

Hepatobiliary scintigraphy for early diagnosis of biliary atresia

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ABSTRACT

INTRODUCTION: The aim of this study was to evaluate the validity of ^{99m}Tc-trimethylbromo-iminodiacetic acid hepatobiliary scintigraphy (HS) for the diagnosis of biliary atresia (BA).

METHODS: From January 2005 to December 2009, a total of 47 infants with conjugated hyperbilirubinaemia (> 20 micromol/l total bilirubin of which 20% is conjugated) underwent HS. BA was suspected if no tracer was visualised in the gut 24 hours post-injection. The results of the HSs were compared with the gold standard, laparotomy with antegrade cholangiography findings.

RESULTS: Considering the final diagnosis based on the gold standard, the sensitivity, specificity, positive predictive value and negative predictive value (NPV) of the HS in the diagnosis of BA was 100%, 63.6%, 53.8%, and 100%, respectively. The accuracy was 74.5%. BA patients with non-draining HS had significantly higher levels of gamma-glutamyl transpeptidase (GGTP) than non-BA patients with non-draining HS ($p = 0.019$) or draining HS ($p = 0.0001$).

CONCLUSIONS: HS plays an important role in the diagnostic strategy of infantile jaundice due to conjugated hyperbilirubinaemia. It is a non-invasive method that only seldomly calls for sedation. A high sensitivity and NPV prevent unnecessary surgery. Because of the low specificity of HS in diagnosing BA, it should be part of a multimodality imaging strategy when the result supports a clinical suspicion of BA. In cases with non-draining HS and normal GGTP blood levels, supplemental imaging modalities are especially needed.

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The correct and timely diagnosis of biliary atresia (BA) is important as delay may lead to cirrhosis and impair the effectiveness of the Kasai portoenterostomy (KP) [1]. Nonetheless, jaundice is relatively common in neonates, and BA remains a rare disease with a reported prevalence of 1:12.000 livebirths. A perfect discrimination algorithm has yet to be devised. Hepatobiliary scintigraphy (HS) is a well-established method for diagnosing hepatobiliary diseases, but not all centres regard it as a first-line diagnostic tool in BA because of its low specificity. In Denmark, the Danish Health Authority states that a HS should be performed within three days after conju-

gated hyperbilirubinaemia has been demonstrated [2]. Since this non-invasive imaging modality functions as a gate keeper at a very early diagnostic stage of conjugated hyperbilirubinaemia in Denmark, our aim was to estimate the sensitivity and specificity of the HS in excluding BA. Furthermore, we wanted to evaluate whether other variables could support the diagnosis of BA in those with non-draining scintigraphies and to explore which other diagnoses apart from BA that could result in a non-draining scintigraphy.

METHODS

This was a retrospective review of infants admitted to Rigshospitalet between January 2005 and December 2009. Rigshospitalet is a tertiary referral centre for BA in Denmark, Greenland and the Faeroe Islands. All infants who had a ^{99m}Tc-mebrofenin HS performed had conjugated hyperbilirubinaemia (> 20 micromol/l total bilirubin of which 20% is conjugated).

The European Association of Nuclear Medicine's paediatric dosage card was used, and the mean dose of ^{99m}Tc-mebrofenin given was 28.5 (standard deviation: ± 8.2) MBq. Infants fasted for 2 hours before injection and most were treated with phenobarbital 5 mg/kg/day and ursodeoxycholic acid 100 mg/kg/day. After injection of tracer, the infant was placed in the supine position under a two-headed gamma camera equipped with a high-resolution parallel-hole collimator. A dynamic scintigraphy was performed one minute per frame during one hour. If no tracer was visualised in the gut, additional static images were performed at three, six and 24 hours after tracer injection. A HS with no visible tracer in the gut 24 hours post-injection supported the clinical suspicion of BA.

Laparotomy with antegrade cholangiography (if possible) and an intraoperative liver biopsy was then performed in order to confirm or exclude the diagnosis. All the operations were performed by the same surgeon (NK). Other diagnostic results were noted, including results for metabolic, endocrine, infectious and genetic diseases. Blood chemistry results of gamma-glutamyl transpeptidase (GGTP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were noted. Imaging results from ultrasonography, magnetic resonance cholangiopancreat-

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icography (MRCP) and magnetic resonance imaging were also registered. All HSs were subjected to blinded evaluation by a specialist in paediatric nuclear medicine (LB).

Data analysis

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and the accuracy of HS in the diagnosis of BA were calculated. Non-parametric tests were used to compare ages at the time of the HS, gestational ages, birth weights, and blood levels of GGT, AST, ALT and ALP among the patients. Data were quoted as median (range). A p-value of less than 0.05 was regarded as significant. IBM SPSS Statistics version 19 was used for the statistical calculations. The personal data processing was approved by the Danish Data Protection Agency (record number 30-1254).

