UK National Screening Committee

Screening for Biliary Atresia

External review against programme appraisal criteria for the UK National Screening Committee (UK NSC)

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The UK NSC advises Ministers and the NHS in all four UK countries about all aspects of screening policy. Its policies are reviewed on a 3 yearly cycle. Current policies can be found in the policy database at http://www.screening.nhs.uk/policies and the policy review process is described in detail at http://www.screening.nhs.uk/policyreview

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Introduction

Biliary atresia is a rare condition of infancy where there is destructive inflammation and blockage of variable lengths of the extrahepatic bile ducts,[1] preventing the flow of bile from the gall bladder to the intestine. If untreated, the condition rapidly leads to cholestasis, growth failure, cirrhosis, and will result in death from end-stage liver disease within the first 2 years of life.[1,2]

The condition is classified according to the site of blockage in the extrahepatic biliary system, which comprises the common hepatic duct and accessory biliary apparatus (gallbladder and cystic duct):

- Type 1: atresia of the common bile duct with proximal patency (about 5% of cases[1])
- Type 2: atresia of the common hepatic duct (about 2% of cases[1])
- Type 3: atresia of the most proximal part of the extrahepatic biliary tract, with atresia of both right and left hepatic ducts (the most common type, accounting for over 90% of cases[1])

Persistent neonatal jaundice is the first clinical symptom of biliary atresia. Neonatal jaundice is common, affecting approximately 60% of term infants and 80% of preterm infants during the first week of life, with about 10% of breastfed infants still being jaundiced at 28 days.[3] The vast majority of these infants will have elevated unconjugated bilirubin levels; biliary atresia is one of several causes of cholestatic jaundice, where there are raised levels of conjugated bilirubin. Therefore identifying the small minority of infants who have cholestatic jaundice is key for early diagnosis and treatment. Good outcomes in biliary atresia rely on timely Kasai portoenterostomy to allow biliary drainage. Failure to establish bile flow indicates liver transplant, and biliary atresia is reported to account for 75% of all liver transplants performed in those aged under 2 years.[1]

There is no validated screening test for biliary atresia in the UK. Currently early detection of the condition relies upon prompt recognition of prolonged jaundice, which NICE define as jaundice lasting for more than 14 days in term infants, or more than 21 days in preterm infants.

In 1999 Mushtaq et al. evaluated the potential for a screening test that used tandem mass spectrometry to measure conjugated bile acids in dry blood spots on the Guthrie screening card.[4] However, at that time this method was not shown to be a reliable indicator of neonatal cholestatic liver disease. This report reviews the literature post-1999, examining whether any progress has been made on dry blood spot screening or alternative screening methods for biliary atresia. In countries where screening programmes have been introduced, we examine whether there is evidence that screening facilitates earlier detection and treatment, and whether this influences later outcomes. The report also identifies whether we are any closer to understanding the epidemiology and aetiology of biliary atresia, and whether there have been any treatment developments or prognostic improvements.

Appraisal against UK NSC Criteria

These criteria are available online at http://www.screening.nhs.uk/criteria.

1. The condition should be an important health problem

In 1999 the management of all infants with biliary atresia in England and Wales was centralised to three centres: King's College Hospital, London; Birmingham Children's Hospital; and St James's University Hospital, Leeds.[5,6] This national policy has allowed

evaluation of the incidence, characteristics and outcomes of all infants with biliary atresia diagnosed and treated in England and Wales.

Livesey et al. (2009) used the national data registry to examine the incidence of biliary atresia in England and Wales between January 1999 and December 2006.[6] During this period 302 infants were histologically confirmed to have biliary atresia. Using published data on the number of live births by administrative region and place of maternal origin, the overall incidence of biliary atresia in England and Wales was estimated at 0.58 per 10,000 live births (1 in 17,049), though there was marked regional variation (varying from 0.78 per 10,000 live births in south-east England to 0.38 per 10,000 in north-west England).[6]

In a more recent study, Davenport et al. (2011) used the national registry to establish a national benchmark for the management and outcomes of all infants with confirmed biliary atresia born in England and Wales between January 1999 and December 2009.[5] Outcomes were assessed using three performance indicators: age at time of Kasai procedure, post-operative jaundice clearance, and 5- and 10-year overall survival and survival with native liver (survival in children who have not had a transplant).

Key data for all infants with biliary atresia born in England and Wales, 1999-2009, are as follows:[5]

- 443 infants had histologically confirmed biliary atresia:
    - 359 had isolated biliary atresia
    - 84 had other significant anomalies, 75% of whom had biliary atresia splenic malformation syndrome (BASM)
- 4 infants died prior to any intervention
- 424 infants underwent the Kasai procedure:
    - 406 infants (96%) had type 3 disease, 12 had type 1, and 6 had type 2
    - 40 infants (9%) had cystic change
- 15 infants underwent primary liver transplant (performed at 0.64 years)
- Median age at Kasai procedure was 54 days (range 7 to 209), and 10% of infants (n=44) were ≥90 days of age at time of procedure. There was variation in age at procedure between centres: 50 days at Birmingham, 51 days at Leeds and 59 days at Kings College London
- Status of infants in January 2010:
    - 50% alive with native liver (n=221)
    - 41% alive with transplanted liver (n=181)
    - 9% dead (n=41)
        - 10% (n=4) died prior to any intervention
        - 58% (n=24) died post-Kasai due to failure and end stage liver disease
        - 32% (n=13) died post liver-transplant due to multiorgan failure, often following re-transplant
- Of those who underwent Kasai procedure 55% cleared their jaundice (total bilirubin <20µmol/l) within 6 months (n=232)
- Estimated 5-year overall survival: 90% (95% CI 88 to 93)

- Estimated 5-year survival with native liver: 46% (95% CI 41 to 51)
- Estimated 10-year overall survival: 89% (95% CI 86 to 93)
- Estimated 10-year survival with native liver: 40% (95% CI 34 to 46)

The European Biliary Atresia Registry (EBAR) was established in 2001-05 to improve data collection of and develop a pan-national strategy to improve clinical outcomes.[7] By 2006 it had received complete enrolment from 60 centres from 20 countries with 514 cases reported over a 5-year period. Based on an estimated incidence in Europe of 1 in 18,000 live births this is believed to represent 35% of the expected cases. Follow-up by each individual centre has been variable and inconsistent, but overall survival figures across countries have ranged from 41 to 92%, and survival with native liver has ranged from 14 to 75%.[7]

The incidence for England and Wales - 0.58 per 10,000 live births[6] – is comparable to that observed in national series of other western countries: 0.51 per 10,000 in metropolitan France,[8] 0.53 per 10,000 in Canada ,[9], 0.5 per 10,000 in Finland,[10] 0.71 per 10,000 in Sweden,[11] 0.52 per 10,000 in Germany,[12] and 0.56 per 10,000 in Switzerland.[13]

The incidence is higher in east asian countries. A national stool colour card screening programme was introduced in Japan in the 1990s, and more recently introduced nationwide in Taiwan in 2004. A recent study reported that in Taiwan between 1996 and 2008, an average of 36.3 cases were diagnosed each year (varying from 17 to 50 across the 13-year period), with an overall incidence of 1.48 per 10,000 live births over the 13-year study period (varying from 0.74 to 1.99 per 10,000), and a female-to-male ratio of 1:06.[14] An earlier study from Japan estimated the incidence there to be 1.04 per 10,000, though this national estimate was based on voluntary registry data.[15]

Summary: Criterion 1 Met

Biliary atresia is an important health condition. It is a rare condition, but one that is fatal without treatment. The national registry of England and Wales has allowed for a valuable overview of all infants diagnosed and treated between 1999 and 2009. All affected infants require surgery to establish bile flow. Ninety-six per cent of affected infants underwent a Kasai procedure, 3% received a primary liver transplant and 1% died prior to receiving any treatment. Almost 90% of affected infants in England and Wales will still be alive after 10 years, but only an estimated 40% will survive without a transplant.

2. The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, disease marker, latent period or early symptomatic stage

Hartley et al. report that although biliary atresia is the most common cause for liver transplant in children, the epidemiology and pathophysiology remain unknown.[1,16]

Epidemiology of infants with biliary atresia in England and Wales

The national data registry has provided an opportunity to study the epidemiology all infants with biliary atresia in England and Wales. Of the 302 infants histologically confirmed to have biliary atresia from January 1999 to December 2006, 133 were male (44%). Almost three quarters of affected infants had isolated biliary atresia (IBA, n=219, 73%) and a quarter had

developmental biliary atresia (DBA, n=76, 25%), which included cystic biliary atresia, BASM and any other syndromic relation.[6] The mean gestational age of affected infants was 38.7 weeks and mean birth weight 3,170g, with no difference between subtypes. Infants in both subtypes failed to thrive with a significant reduction in their weight percentile at the time of first hospital admission. Three-quarters (75%) of affected infants (n=228) were white, 12% (n=46) were of south Asian origin (Indian, Bangladeshi and Pakistani) and the remainder were Afro-Caribbean (n=7), Chinese (n=1) and not identified/mixed race (n=20). Infants with DBA were more likely to be of white origin, and to be female.[6] Other observations were made on the frequency of IBA and DBA according to birth order and predominant mode of feeding, though these differences may be influenced by small sample size. There was no significant evidence for seasonal variation in births.[6]

The marked regional variation in incidence in England and Wales, along a north-west/south-east axis, was notable. It is possible that this could be attributed to variation in susceptibility between ethnic groups; ethnic variation in incidence has been observed since the 1970s.[6] The slight female predominance in this England and Wales cohort has also been demonstrated in other past case series of biliary atresia patients.[12,17]

Influence of phenotype

In the later evaluation by Davenport et al. of all cases of biliary atresia diagnosed and treated in England and Wales between January 1999 and December 2009, 84 of the 443 infants (19%) had associated anomalies, 63 of these were defined as BASM and 21 as significant other anomalies (including congenital malformations, cleft palate and oesophageal atresia).[5] The 84 infants with these developmental anomalies were surgically treated at a lower median age than infants with IBA (49 days vs. 54 days) and observed to have poorer outcomes than infants with IBA (post-operative jaundice clearance 43% vs. 57%, and decreased overall 5-year survival: 72% vs. 94%).[5]

Over the past decades one of the most frequent observations in cohorts of biliary atresia patients is that infants who have biliary atresia splenic malformation (BASM) and other anatomical malformations tend to have poorer outcome than infants with isolated biliary atresia (IBA).[18] It has been speculated that the reason for this may be that the pathological obliterative process begins in the embryonic phase in these infants, compared to occurring perhaps during the later perinatal period in infants with IBA.[1] Similar to the UK study, other cohorts have observed that such developmental anomalies affect between 3 and 20% of infants with biliary atresia.[1,18]

National studies that have also observed poorer outcome in infants with BASM or other congenital malformations compared to infants with IBA:

- The French national registry of all 743 infants with biliary atresia born between 1986 and 2002 observed significantly greater 4-year survival with native liver among infants with IBA compared to those with biliary atresia associated with polysplenia. Analysis was in two cohorts depending on year of diagnosis. In cohort A (382 infants with IBA and 35 with polysplenia born between 1986 and 1996) 4-year survival with native liver was 42.1% vs. 18.4%, respectively; in cohort B (223 infants with IBA and 19 with polysplenia born between 1997 and 2002) 4-year survival with native liver was 45.0% vs. 19.2%, respectively[8]
- Schneider et al. observed 104 infants diagnosed and treated at 9 US centres between 1997 and 2000. The overall two-year outcome (post-operative jaundice clearance and survival with native liver) was significantly poorer in the 11 infants who had BASM[17]

- Leonhardt et al. observed 183 infants diagnosed and treated in Germany between 2001 and 2005. Two-year overall survival was poorer in the 23 infants who had BASM (though survival with native liver was no different)[12]
- Superina et al. observed 244 surgically-treated infants enrolled between 2004 and 2010 in the Prospective Study of Biliary Atresia Epidemiology (PROBE), which had the primary aim of investigating the factors associated with successful Kasai procedure (total bilirubin <2.0mg/dL within the first three months). BASM (n=19) had no effect on the chance of the primary outcome, though none of the 5 infants with non-BASM abnormalities achieved successful drainage. BASM was associated with increased risk of liver transplant or mortality with native liver[19]

