolism due to hepatocyte dysfunction, possibly exacerbated by ethnicity and even season of birth.

**Intravenous immunoglobulin therapy**

The inflammatory component of BA has already been highlighted and is a clear target for adjuvant therapy. Whereas steroid use has been studies for many years and come to placebo-controlled trials, the use of immunoglobulins in has not. Intravenous immunoglobulin therapy is known to reduce inflammatory cytokines and increase anti-inflammatory regulatory T cells (63) and is an established part of treatment in multiple autoimmune, immunodeficiency and inflammatory conditions (64). Fenner et al. (65) using a rhesus-rotavirus model of BA in mice showed that there was diminished bilirubin levels together with less expression of VCAM-1 on portal epithelium and cytokine production by T cells using high-dose IgG therapy. In North America the Safety Study of Intravenous Immunoglobulin (IVIG) Post-Portoenterostomy in Infants with Biliary Atresia (PRIME) trial is currently underway (https://clinicaltrials.gov/ct2/show/NCT01854827). This is an open-label multicentre phase 1 & 2 trial assessing the feasibility, tolerability and safety of intravenous IG post-KPE. Infants will receive three doses of IV IgG over a 60 days period and be followed up for 360 days.

**Chinese herbs and Kampo medicine**

BA is characterised by relatively early-onset aggressive hepatic fibrosis which leads ultimately to cirrhosis. This may lead later on to clinically significant portal hypertension, the development of varices, and less commonly ascites. Modulation or even abbreviation of this pathological process would have immense benefit but so far seems elusive.

Both Japanese and Chinese centres routinely prescribe the Chinese herb mixture “Inchinko-to” to infants post-KPE and one of the claimed benefits includes inhibition of apoptosis and inhibition of liver fibrosis (66). For instance, Tamura et al. (67) reported a prospective study of 21 children post-KPE who had cleared their jaundice.
but who had persistent elevated liver enzymes and GGT. Inchinko-to was given to 12 for up to 3 years while the remainder persisted in their standard regimen without any herb. Liver enzymes, bile acids and markers of liver fibrosis were measured sequentially. There were no side effects of treatment. In the Inchinko-to group, markers of liver fibrosis (e.g., hyaluronic acid) were significantly decreased at 1 and 3 years without change in liver enzymes, bile acids or bilirubin.

**Miscellaneous**

Phenobarbitone and its derivatives have a long history of empirical use in paediatric hepatology and have been within our Kings College Hospital protocol since the 1970s (prescribed as phenobarbitone 15 mg daily increasing to 45 mg in steps of 15 mg/week). Similarly the bile acid sequestrant, cholestyramine (1 sachet t.d.s.) is also part of our protocol, again empirically without any real evidence of benefit. Its use probably started following publication of a randomized French trial of 80 infants post-KPE looking at both agents individually together with a comparative control group (68). Ironically, no significant differences were identified which was obviously lost in translation!

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None.

**Footnote**

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

**References**