Trial registration: not relevant.

RESULTS

The study included 47 infants with a possible diagnosis of BA. Infants were subdivided into four groups based on the retrospective interpretation of the HS and the findings at laparotomy and antegrade cholangiography (Table 1). Figure 1 shows a normal HS and Figure 2 a non-draining HS indicative of BA.

Demographic characteristics

Table 2 illustrates the demographic characteristics of the 47 patients (20 females and 27 males) included in groups I-III. Overall, the median age was 48 (4-160) days. BA patients (group I) had significantly higher levels of GGTP (compared with group II; $p = 0.019$ and group III; $p = 0.0001$). There was no significant difference in GGTP blood levels between patients in groups II and III ($p = 0.734$ and $p = 0.115$, respectively). No significant statistical difference was evident in gestational age, age at HS, birth weight or blood levels of AST, ALT and ALP (Table 2).

Hepatobiliary scintigraphy results and antegrade cholangiography findings

The scintigraphies showed that 21/47 (45.7%) infants had drainage to the intestines at < 24 hours. A total of

26 infants (55.3%) had no drainage either on early or late images. In this group, 24 had a laparotomy, antegrade cholangiography and liver biopsy performed. The diagnosis of BA was confirmed in 14 (58.3%) and refuted in ten (41.7%). Two infants with non-draining HS did not have an antegrade cholangiography performed. One had an MRCP performed, which showed patent bile ducts. The other patient's clinical status improved with normalisation of liver biochemistry. The clinical suspicion of BA was therefore abandoned and no cholangiography was performed.

Hepatobiliary scintigraphy validity for diagnosis of biliary atresia

The sensitivity, specificity, PPV and NPV of the HS in the diagnosis of BA were 100%, 63.6%, 53.8%, and 100% respectively. The accuracy was 74.5%.

Final diagnoses of group I

(no drainage and biliary atresia, $n = 14$)

A total of 14 patients had BA.

Final diagnoses of group II

(no drainage, but patent bile ducts, $n = 12$)

Four patients had mechanical obstruction of non-BA aetiology: fusiform choledochal malformation ($n = 1$), obstruction of bile flow due to gut anastomosis problems ($n = 2$) and bile duct stenosis ($n = 1$), respectively. The two infants with anastomosis problems had undergone abdominal surgery prior to the HS due to pancreas annulare and duodenal atresia with incomplete malrotation, respectively. In both cases, the cholangiography revealed that bile was displaced upwards and into the ventricle instead of through the gut anastomosis. The displacement of tracer to the ventricle could not be acknowledged on either of the patients' planar HS.

Seven infants were shown to have intrahepatic cholestatic conditions due to: idiopathic neonatal hepatitis ($n = 2$), autoimmune hepatitis ($n = 1$), cytomegalovirus hepatitis ($n = 1$), total parenteral nutrition cholestasis ($n = 2$) and alpha-1-antitrypsin deficiency ($n = 1$), respectively.

One patient had idiopathic acute liver failure.

Several tests ruled out bile duct obstruction, infections

TABLE 1

Division of the 47 infants with conjugated hyperbilirubinaemia into four groups based on the retrospective interpretation of the hepatobiliary scintigraphy and the findings at laparotomy and antegrade cholangiography, if performed.

Group	n	^{99m} Tc-mebrofenin hepatobiliary scintigraphy	Surgical cholangiography
I	14	No drainage after 24 h	No extrahepatic bile ducts
II	12	No drainage after 24 h	Patent extrahepatic bile ducts
III	21	Drainage before 24 h	Not performed: patent extrahepatic bile ducts
IV	0	Drainage before 24 h	Not performed: no extrahepatic bile ducts

and metabolic or endocrine disorders as the cause. The patient's HS showed no drainage to the gut, but an MRCP confirmed patent bile ducts.