However, Guttman et al. (2011) conducted a focused examination on the influence of the congenital/developmental malformations associated with biliary atresia, and came to the conclusion that there was no difference in prognosis between those with IBA and those with associated malformations.[18] They evaluated the outcome of all Canadian infants with extrahepatic congenital malformations born between January 1985 and December 2002. Among 329 infants with confirmed biliary atresia, 44 (13%) had ≥1 malformations. These included: [18]

- Intra-abdominal anomalies: polysplenia (n=25), abnormal abdominal situs (n=9), intestinal malrotation (n=19), portal vein anomaly (n=12), hepatic artery anomaly (n=3), inferior vena cava interruption (n=20)
- Cardiac anomalies: pulmonary stenosis (n=11), ventricular septal defect (n=10), atrial septal defect (n=7), total anomalous pulmonary venous return (n=3), double outlet right ventricle (n=3), tetralogy of Fallot (n=2), atrioventricular canal (n=2), dextrocardia (n=2), bicuspid aortic valve (n=2), hypoplastic aortic arch (n=1) and partial anomalous pulmonary venous return (n=1)

In this Canadian study – the largest North American description of the subgroup of infants with biliary atresia and congenital malformations – these infants were observed to have comparable outcomes to infants with IBA: comparable age at referral, age at Kasai operation, overall survival, survival with native liver, and outcomes following liver transplant.[18] There was also no difference in the female:male ratio among infants with congenital malformations compared to those with IBA. Guttman et al. suggest that the term BASM should be redefined as biliary atresia *structural* malformation, as splenic malformation assumes that an abnormality of the spleen is invariably present. They conclude that though the wide consensus is that infants with BASM fair worse than those with IBA, this may be related to the current classification impeding comparison between subgroups of infants with IBA and those with all congenital malformations.[18]

Anatomical type of biliary atresia has also been observed to have an influence on outcome. Infants with type 1 biliary atresia, where there is patency down to the common bile duct and/or proximal cystic biliary duct, are often observed to have better outcome than those with types 2 or 3 biliary atresia. Among the 443 infants with biliary atresia diagnosed and treated in England and Wales between 1999 and 2009, 40 had cystic change. These infants were operated at lower median age than those without cysts (44 days vs. 55 days), and observed to have improved 5-year survival with native liver (80% vs. 44%).[5] Other studies observing improved outcome in infants with type 1 biliary atresia and/or cystic change compared to infants with types 2 or 3 are those by Serinet et al. (improved 4-year survival with native liver)[8] and Superina et al. (reduced risk of liver transplant or mortality with native liver)[19]

Possible pathophysiological factors

Possible factors that have been considered in the pathophysiology of biliary atresia are genetic, infective, inflammatory and even toxic insult.[1] However, upon reviewing the literature retrieved in the update search, there appears to have been little concrete development in these areas over the past decade.

There is little evidence that biliary atresia is hereditary, with only exceptional reports where siblings or mother-child pairs have been affected.[1] However, the condition has been associated with certain genetic abnormalities including trisomy 18 and 21.[1] The update search identified an additional piece of preliminary research investigating a possible genetic susceptibility. Based on the observation that abnormalities of the primary cilia are the cause of certain renal syndromes where renal cyst formation is associated with liver disease, Hartley et al. (2011) investigated children with biliary atresia treated at Birmingham Children's Hospital who had developed renal cysts.[16] Of 355 children diagnosed with biliary atresia, 9 developed renal cysts, 8 of whom had undergone liver transplant. The incidence of simple renal cysts in the general child population is 0.1%, giving an increased incidence (2.5%) among this population with biliary atresia.[16] To further investigate primary cilia, the researchers looked for mutations in the *PKHD1* gene and examined liver sections for the presence of fibrocystin, which localises to the primary cilia and basal bile ducts. In one child with renal cysts a single gene mutation was found, and in all children in the cohort – those with and without renal cysts – liver sections were negative for fibrocystin. This contrasts with positive fibrocystin staining in samples of healthy liver tissue, and in tissue samples from individuals with other forms of liver disease.[16] This preliminary research may lead to further study examining the genes involved in ciliary development and laterality (e.g. the abnormality of situs inversus which is determined by primary cilia).

The possible multifactorial contribution of viral infection, immune dysregulation and proinflammatory genes in the pathophysiology of biliary atresia has also been considered.[1] A 2009 non-systematic review noted recent investigations that have been made into the role of innate and adaptive immunity in bile duct damage, yet concluded that the cause and mechanisms of the inflammatory response targeting the biliary system are poorly understood.[20] Hepatotropic viruses including rotavirus, cytomegalovirus, reovirus and the hepatitis viruses have also been examined in murine models[21] and in small studies of human biopsy specimens,[22,23] but provide no conclusive evidence on aetiology.

Summary: Criterion 2 partly met

The epidemiology of the condition (incidence and prevalence) is well understood and documented, but the natural history remains less well understood. It is also unclear whether the pathophysiological process may differ between infants with different anatomical type of biliary atresia or those who have associated syndromes. There have been observations that type 1 biliary atresia and/or cystic change is associated with improved outcomes compared to type 2 or 3 disease involving more extensive parts of the extrahepatic biliary system. Associated congenital malformations – the most common is splenic malformation syndrome – affect up to 20% of infants with biliary atresia, and have also frequently been observed to be associated with poorer outcome in some studies.

Infants with biliary atresia are currently diagnosed due to the persistence of jaundice beyond 2 weeks and/or detection of pale coloured stool. There is no clear understanding of the stage at which biliary atresia develops. It has been speculated that the presence of congenital malformations is associated with poorer outcomes because pathological changes in these infants occurs earlier, during the developmental phase, rather than in the

perinatal/postnatal period as may be the case in infants with isolated biliary atresia. The possible influence of genetics, viral infection and inflammation in the pathophysiology of biliary atresia continue to be investigated.

3. All the cost-effective primary prevention interventions should have been implemented as far as practicable

Not applicable. There are no primary preventative interventions for biliary atresia.

4. If the carriers of a mutation are identified as a result of screening the natural history of people with this status should be understood, including the psychological implications.

Not applicable. Biliary atresia is not known to be associated with carriage of a particular genetic mutation.

5. There should be a simple, safe, precise and validated screening test

One key study was published in 1999 that assessed the feasibility of screening for cholestatic hepatobiliary disease using tandem mass spectrometry (TMS) to measure conjugated bile acids in dry blood spots on the Guthrie screening card.[4] The NHS Newborn Blood Spot Screening Programme currently offers collection of dry blood spot on the Guthrie screening card at 7 to 10 days for the screening of certain metabolic diseases. Therefore the current screening programme offers the potential to extend screening to other diseases. However, this study found that there was considerable overlap in bile acid concentration between the few infants with cholestatic hepatobiliary disease and the majority of the healthy infant population, meaning that the accuracy of this test was too low to support mass screening for biliary atresia using this single test alone. The update search identified no progress in dry blood spot screening since 1999, and as such the findings of this key study are presented here to provide background on dry blood spot screening. Later studies have demonstrated that conjugated and direct bilirubin levels are accurate predictors of cholestatic hepatobiliary disease in general, and of biliary atresia in particular, but so far this has only been tested in liquid blood samples, and the limitations of this approach mean that little progress could be made unless methods are adapted to allow for testing of conjugated bilirubin in dry blood spots, which is the simpler test suitable for a screening programme. An alternative approach to screening is the stool colour card programme, and this was introduced nationwide in Taiwan in 2004. This involves selection of the most appropriate colour match on a six-colour card and notification to the national infant stool colour registry if abnormal stool is identified. Reports of the progress of this programme are also presented here.

Measurement of conjugated bile acids in dry blood spots

Mushtaq et al. (1999) evaluated the screening test in 177 children with cholestatic hepatobiliary disease identified from King's College London and Birmingham Children's Hospitals.[4] Eligible cases had been born in the UK, referred for conjugated hyperbilirubinaemia at between 14 days and 1 year of age, and had a final diagnosis (61 had biliary atresia). The study used unused blood spots originally collected at around 7-10 days of age for phenylketonuria screening. Cases were compared with two anonymised screening

cards stored on either side of each index card (n=708). Concentrations of the following conjugated bile acids were measured and receiver operator curves (ROC) plotted to determine which parameter or combination of parameters would produce optimal accuracy:[4]

- glycodihydroxycholanoates
- glycotrihydroxycholanoates
- taurodihydroxycholanoates
- taurotrihydroxycholanoates

The sensitivity and specificity of different cut-off values for predicting cholestatic hepatobiliary disease and biliary atresia was calculated for each bile acid, and for total bile acid (TBA) concentration. The upper limit of 'normal' for TBA concentration in the control group (based on the 97.5th centile value) was 33µmol/l (95% confidence interval 31 to 42µmol/l). TBA concentration was >33µmol/l in 59% of all cases (n=104), and in 77% of those with biliary atresia (47 cases). Of the remaining cases with biliary atresia, TBA was 20-33µmol/l in 7 cases, and <20µmol/l in 7 cases.[4]

Using a TBA cut-off of 30µmol/l gave:[4]

- sensitivity of 61.5% and specificity of 96.3% for all cholestatic hepatobiliary disease
- sensitivity of 78.7% and specificity of 96.3% for biliary atresia

Sensitivity, specificity and positive and negative predictive values for cholestatic hepatobiliary disease (Figure 1) and biliary atresia (Figure 2) at different TBA cut-offs are presented below. The authors consider that a recall rate of 2% or higher for diagnostic testing among false positives (i.e. specificity below 98%) would be unlikely to gain acceptance as a screening test. As can be seen, raising the cut-off to improve specificity and reduce the false positive rate gives a rapid decline in sensitivity. At a cut-off of 35µmol/l specificity is 97.8% and sensitivity for biliary atresia is 70.5%; raising the cut-off to 48.5µmol/l to give a specificity of 99.0% decreases the sensitivity to only 50.8%.

			Prevalence of disease			
			1 in 500		1 in 2500	
Cut off (µmol/l)	% Sensitivity (95% CI)	% Specificity (95% CI)	PPV %	NPV %	PPV %	NPV %
25.0	65.9 (59.6 to 71.2)	94.0 (92.0 to 94.7)	2.14	99.93	0.43	99.99
30.0	61.5 (55.4 to 67.9)	96.3 (94.9 to 97.2)	3.22	99.92	0.66	99.98
35.0	53.3 (47.1 to 59.8)	97.8 (97.2 to 99.1)	4.63	99.90	0.96	99.98
48.5	35.2 (29.3 to 41.1)	99.0 (98.1 to 99.5)	6.83	99.87	1.44	99.97
69.0	12.1 (8.4 to 16.9)	99.5 (98.7 to 99.8)	4.22	99.82	0.87	99.96
79.0	7.1 (4.3 to 11.1)	100.0 (99.6 to 100.0)	100	99.81	100	99.96

Figure 1: Sensitivity and specificity of measurements of total bile acid concentration in prediction of cholestatic hepatobiliary disease, and positive and negative predictive values, assuming a prevalence of cholestasis of 1 in 500 or 1 in 2,500[4]

			Prevalence of extrahepatic biliary atresia			
			1 in 10 000		1 in 20 000	
Cut off (µmol/l)	% Sensitivity (95% CI)	% Specificity (95% CI)	PPV %	NPV %	PPV %	NPV %
25.0	85.3 (75.5 to 92.0)	94.0 (92.3 to 95.3)	0.14	100.00	0.07	100.00
30.0	78.7 (68.1 to 86.9)	96.3 (94.9 to 97.4)	0.21	100.00	0.11	100.00
35.0	70.5 (59.4 to 79.9)	97.8 (96.7 to 98.6)	0.32	100.00	0.16	100.00
48.5	50.8 (39.6 to 62.0)	99.0 (98.2 to 99.5)	0.53	100.00	0.26	100.00
67.0	21.3 (13.1 to 31.9)	99.5 (98.7 to 99.8)	0.39	99.99	0.19	100.00
79.0	9.8 (4.4 to 18.7)	100.0 (99.6 to 100.0)	100.00	99.99	100.00	100.00

Figure 2: Sensitivity and specificity of measurements of total bile acid concentration in prediction of biliary atresia, and positive and negative predictive values, assuming a prevalence of 1 in 10,000 or 1 in 20,000[4]

Total bile acid concentration and taurotrihydroxycholanoate concentration were the best predictors of both cholestatic hepatobiliary disease, and biliary atresia specifically. However, despite the potential for TMS as a method to detect neonatal cholestasis using the current Guthrie screening card system, the overlap in bile acid concentration between infants with cholestatic hepatobiliary disease and those without was too great for this to be used as a single screening test.

Mushtaq et al. suggested that possible ways to enhance performance of the screening test could be to also measure conjugated bilirubin in dry blood spots using TMS. However, conjugated bilirubin is susceptible to photodegradation, which would require blood spots to be protected from the light. Alternatively, Mushtaq et al. also reported on unpublished observations that cholestasis is associated with elevated plasma phospholipid concentrations, and this can be measured in blood spots by TMS. They finally suggest that bile acid analysis could be combined with clinical detection of jaundice.[4]

The update search identified no further research evaluating dry blood spot screening, though there has been study of measurement of conjugated bilirubin in liquid blood samples.

Measurement of conjugated bilirubin in liquid blood samples

Powell et al. (2003) evaluated a community-based screening programme for neonatal cholestatic liver disease that measured conjugated bilirubin in liquid blood samples.[24] The study included 27,654 infants born in the Birmingham Health District between August 1995 and July 1997, for whom spare plasma from routinely collected liquid screening samples (collected at <28 days of life) was available. Samples were stored under appropriate conditions to prevent degradation of bilirubin, and conjugated and unconjugated bilirubin were measured using dry slide chemistry (requiring 50µl of plasma for both tests). An abnormal conjugated bilirubin level was defined as a level >18µmol/l and fraction (percentage of total bilirubin) >20% (based on the 97.5th centile value obtained in a pilot study). Infants with abnormal results had a second heel prick for repeat bilirubin testing and liver function tests, and if these results were abnormal, referral was made to the Liver Unit at Birmingham Children's Hospital. False negatives (infants whose screening result was normal but who developed liver disease within the first year of life) were identified through contact with neonatologists/paediatricians and clinical laboratories in the district at the end of the study.

The main findings were as follows:[24]

- 84.7% of samples were suitable for testing (23,435/27,654), the remainder being unusable due to gross haemolysis or insufficient sample (a further 221 samples were excluded because the baby was over 27 days old)
- Conjugated bilirubin level exceeded 18µmol/l in 3.8% of tested samples (878/23,214)
- Conjugated bilirubin fraction exceeded 20% in 16.1% of samples (3748)
- Both cut-offs were exceeded in 0.46% of samples (107)
- 11% of infants with a positive screen test remained abnormal on repeat testing (12/107)
- 11/12 with abnormal repeat tests had clinical liver disease: 2 had biliary atresia, 6 had neonatal hepatitis, 1 had hypopituitarism, 1 had Alagille syndrome and 1 had

alpha-1-antitrypsin deficiency (the 1 false positive was a carrier for alpha-1-antitrypsin deficiency)

- Of the 23,107 infants with a normal test result, none had clinical liver disease

In this study, testing conjugated bilirubin levels in neonatal liquid blood samples taken before 28 days of age had a sensitivity of 100% (95% CI 76.2 to 100) and specificity of 99.59% (95% CI 99.5 to 99.67) for neonatal liver disease.[24] The positive predictive value was 10.3% (95% CI 4.5 to 16.0). Raising the conjugated bilirubin cut-off to >19µmol/l while keeping the conjugated bilirubin fraction at >20% increased specificity to 99.67% and positive predictive value to 12.5% with no effect on sensitivity. However, among the 4,219 samples (15.3% of the cohort) that were unsuitable for testing due to haemolysis of insufficient sample, there were 6 cases of liver disease, including 1 case of biliary atresia.[24] Therefore though the testing of liquid blood samples appeared to be accurate, this highlights its lack of feasibility as a screening test, considering the high recall for testing that would be needed.

Testing in a separate sample of 2,425 infants who were still hospitalised at the time of testing found a positive screen result (on repeat testing) in 2.0% of samples (43 infants). However, only 6 of these infants (14%) had primary liver disease (none biliary atresia); the remaining positive tests were among infants with liver dysfunction secondary to another condition. There was also one false negative test in a hospitalised infant who was later diagnosed with neonatal hepatitis at 9 weeks of age.[24]

Powell et al. concluded that conjugated bilirubin is an effective marker for neonatal liver disease and a community screening programme could improve referral age.[24] However, the problem of haemolysis and insufficient sample collection was significant and a repeat testing rate of 15% would be unacceptably high. Further, modification of protocol would be required for infants hospitalised at the time of testing.

At the time of this project Powell et al. reported that liquid blood samples were being collected from around 18% of newborns in the UK, but there has since been greater switch to collection of dried blood spots, including in the Birmingham Health District where this study was conducted.[24] As Powell et al. acknowledge, progress in the screening programme would require adaptation to testing conjugated bilirubin in dried blood spots using TMS, and at the time of this update search no further developments have been made.

Further observational studies evaluating biomarkers in liquid blood samples

A case control study conducted by Harpavat et al. (2011) aimed to determine the earliest time that elevated direct bilirubin (DB) or conjugated bilirubin (CB) could be detected in infants with biliary atresia.[25] DB measures the total concentration of CB and the small proportion of CB that is bound to albumin (delta bilirubin).[26] In their study Harpavat et al. considered DB and CB measures to be similar, but not equivalent.

Cases were 61 children with biliary atresia who were born between January 2007 and December 2010 and cared for at Texas Children's Hospital. Controls were the first 75 term infants born at the beginning of each season (April 2010, July 2010, October 2010, and January 2011) in a large county hospital in Houston, where all newborns have DB and total bilirubin measured in the first 24-48 hours of life, regardless of clinical condition.

Retrospective data was collected on birth times, and DB or CB levels.[25]
Fifty-six percent of cases (34/61) had DB or CB measures taken in the first 96 hours of life. In all of these cases, serum DB/CB levels exceeded laboratory norms and rose over time after birth (Figure 3). The mean serum DB level at 0 to 24 hours of life was 0.98mg/dL (+/- 0.17), and at 72-96 hours after birth was 2.5mg/dL (+/- 0.72) in infants with biliary atresia. The

earliest DB measurement, taken at 1 hour after birth, was 1.1mg/dL. Conjugated bilirubin levels demonstrated a similar pattern on graphical representation (Figure 3), with the earliest measurement of 1.0mg/dL taken in one case at 17 hours after birth, rising to a measurement of 3.3mg/dL taken in one case at 80 hours after birth.[25]

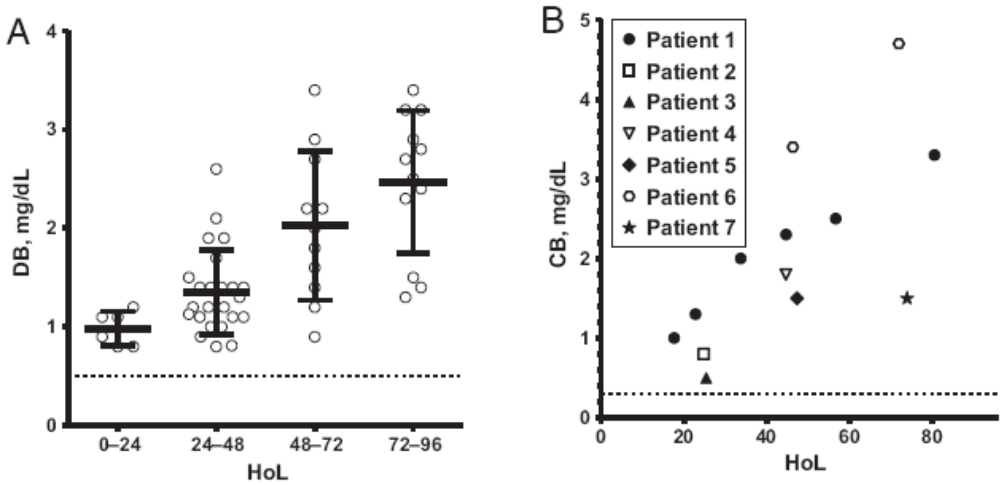

Figure 3: A) Mean DB levels in cases later diagnosed with biliary atresia: at 0-24 hours after birth (n=6), 24-48 hours (n=24), 48-72 hours (n=11) and 72-96 hours (n=12). B) Mean CB levels after birth in 7 cases later diagnosed with biliary atresia. Dashed lines indicated upper limit of norm: 0.5mg/dL for DB and 0.3mg/dL for CB.[25]

Mean serum DB levels at 24 to 48 hours after birth were higher in cases than in controls, with no overlap in values: 1.4mg/dL (+/- 0.43) vs. 0.19mg/dL (+/-0.075) ($p<0.0001$). However, total bilirubin (TB) levels in cases were not excessively high and remained below phototherapy levels for term infants. Also in the majority of cases (79%) the DB:TB ratio was ≤20%, which is considered normal. Only 53% of cases who had bilirubin measures taken in the first hours 96 hours of life (18/34) received follow-up after discharge.[25]
Harpavat et al. demonstrated in this case control that though biliary atresia is believed to be a disease acquired after birth, evidence of disease is already present in the immediate neonatal period. All 34 cases with biliary atresia who had DB or CB measures taken within the first 96 hours of life, had levels significantly higher than controls. Cases with and without measures taken had similar clinical course of disease and outcomes, which suggests that these findings are representative of the general population with biliary atresia.[25] However, the case control is small, and the majority of cases underwent serum testing of direct bilirubin rather than conjugated bilirubin levels.

Another study (Davis et al. 2011) also examined the interpretation of DB and CB levels in neonates, and observed that all infants with biliary atresia have raised levels within the first 14 days of life, but concluded that DB and CB are not interchangeable measurements.[26]

Davis et al. (2011) evaluated a cohort of all term infants born between January 1995 and December 2004 at 12 hospitals within the Northern California Kaiser Permanente Program (NC-KPMCP).[26] Eligible infants had at least one DB or CB level measurement taken within the first 2 weeks of life (n=66,431). Eleven of the 12 NC-KPMCP hospitals measured CB levels (on Vitros instruments using the BuBc slide method) rather than DB, while the remaining hospital measured DB levels (this is reportedly significant as within NC-KPMCP conjugated and unconjugated levels are measured regardless of whether there is suspicion of cholestasis, but DB usually has to be specifically requested). Conjugated bilirubin levels were obtained for 64,095 infants, and direct bilirubin for 2,898. ICD-9 diagnostic codes from inpatient and outpatient visits were obtained for the whole cohort.[26]

The 99th percentile for CB in this cohort was 0.5mg/dL and for DB was 2.1mg/dL, with DB levels considerably higher because of the differences in how the two measures are made. Among 19,906 infants who had CB levels measured within the first 3 days of life, and then repeated at 3-14 days, there was a significant increase in the mean CB level from 0.10 to 0.15mg/dL ($p<0.001$).[26]

The prevalence of biliary atresia within this cohort was around 1 in 10,040, with 27 infants affected. All of these infants had cholestasis in the first 14 days of life with conjugated bilirubin level ≥2mg/dL or direct bilirubin ≥5mg/dL. Though other diseases were more common, biliary atresia was the only condition where 100% of affected infants had abnormally elevated levels. The study found that CB levels between 0.5 and 1.9mg/dL can be associated with bacterial infection, but above this level hepatobiliary disease becomes more likely. Davis et al. report a likelihood ratio (LR) for hepatobiliary disease of 495 (95% CI 267 to 919) with CB level ≥5mg/dL; LR of 80.6 (95% CI 49.9 to 130.0) with CB level 2.0 to 4.9 mg/dL; and LR of 2.77 (95% CI 1.85 to 4.15) with CB level 0.5 to 1.9mg/dL. With a CB level ≥5mg/dL, 47% of infants have biliary disease and 43% have liver disease.[26]

Stool colour card screening

An alternative method of screening is using the stool colour card system. This was introduced in Japan in the early 1990s, and more recently introduced nationwide in Taiwan in 2004.