Final diagnoses of group III (drainage and patent bile ducts, n = 21)

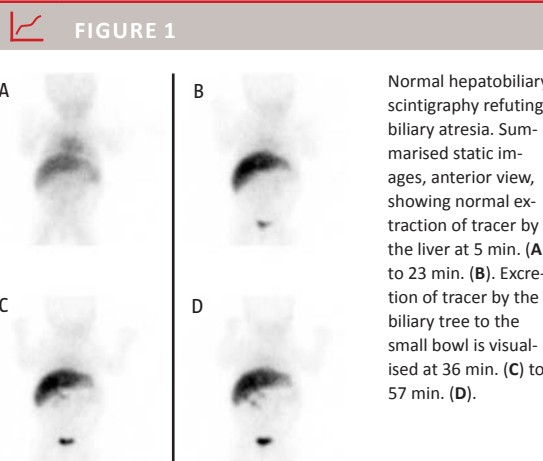
Ten patients had transient, conjugated hyperbilirubinaemia of non-specific origin. This was confirmed by normalisation of liver biochemistry, which was evident at subsequent clinical follow-up. Nine infants had intrahepatic cholestasis due to: idiopathic neonatal hepatitis (n = 3), cytomegalovirus hepatitis (n = 1), total parenteral nutrition (n = 4) and drug-induced intrahepatic cholestasis (n = 1), respectively.

One infant of Greenlandic descent had intrauterine growth retardation and dysmorphic facial features. The result of the liver biopsy suggested ductopenia or other structural anomalies such as progressive familial intrahepatic cholestasis. The infant died of complications related to necrotising enterocolitis before further diagnostic genetic procedures were conducted. One patient had an infantile haemangioendothelioma localised in the right liver lobe.

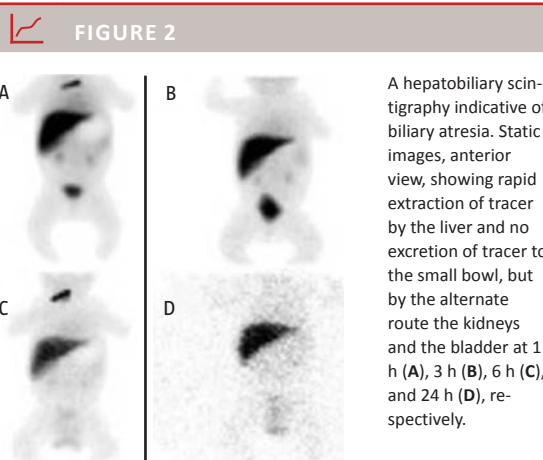
DISCUSSION

Several large studies have reported the sensitivity (84.6-100%) and specificity (61.1-88.6%) for the hepatobiliary scintigraphy in diagnosing BA [3-7]. A recent meta-analysis reported a pooled sensitivity of 98.7% and a pooled specificity of 70.4% [8]. Because of its rather low specificity, hepatobiliary scintigraphy is not implemented as a first-line diagnostic tool in all centres where ultrasound of the liver and the biliary tree in combination with liver histopathology is generally the diagnostic strategy of choice. In Denmark, HS is presented as a first-line diagnostic procedure in order to exclude BA. The procedure is non-invasive and seldomly requires sedation. Given the high sensitivity and NPV of HS in excluding BA, more invasive first-line diagnostic procedures, e.g. liver biopsy, are avoided in patients with patent bile ducts.

To increase the specificity of HS, it is important to use a tracer with high hepatic uptake. In the majority of the mentioned studies (as in our study), the tracer used was ^{99m}Tc -mebrofenin. ^{99m}Tc -mebrofenin has a better hepatic uptake than other iminodiacetic derivatives, even in the case of hyperbilirubinaemia [9-12]. The use of pre-medication is also used to increase the specificity of the HS. Despite the fact that we used two pre-medications (phenobarbital and ursodeoxycholic acid) before HS compared with the studies where only one pre-medication was used, we found a lower specificity [3, 4, 6]. In part, this low specificity is explained by the fact that a third of our patients with patent biliary ducts but a non-draining scintigraphy had a mechanical obstruction. It is



Normal hepatobiliary scintigraphy refuting biliary atresia. Summarised static images, anterior view, showing normal extraction of tracer by the liver at 5 min. (A) to 23 min. (B). Excretion of tracer by the biliary tree to the small bowel is visualised at 36 min. (C) to 57 min. (D).



A hepatobiliary scintigraphy indicative of biliary atresia. Static images, anterior view, showing rapid extraction of tracer by the liver and no excretion of tracer to the small bowel, but by the alternate route the kidneys and the bladder at 1 h (A), 3 h (B), 6 h (C), and 24 h (D), respectively.

important to keep in mind that HS is a method for assessing the patency of the biliary tree, why mechanical obstruction of any kind will result in no visible tracer in the gut. In the studies mentioned above, the underlying diseases of mechanical obstruction of non-BA origin were not a frequent cause of the non-draining scans [3-5]. In the case of mechanical obstruction, the choleric effect of phenobarbital and ursodeoxycholic acid and ^{99m}Tc -mebrofenin's high hepatic uptake are not efficient. Interestingly, some studies have found that combining HS with single-photon emission computed tomography (SPECT) resulted in an increase in the specificity and accuracy in diagnosing BA [13]. We believe that adding SPECT in cases of no drainage or suspicion of atypical drainage could be a useful tool for evaluating the precise localisation of the tracer and thereby diagnosing mechanical obstruction of non-BA origin, for instance.