The pilot scheme was first introduced to 49 hospitals in northern and central Taiwan in March 2002, with extension of the scheme to a further 96 hospitals in southern and eastern Taiwan in 2003.[27] An infant stool colour card was designed with seven photographs of different coloured stool samples: three colours labelled abnormal (clay-coloured, white to pale/light yellowish) and four labelled normal (yellowish to brown to greenish). Parents were asked to observe their infant's stool colour, and medical staff checked the picture number chosen at one month of age during the routine health check-up and sent this card to the stool colour card registry centre (abnormal colour selections were faxed to the centre within 24 hours). Abnormal acholic colours were followed until a definitive diagnosis was made. During the second year of the pilot the labelling of 'normal' and 'abnormal' were removed from the stool colour card in case labelling had an influence.[27]

The average return rate of stool cards was 65.2% (78,184 infants of 119,973 live births at participating hospitals). There were a total 94 infants with abnormal stool colours, and 29 of these (30.9%) had biliary atresia, giving an incidence of biliary atresia in these screened infants of 3.7 per 10,000. Of the remaining 65 infants with abnormal colour, 11 had other pathology (neonatal hepatitis in 8 infants, and one case each of progressive familial intrahepatic cholestasis, choledochal cyst and benign hepatic haemangioma) and 54 infants were found to be normal after >3 months of follow-up. Of the 29 infants with biliary atresia, 26 (90%) were detected by screening prior to 60 days of age, and 17 (58.6%) underwent the Kasai procedure prior to 60 days of age. For the detection of biliary atresia prior to 60 days of age, stool colour card screening had 89.7% sensitivity, 99.9% specificity, 28.6% positive predictive value and 99.9% negative predictive value.[27]

Chen et al. stated that one infant who had not returned a stool colour card was reported to have biliary atresia 'by a paediatrician in another country not participating in this study'. Therefore the authors conclude that there were 30 infants diagnosed with biliary atresia during the study period. However, the study provides no further detail on whether there was any systematic method to identify false negatives among screened infants, or to identify

cases among the 41,789 infants who did not participate in screening. Therefore it is not clear whether there has been exhaustive follow-up of the full 119,973 infants who would have been eligible for screening.[27]

Stool colour card screening was introduced nationwide in Taiwan in 2004, with a revised six-colour stool colour card (three normal colours and three abnormal) integrated into the child health booklet and the national infant stool colour registry card system established. Hsiao et al. reported on the sensitivity and specificity of stool colour card screening for biliary atresia in the first two years following nationwide introduction, including 216,419 infants born in 2004, and 205,854 born in 2005.[28] In 2004 and 2005 there were 75 infants diagnosed with biliary atresia in Taiwan, 63 of whom (84%) were successfully detected by screening prior to 60 days of age. However, the sensitivity of stool colour card screening for detecting biliary atresia prior to 60 days of age differed in the two years: in 2004, 29 of 40 infants were screen detected prior to 60 days giving a sensitivity of 72.5%; in 2005 sensitivity increased to 97.1% (34/35 infants). The 63 infants detected prior to 60 days represented 19.6% of those reported to have acholic stools in 2004, and 26.0% of those reported to have acholic stools in 2005. The majority of abnormal reports (71% in 2004 and 63% in 2005) were only transient pale coloured stool not associated with pathology. Other pathological causes of abnormal reports, aside from biliary atresia, included neonatal hepatitis, TPN (total parenteral nutrition) cholestasis, and progressive familial intrahepatic cholestasis.[28]

Of the total 75 infants diagnosed with biliary atresia in Taiwan in 2004 and 2005:[28]

- Pale stool was first recognised at mean age 27.8 days (+/- 17.6)
- First hospitalisation was at mean age 43.6 days (+/- 22.1)
- Kasai operation was performed at mean age 54.6 days (+/- 17.1)

Hsiao et al. compared the age at operation of infants diagnosed in 2004 and 2005, with the operative age during the stool colour card pilot (2002-2003) and with a historical cohort of 144 infants treated at the National Taiwan University Hospital between 1976 and 2000 (excluding 41 infants who were lost to follow-up at one year). Of infants who received the Kasai procedure:[28]

- In 1976-2000, 47.2% were treated at ≤60 days
- In 2002-2003, 58.6% were treated at ≤60 days
- In 2004, 60.0% were treated at ≤60 days
- In 2005, 74.3% of infants were treated at ≤60 days

Figure 4 displays this data: the number of diagnosed cases, the number operated and age at operation.

	1976–2000*	2002–2003†	2004	2005
Number of cases of BA	144	29	40	35
Received Kasai operation	135 (93.8%)	28 (96.6%)	39 (97.5%)	35 (100%)
≤60 days	68 (47.2%)‡§ |	17 (58.6%)‡	24 (60.0%)§	26 (74.3%) |
61–90 days	45 (31.3%)	8 (27.6%)	15 (37.5%)	9 (25.7%)
≥91 days	22 (15.3%)	3 (10.3%)	0 (0%)	0 (0%)
Without Kasai operation	9 (6.2%)	1 (3.4%)	1 (2.5%)	0 (0%)

Abbreviation: BA, biliary atresia.

* From 1976 to 2000, 185 infants with BA received treatment and follow-up at the National Taiwan University Hospital, and 41 patients were excluded because they were lost to follow-up at 1 year old.

†A pilot regional screening program for BA by the infant stool card covering 4 regions of Taiwan from 2002 to 2003.[4]

‡Relative risk, 1.2; 95% confidence interval (CI), 0.9–1.8; $P = 0.264$, early Kasai operation rate ≤ 60 days in 2002–2003 versus 1976–2000 (1976–2000 group, relative risk of 1.0).

§Relative risk, 1.3; 95% CI, 0.9–1.7; $P = 0.154$, early Kasai operation rate ≤ 60 days in 2004 versus 1976–2000.

\| Relative risk, 1.6; 95% CI, 1.2–2.0; $P = 0.004$, early Kasai operation rate ≤ 60 days in 2005 versus 1976–2000.

Figure 4: Age at surgery among infants with biliary atresia during the stool card screening programme (2002-2005) versus the era prior to stool card screening (1976-2000)[28]

Further Taiwanese studies have been conducted comparing operative age and 3-year outcomes in the years prior to introduction of the stool colour card screening programme with outcomes following introduction of the programme. The findings of these studies are presented below. These findings are later referred to in Section 10, which presents a comparison of these outcomes with those in western countries.

In 2011 Lien et al. reported on the operative age and longer term 3-year outcomes of infants with biliary atresia who underwent the Kasai procedure before and after introduction of stool colour card screening in Taiwan.[29] They considered three cohorts: cohort A, 89 cases diagnosed and treated at the National Taiwan University Hospital 1990-2000, for whom 3-year follow-up was available; cohort B, the 28 cases diagnosed and treated 2002-2003 (the period of the pilot programme); and cohort C, the 74 cases diagnosed and treated 2004-2005 (after introduction of nationwide screening). Data from cohorts B and C was combined to represent the whole era of the stool colour card screening programme (2002-2005). Lien et al. found improvements in the following outcomes after introduction of the screening programme:[29]

- An increase in the proportion of infants undergoing a Kasai operation at <60 days of age: 49.4% of cohort A (44/89) vs. 65.7% of cohorts B and C (67/102) (p=0.02)
- An increase in the proportion of infants jaundice-free (<2.0mg/dL) 3 months after Kasai operation: 34.8% vs. 60.8% (p<0.001)
- An increase in 3-year overall survival rate: 64.0% vs. 89.2% (p<0.001)
- An increase in 3-year jaundice-free-survival-with-native-liver rate: 31.5% vs. 56.9% (p<0.001)
- No before-after difference in 3-year survival with native liver: 51.7% vs. 61.8% (p=0.16)

Tseng et al. also evaluated the effectiveness of stool colour card screening in 2011, this time using claims data from the National Health Insurance Research Database.[14] The medical charts of all inpatients, 1996-2008, whose first, second or third ICD diagnosis was biliary atresia were identified. An ICD Operation (OP) code for Kasai operation or liver transplant was considered as confirmation of diagnosis. In total, 472 infants had 'confirmed' biliary atresia during the study period, 312 patients identified prior to nationwide introduction of the stool colour card screening programme (1996-2003), and 160 identified from 2004 onwards. Of the 472 with confirmed biliary atresia, 440 underwent the Kasai operation, and 32 received primary liver transplant without a prior Kasai procedure.[14] Tseng et al. used a before-after analysis to examine the effect of the screening programme.

The main findings on the effectiveness of the stool colour card screening programme were:[14]

- Median age at first admission for suspected biliary atresia decreased after introduction of screening: 47 days vs. 43 days (p=0.028)
- Median age for patients at time of the Kasai operation decreased after introduction of screening: 51 days vs. 48 days (p=0.051)
    - significance of the before-after difference was removed by excluding any patients referred at >90 days of age (49 vs. 47 days, p=0.18), or treated at >75 days of age (48 vs. 45 days p=0.10)
- There was no significant before-after difference in the proportion of infants undergoing a Kasai operation at ≤60 days of age: 68.9% vs. 73.6% (p=0.31)
- There was no significant before-after difference in the proportion of infants undergoing a Kasai operation at >90 days of age: 9.5% vs. 4.9% (p=0.094)

- Of the 32 infants across the study period who received a primary liver transplant without prior Kasai operation, their median age at time of transplant was 317 days (range 182 to 693 days)

Tseng et al.'s before-after study demonstrated that implementation of the stool colour card screening programme in a country where there is comparatively high incidence of biliary atresia had reduced the age at first admission for suspected biliary atresia by a median of 4 days, and reduced the age at time of Kasai operation by a median 3 days.[14] However, unlike the reports of Hsiao et al.[28] and Lien et al.[29] they found no significant difference in the proportion of infants treated prior to 60 days. As the study is based on analysis of claims data, it is not possible to determine in this study how many infants were diagnosed with biliary atresia as a result of detection through stool card screening.
No randomised controlled trials have been conducted examining the introduction of stool colour card screening.

The update search identified only one western publication evaluating stool colour screening; and this was a UK community project by Crofts et al. in 1999.[30] NICE evaluated this study when making the recommendation in their Neonatal Jaundice guideline (2010) that infants with prolonged jaundice and/or pale stool and/or dark urine require further investigation.[3] Crofts et al. conducted a 3-phase study in Sheffield during 1995-96. The first phase involved devising a 25-colour chart (20 normal colours, 5 abnormal) based on inspection of stool from healthy infants and those with cholestatic liver disease. This was then tested on 109 parent-infant pairs. In 5,053 stool observations made over 12 days all 20 normal colours were selected and none of the abnormal colours. In the second phase the six colours most commonly selected by parents were used, alongside three abnormal colours, to develop a simplified chart. The third phase included 3,629 parent–infant pairs, resident in Sheffield, who were administered the colour chart at the first health visitor contact at 10-14 days. Information was then re-collected by the health visitor when the infant was 28 days old. The breastfeeding rate at this time was 37%.[30]

In total, 127 babies were jaundiced at 28 days of age, 125 of whom were breastfed. The incidence of jaundice at 28 days among breastfed infants was 9.2%. Blood samples were collected from 107 infants, and the mean bilirubin level was 131µmol/l and conjugated bilirubin 14µmol/l. In 60 infants for whom liver function tests were available, gamma glutamyl transferase and alanine aminotransferase were elevated in 23, and alkaline phosphatase elevated in 42.[30] Though abnormal liver function was common among jaundiced infants, none of them had presented with abnormal stool colour and none were found on further investigation to have liver disease. Four non-jaundiced infants were reported to pass pale stools, but they were not investigated as stool returned to normal colour and all were thriving at the 6 months follow-up. [30]

Croft et al. concluded: [30]
"The debate about screening for extrahepatic biliary atresia and other liver disease in infancy remains unresolved. At present, an active community-wide search for babies with prolonged jaundice seems hard to justify, since it is a very non-specific marker. We have shown that the stool colour chart has high specificity, but clinical data suggest that it would not be 100% sensitive. Whether or not its routine use in the Personal Child Health Record could reduce the mean age of referral and the number of late-operated cases is a difficult question to answer…We now need to compare the costs and benefits of three possible policies: actively seeking and investigating every baby with prolonged jaundice; a selective

policy that relies on the colour chart as a screening mechanism in well breastfed babies with prolonged jaundice; and a purely reactive approach dependent on clinical presentation."