Combining SPECT with HS is not done routinely in Denmark yet, but our limited experience shows promising results.

 TABLE 2

Baseline clinical and laboratory parameters of the 47 infants with conjugated hyperbilirubinaemia divided into the three groups based on the retrospective interpretation of the hepatobiliary scintigraphy and the findings at laparotomy and antegrade cholangiography, if performed. The values are median (range).

Parameter [reference interval]	Group I: no drainage after 24 h and no extrahepatic bile ducts (n = 14)	Group II: no drainage after 24 h and patent extrahepatic bile ducts (n = 12)	Group III: drainage before 24 h and patent extrahepatic bile ducts (n = 21)
Age at hepatobiliary scintigraphy, days	66 (24-160)	48 (4-118)	44 (17-94)
Gestational age, weeks	40 (35-42)	36.5 (26-41)	37 (26-41)
Birth weight, g	3,400 (1,150-4,200)	2,427 (790-4,686)	2,840 (940-4,200)
GGT, U/l [50-250]	387 (94-1,572)*	122 (28-776)	57 (14-272)
AST, U/l [15-65]	246 (125-722)	76 (36-395)	282 (25-503)
ALT, U/l [10-45]	139 (53-763)	89 (12-288)	179 (15-473)
ALP, U/l [55-425]	597 (407-1,299)	521 (171-999)	466 (198-843)

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transpeptidase.

*) p < 0.05 vs group II and group III.

The value of HS is its high sensitivity for exclusion of BA. Interestingly, Shah et al found that six BA patients had draining scans [7]. It is notable that only static images were performed. The dynamic scintigraphy gives an overview of the tracer excretion path, e.g. to the small bowel or the alternate route by the kidneys to the bladder which can be seen in BA. It is important not to interpret kidney or bladder tracer accumulation as bowel excretion. In case of doubt, lateral images can be performed. Indeed, misinterpretation of the scan is believed to be the cause of false-negative results as stated in the mentioned meta-analysis [8]. Our study confirms the high sensitivity (100%) and it thereby confirms the explicit value of the hepatobiliary scintigraphy in the exclusion of BA. We acknowledge that our reported high NPV can, in part, be explained by the fact that the prevalence of BA in our study population must be somewhat higher than the general prevalence since it originates from a tertiary referral centre for BA. The evaluations of both the hepatobiliary scintigraphies and performance

of the diagnostic laparotomies with the antegrade cholangiographies were performed by an experienced paediatric nuclear medicine physician and paediatric surgeon, respectively, why our study is comparable with other studies performed at tertiary referral centres.

There is no doubt that the challenging group of patients is those with patent bile ducts and a non-draining HS. Our results showed that this group had significantly lower levels of GGTP than the BA patients. Although Arora et al suggest an algorithm that includes GGTP blood levels > 150 U/l, no absolute cut-off value has generally been accepted as diagnostic of BA [14-16]. Since a high activity of plasma GGTP is indicative of disease at a biliary canaliculi level, normal or low GGTP in an infant with a non-draining HS is not common for BA. We acknowledge that further studies are needed on the subject and emphasise that if the patient is approximately 90 days of age, the laparotomy with antegrade cholangiography should not be delayed. If time allows it, other less invasive imaging modalities, e.g. the MRCP, could be considered.

In line with other studies, we found no diagnostic value of AST, ALT and ALP [14, 17]. In general, it appears that no single biochemical test can discriminate BA from other causes of conjugated jaundice.

Studies have reported somewhat divergent results regarding the age of BA patients compared with the age of patients with intrahepatic cholestasis [3, 4, 18]. The BA patients in our study were approximately the same age as the other patients at the time the HS was performed. A large retrospective study carried out by NK et al at Rigshospitalet found a median age at the time of Kasai operation of 59 days, which correlates well with our study [19]. Age at disease manifestation or gestational age does not seem to be a useful parameter to support the clinical suspicion of BA in cholestatic infants [20].

Even neonates who need continuous monitoring of vital parameters lying in an incubator can be scanned by the two-headed gamma camera.



CONCLUSIONS

Many diseases can result in conjugated hyperbilirubinemia in infants. It is a clinical challenge to find those patients that need KP. We believe that HS, which is a non-invasive examination that seldomly calls for sedation, prevents unnecessary invasive procedures and surgery because of its high sensitivity and NPV in refuting BA. This is why the HS still has an early place in the diagnostic strategy of jaundiced infants with clinical suspicion of BA.

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