Based on the findings of this study the NICE guideline development group concluded that there is no evidence to show that the examination of stool colour is helpful in the recognition of jaundice in babies.[3] NICE consider that though prolonged jaundice is common in breastfed infants, this is rarely found in combination with persistently pale stools and/or dark urine. NICE recommends that the combination of these signs suggests pathology and indicates immediate referral for investigation.[3]

Other potential screening methods

One pilot study was identified that evaluated urinary sulfated bile acid (USBA) analysis for the early detection of biliary atresia.[31] The study enrolled 1,148 term infants born at the affiliated hospitals of two Japanese universities between 2006 and 2007. Parents were instructed to collect their infant's urine at a randomly assigned time point, ranging from 10 to 40 days after birth. USBA-to-creatinine ratio was measured and a cut-off of 55µmol/g creatinine was used (based on the 99th percentile observed in a prior study measuring USBA/creatinine at one month of age in 91 infants). Infants were followed up to the 3 and 4 month routine postnatal health checks.

At first analysis 47 infants (4.1%) had a USBA/creatinine level above the cut-off. Of these, only 2 were eventually found to have liver disease, 1 of whom had biliary atresia. Of the remaining 45, 13 were found to be without jaundice or pale coloured stool and did not undergo re-test; 27 were below the cut-off at re-test; 5 who remained above the cut-off at re-test were not found to have liver disease following clinical health checks and blood tests. All 1,101 infants who were below the cut-off were confirmed to be free of biliary atresia at the 3-4 month health check. For the detection of cholestatic hepatobiliary disease this study found USBA testing to have a sensitivity of 100%, specificity of 96%, positive predictive value of 4% and negative predictive value of 100%.[31] Potential advantages of this test include the non-invasive nature, and that it does not rely upon subjective parental judgment like stool colour card screening; however, as the authors of this pilot acknowledge, the high false positive rate is a significant obstacle to use of this method.[31]

Summary: Criterion 5 Not Met

There is no simple, safe, precise and validated screening test for biliary atresia. Several have been proposed but none is ideal. In 1999 Mushtaq et al. evaluated the potential for a screening test that used tandem mass spectrometry to measure conjugated bile acids in dry blood spots on the Guthrie screening card.[4] Though this would be a simple and safe method, as the NHS Newborn Blood Spot Screening Programme is already offered to all infants in the first days of life for the screening of certain metabolic disorders, this method alone was not accurate for the detection of biliary atresia due to the considerable overlap in bile acid concentration between the few infants with cholestatic hepatobiliary disease and the majority of the healthy infant population. Reviewing the studies retrieved in the 2011 update search, there appear to have been no further developments in dry blood spot screening for biliary atresia since this 1999 study.

Powell et al. (2003) demonstrated that an elevated conjugated bilirubin (level >18µmol/l and fraction >20%) in neonatal liquid blood samples had a sensitivity of 100% and specificity of 99.59% for detection of neonatal liver disease. [24] In this study 11 of 12 infants detected by the test had clinical liver disease, 2 of whom had biliary atresia. Further observational studies have also demonstrated that elevated direct or conjugated bilirubin levels in

neonatal liquid blood samples are a reliable indicator of biliary atresia, and exceed laboratory norms within the first 24-48 hours of life, with levels increasing with age.[25,26] However, despite this apparent accuracy, measuring conjugated bilirubin in liquid blood samples has two important limitations which restrict its potential value as a screening test: firstly, liquid blood samples are not routinely collected from neonates in the UK; and secondly, even if obtained, Powell et al. observed that 15.3% of liquid blood samples were unsuitable for testing, among these untested infants being 6 cases of liver disease, including 1 case of biliary atresia, indicating a need for a high rate of recall testing. Therefore screening for biliary atresia through testing of conjugated bilirubin would have limited potential unless it could be adapted to dry blood spot screening. Reviewing the update search there appears to have been no progress adapting methods to allow accurate screening for biliary atresia using TMS to measure conjugated bilirubin in dry blood spots.

Stool colour card screening was introduced nationwide in Taiwan in 2004. The six-colour card has been integrated into the child health booklet and medical personnel/parents or guardians are required to report within 24 hours to the national infant stool colour registry if abnormal stool is identified. The stool colour card pilot study demonstrated the system to have 89.7% sensitivity and 99.9% specificity for detecting biliary atresia prior to 60 days of age, but a positive predictive value of only 28.6%.[27] Before-after studies have been conducted analysing age at first hospital admission for suspected biliary atresia and age at Kasai procedure, prior to and after introduction of screening in Taiwan. The largest of these studies observed that prior to nationwide introduction in 2004 the median age at admission was 47 days and at treatment was 51 days, decreasing to 43 days and 48 days, respectively, after nationwide introduction of screening.[14] This study did not observe a significant increase in the proportion of infants treated prior to 60 days of age, or significant decrease in those treated after 90 days of age.

No European or North American studies evaluating stool colour card screening have been published since 1999. Existing NICE guidance concludes that there is no evidence to show that the examination of stool colour is helpful in the recognition of jaundice in babies, though the combination of these signs suggests pathology and indicates immediate referral for investigation.[3]

6. The distribution of test values in the target population should be known and a suitable cut-off level defined and agreed

The 1999 study by Mushtaq et al. assessed the diagnostic accuracy of using tandem mass spectrometry (TMS) to measure conjugated bile acids in dry blood spots on the Guthrie screening card (see Section 5). It did not find a suitable cut-off level as there was too much overlap between the few affected infants and the majority of the healthy infant population. The upper limit of normal for total bile acid concentration in the control group (based on the 97.5th centile value) was 33µmol/l. A cut-off of 30µmol/l had a sensitivity of 78.7% and specificity of 96.3% for biliary atresia. The authors consider that a recall rate of 2% or higher for diagnostic testing among false positives (i.e. specificity below 98%) would be unlikely to gain acceptance as a screening test. Raising the cut-off to improve specificity and reduce the false positive rate gives a rapid decline in sensitivity. At a cut-off of 35µmol/l specificity is 97.8% and sensitivity for biliary atresia is 70.5%; raising the cut-off to 48.5µmol/l to give a specificity of 99.0% decreases the sensitivity to only 50.8%.[4]

Powell et al. in 2003 demonstrated that testing conjugated bilirubin level in liquid blood samples using a cut-off level >18µmol/l and conjugated bilirubin fraction >20% had a 100%

sensitivity and 99.59% specificity for neonatal liver disease.[24] Raising the conjugated bilirubin cut-off to >19µmol/l while keeping the conjugated bilirubin fraction at >20% further increased specificity to 99.67% with no effect on sensitivity. This would suggest that if testing conjugated bilirubin levels in blood met other criteria it could be a highly accurate test for neonatal liver disease, but less so for biliary atresia alone. However, as discussed in Section 5, measurement of conjugated bilirubin could have little further potential as a screening test unless methods could be adapted to allow measurement of conjugated bilirubin levels in dry blood spots. So far, there appears to have been no progress in this area.

Summary: Criterion 6 Not Met

7. The test should be acceptable to the population

If it were possible to screen for biliary atresia using dry blood spots then this would be likely to be an acceptable method as the NHS Newborn Blood Spot Screening Programme already offers screening for phenylketonuria, congenital hypothyroidism, sickle cell disease, cystic fibrosis and medium-chain acyl-CoA dehydrogenase deficiency (MCADD) using this method of heel prick at around 7-10 days. However, as presented in Section 5, an accurate screening test for biliary atresia using dry blood spot has not yet been adapted.

In Taiwan where biliary atresia screening has been introduced nationwide, the stool colour card has been integrated into the child health booklet. The update search identified no research investigating the acceptability of stool colour card screening to parents in Taiwan, or in western countries. The 1999 UK community project by Crofts et al. evaluated the parental anxiety caused by assessment for prolonged jaundice and stool colour,[30] and this is discussed in Section 15.

Summary: Criterion 7 Unknown

8. There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals

Current methods of diagnosis of biliary atresia

NICE clinical guideline 98, Neonatal Jaundice (2010) currently recommends that infants with prolonged jaundice (>2 weeks in term infants) and/or pale, chalky stools and/or dark urine have measurement of conjugated bilirubin, full blood count, blood group determination, direct antiglobulin test (DAT), urine culture and routine metabolic screening. NICE advise that expert advice is obtained for all infants with a conjugated bilirubin level greater than 25µmol/l.[3]

The update search also identified a 2009 systematic review evaluating the evidence pertaining to the investigation and diagnosis of neonatal cholestatic jaundice.[2] Benchimol et al. conclude that total and direct bilirubin levels should be measured in any infant who is still jaundiced at 2-3 weeks of age. Cholestatic jaundice is indicated by:

- Direct reacting serum bilirubin levels >17µmol/L (1.0mg/dL)
- Direct reacting bilirubin >20% of the total serum bilirubin concentration, if total bilirubin is >85µmol/L (5.0mg/dL)

Any infant with cholestatic jaundice should then be referred to a paediatric gastroenterologist.

The typical blood profile of an infant with biliary atresia will be elevated levels of the bilirubin, alkaline phosphatase and gamma-glutamyltransferase (GGT typically being higher in biliary atresia than in other causes neonatal cholestasis); mildly elevated alanine aminotransferase and aspartate aminotransferase; and normal albumin and normal prothrombin time.[1] Liver enzymes concentrations, however, are reported to be poor predictors of liver disease aetiology (sensitivity 68% and specificity 43% for biliary atresia), but when combined with suggestive clinical features (e.g. pale, acholic stool, failure to thrive and ascites) may be useful for guiding further investigation.[2]

Diagnostic investigations specifically recommended by Benchimol et al. for the further evaluation of suspected biliary atresia:[2]

- **Ultrasound**: assessment of presence, size, and appearance of gallbladder; evidence of cirrhosis and portal hypertension, polysplenia, or asplenia. The 'triangular cord sign' at the porta hepatis has been reported in studies to have 73-100% sensitivity and 98-100% specificity for biliary atresia[2]
- **Hepatobiliary scintigraphy**: Uptake of the tracker by hepatocytes should be followed by its excretion in bile into the intestine within 24 hours; absent excretion has been demonstrated in two studies to have a sensitivity of 83% and 100% for biliary atresia, but much lower specificity - 33% to 80%[2]
- **Liver biopsy:** This provides evidence of extrahepatic biliary obstruction and is reported to be the usual diagnostic method of choice in the UK[1]
- **Intraoperative cholangiogram:** Though most infants will have a preoperative diagnosis, this may be performed prior to Kasai procedure and is reported to be the gold standard definitive investigation in the diagnosis of biliary atresia[1]

Summary: Criterion 8 Not Met

No studies were identified that have specifically evaluated the further diagnostic evaluation of infants detected through screening programmes. Diagnostic procedures are, however, in place for those identified without screening. The update search did identify retrospective studies that have compared the value of different diagnostic techniques for the diagnosis of biliary atresia, but these have not been evaluated by this review.

9. If the test is for mutations the criteria used to select the subset of mutations to be covered by screening, if all possible mutations are not being tested, should be clearly set out

Not applicable. Biliary atresia is not known to be associated with carriage of a particular genetic mutation.

10. There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment

The standard treatment for infants with biliary atresia is a Kasai portoenterostomy which attempts to restore bile flow. The time to Kasai procedure has consistently been indicated as an issue of paramount importance: liver fibrosis is considered to be 'time dependent', and even infants who have congenital biliary atresia have been demonstrated to have a normal

liver at the time of birth.[5] Whether earlier treatment is associated with improved outcomes is something that has been evaluated in numerous retrospective national series and single centre studies. This section compares the age at Kasai procedure and survival outcomes in England and Wales with that of other European and North American national series, and also with outcomes reported in Taiwan and Japan where stool colour card screening has been introduced. This section also reports on the findings of national studies that have specifically focused upon the question of whether later age at surgery is associated with poorer outcome, and therefore whether this suggests a need for biliary atresia screening.

Age at Kasai procedure and survival outcomes reported in retrospective national series

In England and Wales the median age at Kasai procedure, among all 424 infants who underwent this procedure during 1999-2009, was 54 days.[5] As demonstrated in Table 1, this is the shortest time to surgery reported in all recent national studies conducted in Europe and North America where screening is not currently performed.

Time to surgery in England and Wales also compares favourably with that in Taiwan, even following the introduction of their stool colour card screening programme. In their 2011 study Davenport et al. report that our time to surgery without screening is currently shorter than that in Taiwan.[5] However, this claim is based on the findings of the 2008 study by Hsiao et al. who reported a median age of 54.6 days at time of surgery among the 74 cases diagnosed and treated immediately following nationwide introduction of the screening programme (2004-05).[28] In 2011 Tseng et al. subsequently reported on the 160 cases diagnosed and treated between 2004 and 2008. These infants had a median age at surgery of 48 days, which was a borderline significant (p=0.051) improvement on the 51 day median in the years prior to screening (1996-2003).[14] It is also a 6-day improvement on our current average of 54 days in England and Wales. However, as discussed in Section 5, Tseng et al. found no significant pre-post screening difference in the number of infants treated prior to 60 days (68.9% to 73.6%, p=0.31).

In England and Wales overall survival for infants diagnosed with biliary atresia during 1999-2009 was 90% at 5 years and 89% at 10 years. Survival with native liver was roughly half this: 46% at 5 years and 40% at 10 years.[5] As demonstrated in Table 1, these are, again, the best survival outcomes reported in all recent national studies conducted in Europe and North America where screening is not currently performed.

In Asian countries, Tseng et al. (2011) did not report on survival, but another 2011 study by Lien et al. reported significantly improved 3-year overall survival (89.2%) among infants treated during 2002-05 (the era of the screening programme pilot and nationwide introduction) compared to those treated 1990-2000 (64.0%; p<0.001). However, they found no significant pre-post screening difference in 3-year survival with native liver (51.7% vs. 61.8%; p=0.16)*.[29] A notable observation from these results is that, at 89-90%, the overall 5- and 10-year survival in England and Wales is currently comparable to the 3-year survival in post-screening Taiwan. Overall 5- and 10-year survival in England and Wales is also superior

* Lien et al. do report significantly improved 5-year outcomes, but this is only comparison between those treated 1990-2000 and the 28 infants treated during the pilot (2002-03). Five-year overall survival for these 28 infants was 89.3% and survival with native liver, 64.3%. These are significant improvements on 5-year survival among those treated 1990-2000 (55.7% overall and 37.5% with native liver), but are not the focus of this discussion due to the small sample of infants treated 2002-03. The main body of discussion is given to the 3-year outcomes of the 102 infants treated 2002-05.

to the most recent data identified from Japan where screening was introduced early in the 1990s. Survival with native liver at 3 years in Taiwan (61.8%) and at 5- and 10-years in Japan (59.7 and 52.8%), does, however, appear to be slightly superior to 5-and 10-year survival with native liver in England and Wales (46 and 40%).

Post-operative clearance of jaundice is considered to be a marker of success of the Kasai procedure. Clearance of jaundice was achieved by 55% of operated patients in England and Wales, which was superior to that of all other European and North American series, but was marginally less than that reported by Hsiao et al. for the 74 Taiwanese infants treated 2004-05 (60%),[28] and reported by Lien et al. for the 102 Taiwanese infants treated 2002-05 (61%).[29]

Table 1: Time to surgery and survival outcomes in retrospective national series

Author and year	Country and study period	Cases of biliary atresia (BA)	Primary treatment	Median age at Kasai procedure	Post-operative jaundice clearance (total bilirubin ≤20µmol/L)	Overall survival (all cases unless otherwise stated)	Survival with native liver (all cases unless otherwise stated)
European and North American countries where screening is not currently performed							
Davenport et al. 2011[5]	England and Wales 1999-2009 Cover: centralised care in 3 centres	443	Kasai: 424 Transplant: 15 Died without treatment: 4	54 days	55% (232/424) at 6 months	5-year: 90% 10-year: 89%	5-year: 46% 10-year: 40%
Serinet et al. 2006[8]	France Cohort A: 1986-1996 Cohort B: 1997-2002 Cover: all 45 centres providing care to patients with BA	Total 743 Cohort A: 472 Cohort B: 271	Kasai: 695 (A=440, B=255) Transplant: 35 (A=20, B=15) Died without treatment: 12 (A=12, B=0)	Overall 60 days A=61 days B=57 days	Overall 36.2% (242/668 with follow-up; time period not reported) A=34.4% (144/418) B=39.5% (98/250)	5-year: A=72.0%, B=87.1% 10-year: A=70.2%, B not available	5-year: A=35.0%, B=35.1% 10-year: A=29.7%, B not available
Schreiber et al. 2007[9]	Canada Cohort A: 1985-1995 Cohort B: 1996-2002 Cover: all 12 centres providing	Total 349 Cohort A: 199 Cohort B: 150	Kasai: 312 (a=173, B=139) Transplant: 29 (A=20, B=9) Died without treatment: 8	Overall 65 days A=65 days B=65.5 days	Not reported	Overall 4-year: 77% (A=74%, B=82%) Overall 10-year: 75%	Overall 4-year: 33% (A=31%, B=36%) Overall 10-year: 24%

Author and year	Country and study period	Cases of biliary atresia (BA)	Primary treatment	Median age at Kasai procedure	Post-operative jaundice clearance (total bilirubin ≤20µmol/L)	Overall survival (all cases unless otherwise stated)	Survival with native liver (all cases unless otherwise stated)
	care to patients with BA		(A=6, B=2)				
Shneider et al. 2006[17]	United States, 1997-2000 Cover: 9 centres that make up the Biliary Atresia Research Consortium (BARC, established 2002 to study the epidemiology and management of BA). The study only included those who underwent Kasai procedure.	104 (treated infants)	Kasai: 104	61 days	Not reported	2-year: 91%	2-year: 56%
Leonhardt et al. 2010[12]	Germany 2001-2005 Cover: 29 centres	Total 183 137 from EBAR 46 who	Kasai: 159 Transplant: 21 Died without	57 days	Not reported	2-year: 83% (139/167 with follow-up data)	2-year: 20% (34/167)

Author and year	Country and study period	Cases of biliary atresia (BA)	Primary treatment	Median age at Kasai procedure	Post-operative jaundice clearance (total bilirubin ≤20µmol/L)	Overall survival (all cases unless otherwise stated)	Survival with native liver (all cases unless otherwise stated)
	registered with EBAR (European BA Registry) plus BA patients who underwent transplant at 4 centres not registered with EBAR	underwent transplant at unregistered centres	treatment: 3				
Lampela et al. 2012[10]	Finland 1987-2010 Cover: 1 centre in Helsinki where BA care has been centralised since 2005; 5 centres prior to this; plus the Register of Congenital Malformations which covers all of Finland	72	Patients receiving operative treatment: 64 (57 Kasai and 7 other procedures) Died without treatment: 8	64 days	33% at 3 months, 31% at 6 months	>2-year: 68% (38/56 operated patients followed to >2 years)	>2-year: 38% (21/56)
Wildhaber et al. 2008[13]	Switzerland 1994-2004	48	Kasai: 43	68 days	39.5% (time period not	4/5-year: 91.5%	4/5-year: 32.7%

Author and year	Country and study period	Cases of biliary atresia (BA)	Primary treatment	Median age at Kasai procedure	Post-operative jaundice clearance (total bilirubin ≤20µmol/L)	Overall survival (all cases unless otherwise stated)	Survival with native liver (all cases unless otherwise stated)
	Cover: all 7 centres involved in the care of BA patients		Transplant: 5 Died without treatment: 0		reported)		
De Vries et al.2011[32]	Netherlands 1977-1988 (Aim of the study to examine 20-year outcomes) Cover: Dutch national database of all BA patients	104	Not reported	60 days among 49 treated 1977-82; 58 days among 55 treated 1983-88	Not reported	20-year: 43%	20-year: 27%
Asian countries where Stool Colour Card Screening has been introduced							
Hsiao et al. 2008[28]	Taiwan, 2004-2005 Cover: national registry	75	Kasai: 74	54.6 days	59%	Not reported	Not reported
Lien et al. 2011[29]	Taiwan Cohort A: 1990-	Cohort A: 89 (treated infants)	Not applicable	Not reported	A=34.8%; B+C= 60.8%	3-year: A=64.0%;	3-year: A=51.7%;

Author and year	Country and study period	Cases of biliary atresia (BA)	Primary treatment	Median age at Kasai procedure	Post-operative jaundice clearance (total bilirubin ≤20µmol/L)	Overall survival (all cases unless otherwise stated)	Survival with native liver (all cases unless otherwise stated)
	2000 Cohorts B+C: 2002-2005 (the era of the screening programme). Cover: cohort A treated at 1 centre; cohorts B+C, national registry	Cohort B+C: 102 (treated infants)			at 3 months	B+C=89.2% 5-year outcomes presented only for cohort B, which includes the 28 infants treated 2002-03: A=55.7%; B=89.3% (25/28 treated 2002-03)	B+C=61.8% 5-year outcomes presented only for cohort B, which includes the 28 infants treated 2002-03: A=37.5%; B=64.3% (18/28 treated 2002-03)
Tseng et al. 2011[14]	Taiwan, 1996-2008 Cohort A: 1996-2003 Cohort B: 2004-2008 (post-nationwide introduction)	Total 472 Cohort A: 312 Cohort B: 160 (A diagnosis of biliary	Kasai: 440 Transplant: 32 Died prior to transplant: 0	Cohort A: 51 days Cohort B: 48 days	Not reported	Not reported	Not reported

Author and year	Country and study period	Cases of biliary atresia (BA)	Primary treatment	Median age at Kasai procedure	Post-operative jaundice clearance (total bilirubin ≤20µmol/L)	Overall survival (all cases unless otherwise stated)	Survival with native liver (all cases unless otherwise stated)
	Cover: claims data from the National Health Insurance Research Database	atresia was confirmed by treatment with the Kasai operation or with liver transplant)					
Nio et al. 2003[15]	Japan 1989-1999 Cover: the Japanese BA Society nationwide registry (JBAR)	1,381	Not reported	Not reported	57% ('disappearance of jaundice', not further defined)	5-year: 75.3% (553/734 registered in or before 1994) 10-year: 66.7% (72/108 registered in 1989)	5-year: 59.7% (of 734) 10-year: 52.8% (of 108)

National series that have examined associations between treatment age and outcomes

No evidence for an effect of treatment age upon survival has been identified in studies from England and Wales. In their 2011 study Davenport et al. examined the effect of age at time of surgery upon survival with native liver for the 318 infants with isolated biliary atresia who underwent a Kasai procedure 1999-2009.[5] Examining treatment ages of <44 days, 44-55 days, 56-69 days, and ≥70 days they found no significant effect of treatment age upon 5- or 10-year survival with native liver, and no clear trend.[5]

In an earlier 2004 publication Davenport et al. had examined the outcome of older infants treated at ≥100 days of age, addressing the perception that such infants are 'irretrievable' and require primary treatment with liver transplant.[33] This study also concluded that this is not the case.

Davenport et al. identified a total 422 infants who were diagnosed with biliary atresia between 1980 and 2000 and treated at King's College London. Within this cohort 35 infants (8%) underwent surgery at ≥100 days of age, with a median age at surgery of 133 days.[33] Reasons for delay in 17 cases with available data were misdiagnosis in the referring hospital (7), delay in initial GP referral (6), misdiagnosis at the tertiary centre (3), and failure of parents to attend health checks/raise awareness about persistent jaundice (1). At a median 2.2 years of follow-up, overall survival was 63% (22 infants) and survival with native liver was 34% (12 infants). Actuarial 5- and 10-year survival with native liver was 45% and 40%, respectively.[33] There was no difference in survival according to type of biliary atresia or the presence of preoperative cirrhosis.[33] These transplant-free survival figures for infants treated at ≥100 days are comparable to those for the 1999-2009 England and Wales cohort.

Other national series and single centre studies have had divergent findings about whether later treatment age has a detrimental effect on outcomes in biliary atresia.

The largest of these studies was conducted by Serinet et al., who in a more recent publication have examined the same cohort of infants as presented in their 2006 publication (743 infants diagnosed and treated in France 1986-2002),[8] but with the specific aim of examining the relationship between increased age at surgery and outcomes in adolescence.[34] They observed that for all follow-up periods (2-, 5-, 10- and 15 years) there was a trend for decreased survival with native liver with increased age at Kasai procedure. This was independent of anatomical type of biliary atresia, or the presence of congenital malformations. There was a 12.1% difference in 15-year survival with native liver between those treated at ≤45 days compared to those treated at 46 days or greater.[34] Optimal outcomes were achieved in those treated prior to 30 days of age, though in deference to the conclusions of Davenport et al.[33] they noted that even in those treated at >90 days, 15-year survival with native liver was still 13% and so Kasai procedure should not be denied in older infants.[34]

Serinet et al. estimated that if every infant with biliary atresia was treated before 46 days of age, then this would spare 4.5 liver transplants in France each year - 5.7% of the liver transplants performed annually in France in those aged under 16 years.[34] The authors conclude that their findings 'indicate a rational basis for biliary atresia screening to reduce the need for liver transplantations in infancy and childhood'.[34] Regarding screening options, the focus of their discussion is upon campaigns in France to encourage health professionals and parents to check the colour of infants' stool.

Nearly all retrospective national series have also analysed the relationship between treatment age and outcomes, and observed increased treatment age to be associated with poorer outcome:

- Schreiber et al.: 4-year survival with native liver was 49% when treated at ≤30 days, 36% at 31-90 days, and 23% when treated at >90 days ($p<0.0001$); respective figures for 10-year survival with native liver were 49%, 25% and 15 % ($p<0.0001$)[9]
- Shneider et al.: median treatment age was 64 days in those with poor outcome (defined as death or transplant prior to 24 months) vs. 57 days in those with good outcome (defined as composite survival with native liver and bilirubin <6mg/dL) (non-significant difference)[17]
- Leonhardt et al.: non-significant trend for improved survival with native liver with earlier operative age[12]
- Wildhaber et al.: 4-year survival with native liver 75% when treated at <46 days, 33% at 46-75 days, and 11% >75 days ($p=0.02$)[13]
- De Vries et al.: 20-year survival with native liver was 42% among those who underwent surgery at 60-75 days vs. 11% among those who underwent surgery at >75 days[32]
- Nio et al.: reported poorer post-operative jaundice clearance when treated at >90 days; and reported the age at operation to have significant impact on 5-year survival rate with native liver[15]

Single centre studies retrieved by the update search have given variable results, with some finding older treatment age to be associated with poorer outcomes,[35-40] some finding no clear association between treatment age and outcomes,[41,42] and some conversely observing better outcomes in those treated at greater operative age.[43-46]

Summary: Criterion 10 Not Met

Though there is an established treatment for biliary atresia, there is no conclusive evidence that earlier treatment leads to better outcomes. Retrospective national series have given conflicting results, and significantly, a review of all infants with biliary atresia in England and Wales treated between 1999 and 2009 found no significant effect of treatment age upon survival outcomes. In this series the median age at Kasai procedure in England and Wales was 54 days, and overall survival 90% at 5 years and 89% at 10 years. Survival with native liver was roughly half this: 46% at 5 years and 40% at 10 years.[5] These are the best operative age and survival outcomes reported of all recent national series in Europe and North America. Performance in England and Wales is also fairly comparable with before-after analyses from Taiwan, where screening was introduced nationwide in 2004. In Taiwan between 2004 and 2008 median age at Kasai procedure was 48 days, a borderline significant improvement to their median prior to introduction of screening (51 days); however, there has been no difference in the number of infants treated prior to 60 days.[14] Three-year overall survival data was available for those infants treated in Taiwan during the era of the screening pilot and nationwide introduction (2002-05);[29] at 89.2% this is comparable with the 10-year survival in England and Wales. Three-year survival with native liver in Taiwan (61.8%) is superior to 5- and 10-year survival with native liver in England and Wales, but this is not a significant improvement on their survival with native liver prior to the introduction of screening.

The update search identified no randomised controlled trials evaluating the effectiveness of the stool colour card screening programme upon treatment outcomes.

11. There should be agreed evidence based policies covering which individuals should be offered treatment and the appropriate treatment to be offered

Untreated, biliary atresia is fatal within the first two years of life. Therefore all diagnosed infants would require treatment.

The standard treatment is Kasai portoenterostomy, which is usually performed via laparotomy. During the procedure the entire fibrotic extrahepatic biliary system is excised, the porta hepatis transected and exposed, and a jejunal Roux loop anastomosed to the incised surface, thus establishing bile flow into the intestine.[1,47] Adjuvant therapy following Kasai procedure aims to reduce the risk of the complication of ascending cholangitis, and this centres upon intensive nutritional support, and may also involve the use of corticosteroids, ursodeoxycholic acid and antibiotics.[1,47]

Kasai procedure does not cure biliary atresia, and even with re-established bile flow, the disease process will progress with fibrosis, cirrhosis and portal hypertension in an estimated 70%.[1] Failure to achieve bile drainage with Kasai portoenterostomy, or the development of the various possible complications following an initial successful procedure, may indicate the need for liver transplant. Most infants with failed Kasai portoenterostomy will require transplant within the first two years of life.[1,47]

Summary: Criterion 11 Met

Treatments offered to children identified without a screening programme are the same as those identified through screening.

12. Clinical management of the condition and patient outcomes should be optimised in all health care providers prior to participation in a screening programme

Davenport et al. state that the key stages that can ultimately affect the outcome in biliary atresia are correct diagnosis and time to initial surgery, awareness of the health of the native liver, and timely access to liver transplant as required.[5]

Since 1999 the management of biliary atresia in England and Wales has been centralised to three tertiary centres. Davenport et al. (2011) report that their analysis of infants diagnosed and treated 1999-2009 'illustrates how a country can deal with this health issue by centralisation of surgical and medical resources to obtain arguably the best possible outcome so far reported.'[5] As reported in Section 10, the current 5- and 10-year outcomes for England and Wales are the best reported in all recent national series for Europe and North America. They also demonstrate a slight improvement in survival outcomes for infants treated prior to centralisation of care in England and Wales.

McKiernan et al. (2008) reported on the 13-year outcomes for 93 infants in the UK and Wales diagnosed and treated between March 1993 and February 1995, using data collected by the British Paediatric Surveillance Unit (BPSU).[48] Ninety-one of these cases underwent the Kasai procedure, and like the 1999-2009 cohort, the procedure was performed at a median 54 days and 55% achieved post-operative clearance of jaundice. The 13-year actuarial survival for the whole cohort of 93 infants was 83.8%, 43.8% with native liver. Survival was demonstrated to be better among infants treated in the 2 higher volume centres that treated more than 5 cases per year (89.5%), than in one of the 13 centres that treated fewer than 5 cases per year (75%).[48] Though this difference fell short of significant (p=0.052) the trend supports the move to centralise care in England and Wales to the most experienced centres. Other national series have also highlighted that the level of expertise of the treating centres can have prognostic implications in biliary atresia and are in support of change to a centralised care approach.[8,10,12]

While expertise of the treating centres and timely Kasai procedure may have prognostic significance, another crucial factor that will influence the success of biliary atresia care in a country is access to liver transplant when required. Davenport et al. found an overall 9.3% mortality (41/443) for infants with biliary atresia diagnosed and treated in England and Wales between 1999 and 2009. Notably 24 of these infants (58%) died while on a liver transplant waiting list.[5] In the earlier analysis by McKiernan et al. 15 of the 93 children treated 1993-95 had died 13 years later (16% of the cohort), and 10 of them had died without liver transplant (2 not referred for transplant, 4 unfit for transplant and 4 on a waiting list).[48] Davenport et al. report that 'donor organ shortage is still a major issue and does not appear to have improved over the decade. If there are going to be further gains in survival then this is the areas where change should happen.'[5]

Summary: Criterion 12 Partly Met

Since 1999 biliary atresia care in England and Wales has been optimised through centralisation of care to three tertiary centres. However, timely access to liver transplant when required remains a significant obstacle to improving survival outcomes.

13. There should be evidence from high quality Randomised Controlled Trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (eg. Down's syndrome, cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened

As mentioned in Sections 5 and 10, no randomised controlled trials have been conducted in Taiwan or elsewhere evaluating the effectiveness of the stool colour card screening programme. The only available information on the effects of stool colour card screening upon survival outcomes come from retrospective before-after studies as covered in Section 10.

No randomised controlled trials have been conducted evaluating the alternative methods of screening that have been considered in the England and Wales - measurement of bile acids in dry blood spots or direct or conjugated bilirubin in plasma samples.

Summary: Criterion 13 Not Met

14. There should be evidence that the complete screening programme (test, diagnostic procedures, treatment/ intervention) is clinically, socially and ethically acceptable to health professionals and the public

The update search identified no evidence related to the clinical, social and ethical acceptability of a screening programme for biliary atresia – either dry blood spot screening, stool colour card screening or alternative methods.

Summary: Criterion 14 Not Met

15. The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment)

As discussed in Sections 5 and 6, the study by Mushtaq et al. assessing the feasibility of using tandem mass spectrometry (TMS) to measure conjugated bile acids in dry blood spots, did not find a suitable cut-off level as there was too much overlap between the few affected infants and the majority of the healthy infant population.[4] The authors considered that a recall rate of 2% or higher for diagnostic evaluation in unaffected infants (i.e. specificity below 98%) would be unlikely to gain acceptance as a screening test. Raising the cut-off level to reduce the false positive rate to only 1% reduced the sensitivity of the test to an unacceptable 50.8%. No further developments in dry blood spot screening for biliary atresia appear to have been made since 1999.

The stool colour card pilot study in Taiwan demonstrated a sensitivity of 89.7% and specificity of 99.9% for detecting biliary atresia prior to 60 days of age, but a positive predictive value of only 28.6%.[27] Published studies from Taiwan have not reported on the physical and psychological harms of screening.

No European or North American studies evaluating stool colour card screening have been published since 1999. The 1999 UK community project by Crofts et al. evaluated the anxiety generated by the stool colour chart and parental information page, with the health visitor asking all mothers to complete the Spielberger anxiety scale (short version) at 28 days.[30] Mothers of jaundiced infants were then asked to complete the Spielberger scale again at 'Time 2' and again at 'Time 3'. The study does not clarify when times 2 and 3 relate to, though this may be further investigation. There was no significant difference in the mean anxiety levels at the three time points.[30] However, qualitative data revealed that three parents contacted their health visitor with concerns about jaundice; two parents were concerned about the colour of their infant's stool (though in both cases the stools were found to be normal); and two mothers gave up breastfeeding specifically because of worries about the prolonged jaundice.[30]

Summary: Criterion 15 Uncertain

16. The opportunity cost of the screening programme (including testing, diagnosis and treatment, administration, training and quality assurance) should be economically balanced in relation to expenditure on medical care as a whole (ie. value for money). Assessment against this criteria should have regard to evidence from cost benefit and/or cost effectiveness analyses and have regard to the effective use of available resource

Summary: Criterion 16 Unknown

No studies were identified evaluating the cost effectiveness of screening programmes – either dry blood spot screening, stool colour card screening or alternative methods.

17. All other options for managing the condition should have been considered (eg. improving treatment, providing other services), to ensure that no more cost effective intervention could be introduced or current interventions increased within the resources available

The update search identified one randomised controlled trial related to the management of biliary atresia. Davenport et al. (2007) evaluated whether a course of corticosteroids following Kasai portoenterostomy could improve outcomes in infants with type 3 biliary atresia (non-syndromic).[49] Seventy-three infants were randomised to receive oral prednisolone (2mg/kg/day from days 7 to 21, followed by 1mg/kg/day from days 22 to 28) or placebo. The primary outcomes were clearance of jaundice (total bilirubin ≤20µmol/L) and survival with native liver at 6 and 12 months. There was no significant difference in the proportion of infants in the steroid and placebo groups who achieved clearance of jaundice (47 vs. 49% at 6 months; 50% vs. 40% at 12 months), or who required liver transplant (12 vs. 13% at 6 months; 26% vs. 35% at 12 months).[49]

The update search identified one 2011 systematic review and meta-analysis of steroid therapy for biliary atresia, which reviewed 16 observational studies and the one trial by Davenport et al.[50] This review concluded the lack of a significant effect of steroid therapy over standard therapy.

The update search identified further case series reporting on the outcomes of infants with biliary atresia treated by alternative operative approach (for example, laparoscopic rather than open Kasai portoenterostomy; or hepatic portocholecystectomy – 'Gallbladder Kasai' – rather than standard Kasai for infants with patent distal hepatic ducts) but no further randomised controlled trials of biliary atresia management were identified.

Summary: Criterion 17 Not Met

18. There should be a plan for managing and monitoring the screening programme and an agreed set of quality assurance standards

The update search did not identify any research evaluating how screening programmes for biliary atresia – either dry blood spot screening, stool colour card screening or alternative methods – would be managed and monitored.

19. Adequate staffing and facilities for testing, diagnosis, treatment and programme management should be available prior to the commencement of the screening programme

The update search did not identify any research evaluating how screening programmes for biliary atresia – either dry blood spot screening, stool colour card screening or alternative methods – would be managed and monitored.

20. Evidence-based information, explaining the consequences of testing, investigation and treatment, should be made available to potential participants to assist them in making an informed choice

The update search did not identify any research evaluating how screening programmes for biliary atresia should be introduced to potential participants.

21. Public pressure for widening the eligibility criteria for reducing the screening interval, and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public

The update search did not identify any research related to this issue.

22. If screening is for a mutation the programme should be acceptable to people identified as carriers and to other family members

Not applicable. Biliary atresia is not known to be associated with carriage of a particular genetic mutation.

Conclusions

Biliary atresia is a rare, but life-threatening, condition with an incidence in England and Wales of 0.58 per 10,000 live births.[6] Without surgery to establish bile flow the condition is fatal within the first two years of life. It is the most common cause for liver transplant in children. The pathophysiological process remains poorly understood, though disease change is believed to occur sometime in the developmental or perinatal stage, with studies finding that direct and conjugated bilirubin levels are elevated within the first days of life in affected infants. Currently

diagnosis of biliary atresia in England and Wales relies upon prompt recognition and investigation of prolonged jaundice and/or pale stool. If an accurate screening programme for biliary atresia were available then this could theoretically allow for earlier intervention, thereby reducing the extent of liver damage, which could potentially improve outcomes in biliary atresia.

In 1999 study by Mushtaq et al. assessed the feasibility of using tandem mass spectrometry (TMS) to measure conjugated bile acids in dry blood spots on the Guthrie screening card.[4] The NHS Newborn Blood Spot Screening Programme already offers screening for certain metabolic conditions using this method of heel prick at around 7-10 days, and therefore the current system offers the potential for screening of further disorders. However, this method alone was not accurate for the screening of biliary atresia. It was not possible to find a suitable cut-off level for total bile acid concentration as there was too much overlap between the few affected infants and the majority of the healthy infant population. The upper limit of normal for total bile acid concentration in the control group was 33µmol/l. A cut-off of 30µmol/l had a sensitivity of 78.7% and specificity of 96.3% for biliary atresia; raising the cut-off to reduce the false positive rate to only 1% reduced the sensitivity of the test to 50.8%. Since 1999 there has been no further evaluation of dry blood spot screening.

A 2003 study by Powell et al. demonstrated that an elevated conjugated bilirubin (level >18µmol/l and fraction >20%) in neonatal liquid blood samples is a reliable indicator of neonatal liver disease, with a sensitivity of 100% and specificity of 99.59%,[24] though the study was not evaluating the accuracy of the test for detection of biliary atresia alone. Further observational studies have also demonstrated that elevated direct or conjugated bilirubin levels in neonatal liquid blood samples are a reliable indicator of biliary atresia, and exceed laboratory norms within the first 24-48 hours of life, with levels increasing with age.[25,26] However, despite this apparent accuracy, measuring conjugated bilirubin in liquid blood samples has two important limitations which restrict its potential value as a screening test: firstly, liquid blood samples are not routinely collected from neonates in the UK; and secondly, even if obtained, Powell et al. observed that 15.3% of liquid blood samples were unsuitable for testing due to haemolysis or insufficient sample, among these untested infants being 6 cases of liver disease, including 1 case of biliary atresia, indicating a need for a high rate of recall testing. Therefore screening for biliary atresia through testing of conjugated bilirubin would have limited potential unless it could be adapted to dry blood spot screening. Reviewing the update search there appears to have been no progress adapting methods to allow accurate screening for biliary atresia using TMS to measure conjugated bilirubin in dry blood spots.

The latest data from England and Wales, covering all infants diagnosed and treated between 1999 and 2009 identified that the median age at Kasai procedure in the UK was 54 days, the rate of post-operative jaundice clearance (total bilirubin ≤20µmol/L – considered to be an important indicator of procedural success) was 55% at 6 months, overall survival 90% at 5 years and 89% at 10 years, and survival with native liver 46% at 5 years and 40% at 10 years.[5] These are the best operative age and survival outcomes reported of all recent national series in Europe and North America.

Stool colour card screening for biliary atresia was introduced nationwide in Taiwan in 2004. The stool colour card pilot study demonstrated the system to have 89.7% sensitivity and 99.9% specificity for detecting biliary atresia prior to 60 days of age, but a positive predictive value of only 28.6%.[27] No randomised controlled trials evaluating the effectiveness of the programme have been conducted. A before-after analysis by Tseng et al. indicated that there has been a borderline significant improvement in median age at Kasai procedure from 51 days prior to

2004, to 48 days after 2004.[14] However, this study found no difference in the proportion of infants treated prior to 60 days. A separate study by Lien et al. has evaluated survival outcomes for infants treated in Taiwan during the era of the screening pilot and nationwide introduction (2002-05).[29] Three-year survival in Taiwan since introduction of the programme has been 89.2%, which is comparable with the 10-year overall survival achieved in England and Wales. Three-year survival with native liver in Taiwan (61.8%) is superior to 5- and 10-year survival with native liver in England and Wales, but this is not a significant improvement on Taiwan's survival with native liver prior to the introduction of screening.

Stool colour card screening for biliary atresia has not been evaluated in published studies in Europe or North America since 1999. The 1999 UK community project by Crofts et al. included 3,629 parent-infant pairs who were administered a stool colour chart when their infant was 10-14 days of age.[30] Prolonged jaundice was common in breastfed infants, though not found in combination with persistently pale stools and/or dark urine. Among the few non-jaundiced infants reported to have pale stool, no pathology was found.The study authors concluded that further research was needed to determine whether a stool colour chart in the Personal Child Health Record reduces the mean age of referral and treatment of infants with cholestatic liver disease.[30] NICE evaluated this study when developing their Neonatal Jaundice clinical guideline and concluded that there is no evidence to show that the examination of stool colour is helpful in the recognition of jaundice in babies, though the combination of these signs suggests pathology and indicates immediate referral for investigation.[3]

There have been few treatment developments over the past decade, and only one therapeutic randomised controlled trial has been published, evaluating the value of corticosteroids following Kasai portoenterostomy.[49] Awareness of the health of the native liver, and timely referral and availability of liver transplant when required, remain significant obstacles to outcomes in biliary atresia.

Biliary atresia care in England and Wales is currently achieving the best outcomes of those reported in Europe and North America, and is not far removed from outcomes in Taiwan where stool colour card screening has been introduced. Development of screening methods that allow for measurement of conjugated bilirubin in dry blood spots could potentially allow for earlier diagnosis and treatment and improve post-operative outcomes in England and Wales further. Further research developments in dry blood spot screening and stool colour card screening are awaited.

Implications for policy

The current policy of the National Screening Committee is that systematic population screening for biliary atresia is not recommended. This review of the evidence published since 1999 does not indicate that there should be a change to the current policy.

Implications for research

The evidence update highlights several areas where additional research could be of value:

- **Natural history**. The pathophysiology of biliary atresia remains poorly understood, though it may be multifactorial in nature and involve genetic, infective and inflammatory factors. The update search identified only small case series, animal studies and editorials reviewing these questions. Additionally observations from national cohorts that longer term outcomes may vary depending on the anatomical type of

biliary atresia and whether or not there are associated congenital abnormalities, raises further questions about when in the developmental process pathological changes occur, and whether this may vary between infants with isolated biliary atresia and those with BASM or other abnormalities. Future research that furthers our understanding of the disease process may help to identify potential preventative or therapeutic targets.

- **The Screening Programme.** A validated and accurate method of screening has not yet been identified. In England and Wales there has been interest in a programme that utilises the current method of dry blood spot collection on the Guthrie screening card. The key 1999 study by Mushtaq et al. concluded that measurement of bile acid concentration in dry blood spots using tandem mass spectrometry had insufficient accuracy to be used as a single screening method. Measuring conjugated bilirubin levels in liquid blood samples has on the other hand been demonstrated to be a reliable indicator of neonatal liver disease and biliary atresia. Further research assessing the feasibility of measuring conjugated bilirubin in dry blood spots would be highly valuable. Stool colour card screening was introduced nationwide in Taiwan in 2004. In the UK stool colour card screening has not been evaluated since 1999. Randomised controlled trials evaluating the outcomes of infants detected through stool colour card screening would be valuable. Research into the further diagnostic work-up of screen-detected infants is also needed.
- **Therapy**. Kasai portoenterostomy performed via laparotomy is accepted as the standard therapeutic intervention. The update search identified only one therapeutic randomised controlled trial, which had investigated the value of corticosteroids following Kasai procedure (finding no benefit over placebo in terms of post-operative jaundice clearance or need for liver transplant). Case series have been published reporting on outcomes of infants treated by an alternative operative approach (for example, laparoscopic rather than open Kasai portoenterostomy; or hepatic portocholecystectomy – 'Gallbladder Kasai' – rather than standard Kasai for infants with patent distal hepatic ducts) but no further trials of biliary atresia management were identified. Therapeutic developments could improve post-operative outcomes.
- **Liver transplant.** This review has not focused upon the issue of liver transplant, though biliary atresia remains the most common indication for liver transplant in children. Longer term survival outcomes are likely to be influenced by early awareness of the health of the native liver and timely referral for transplant; availability of liver transplant; and management of post-transplant complications.

Methodology

Search strategy

<Details to be entered by UK NSC>

Quality

This update review included the following:

- Cohort studies related to any screening programme for biliary atresia
- National series that had reliably captured all infants diagnosed with biliary atresia during a fixed time interval, and as such could provide a reliable indication of incidence, therapeutic approach and outcomes for a particular country
- Systematic reviews and cohorts related to epidemiology
- Systematic reviews and randomised controlled trials related to screening, diagnostics or treatment of biliary atresia

The update review excluded:

- Foreign language studies
- Conference reports
- Non-systematic reviews
- Non-national case series reporting on the outcomes of a series of infants diagnosed and treated at a single centre
- Case series comparing different diagnostic or therapeutic approach
- National cohorts updated by more recent publication from the same country
- Cohorts related to longer term developmental, psychological or quality of life outcomes among infants with biliary atresia
- Cohorts related to outcomes following liver transplant and factors associated with outcomes

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