Pediatr Radiol (2013) 43:1365–1375
DOI 10.1007/s00247-013-2704-3

ORIGINAL ARTICLE

Phenobarbital-enhanced hepatobiliary scintigraphy in the diagnosis of biliary atresia: two decades of experience at a tertiary center

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Received: 1 February 2013 / Revised: 25 March 2013 / Accepted: 29 March 2013 / Published online: 11 May 2013

Abstract
Background Hepatobiliary scintigraphy is highly sensitive for diagnosing biliary atresia; however, its specificity has varied in the literature from 35% to 97%.
Objective The purpose of this study was to re-evaluate the accuracy of phenobarbital-enhanced hepatobiliary scintigraphy in differentiating biliary atresia from other causes of neonatal cholestasis.
Materials and methods We retrospectively reviewed all hepatobiliary scans of infants with cholestasis at our institution from December 1990 to May 2011. Per our routine protocol the scans were obtained after pretreatment with phenobarbital (5 mg/kg/day for 5 days) to achieve a serum level of ≥15 mcg/ml. Normal hepatic uptake with no biliary excretion by 24 h was considered consistent with biliary atresia.
Results One hundred eighty-six infants with 210 hepatobiliary scans composed the study group. Forty-three (23%) infants had the final diagnosis of biliary atresia. Hepatobiliary scintigraphy was 100% sensitive, 93% specific and 94.6% accurate in diagnosing biliary atresia. Of the 186, 39/111 (35.1%) term and 2/68 (2.9%) preterm infants had biliary atresia; two of seven children with unknown gestational age also had biliary atresia. Other diagnoses included neonatal hepatitis, total parenteral nutrition cholestasis, Alagille syndrome, cystic fibrosis, choledochal cyst, hypothyroidism, alpha-1 antitrypsin deficiency and persistent cholestasis of unknown etiology.
Conclusion Phenobarbital-enhanced hepatobiliary scintigraphy is highly accurate in differentiating biliary atresia from other causes of neonatal cholestasis. Biliary atresia is rare in premature infants.

Keywords Hepatobiliary scintigraphy · Biliary atresia · Cholestasis · Phenobarbital · Infants

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Introduction

Prolonged cholestatic jaundice in the neonatal period is a major diagnostic concern for pediatricians. The two main causes of neonatal cholestasis are neonatal hepatitis and biliary atresia, which constitute 50–70% of cases [1]. Landing [2] hypothesized that neonatal hepatitis, biliary atresia and choledochal cyst represent variable outcomes of a single process termed infantile obstructive cholangiopathy. Although there might be an association between neonatal hepatitis and biliary atresia, it is extremely important to distinguish between the two for appropriate management. Intrahepatic cholestatic jaundice resulting from neonatal hepatitis, infectious disease, metabolic abnormalities or enzymatic defects can be treated medically or managed conservatively [3]. Biliary atresia, on the other hand, can be a devastating disease in infancy, resulting in cirrhosis, liver failure and death if not corrected surgically in a timely

fashion. This condition is still the most common indication for pediatric liver transplantation [3, 4]. Kasai portoenterostomy before 60 days of life is associated with an 80% chance of successful biliary drainage, whereas the success rate with surgery after 90 days of life falls to 20% [5]. Because early surgical intervention significantly improves the outcome in biliary atresia, it is important to have a reliable noninvasive test to distinguish it from other causes of cholestatic jaundice [6, 7].

Hepatobiliary scintigraphy is one of the oldest and most widely used tests for evaluation of neonatal cholestasis. Other diagnostic tests include ultrasound, magnetic resonance cholangiopancreatography, percutaneous liver biopsy and intraoperative cholangiography. Studies have examined the usefulness of these modalities [8–22]. In 2004, the Cholestasis Guideline Committee of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) examined the value of diagnostic tests commonly used for the evaluation of cholestatic jaundice and how they can be applied to the clinical scenario. About hepatobiliary scintigraphy, these guidelines stated, "Although the high sensitivity for biliary atresia makes this a fairly good single test for detecting disease, it is time-consuming and expensive and does have significant false-positive and false-negative results. The Cholestasis Guideline Committee concludes that hepatobiliary scintigraphy generally adds little to the routine evaluation of the cholestatic infant but may be of value if other means for excluding biliary obstruction are not available" [23].

At our center, we have used phenobarbital-enhanced hepatobiliary scintigraphy routinely for the evaluation of neonatal cholestasis for more than 30 years. As a result of varied data from different centers as well as the NASPGHAN guidelines, we re-examined the role of this diagnostic test in the evaluation of neonatal cholestasis.

Materials and methods

After obtaining approval from the Institutional Review Board, we searched for hepatobiliary scans of infants younger than 130 days of age with cholestasis who were evaluated from December 1990 to May 2011, using a radiology information system search engine (Montage Healthcare Solutions Inc., Philadelphia, PA). The patients' clinical records were reviewed for demographic and laboratory data and final diagnosis. The final diagnoses were based on clinical course, pathology and operative findings. Biliary atresia was diagnosed by atretic extrahepatic biliary system on intraoperative cholangiogram or laparotomy and histopathology. Neonatal hepatitis was diagnosed by liver biopsy or serological studies for various viral infections or the clinical course or a combination of these. Alagille syndrome was diagnosed with evidence of paucity of intrahepatic bile ducts on liver biopsy or the characteristic phenotypic features of Alagille syndrome. Cholestasis related to alpha-1-antitrypsin deficiency was diagnosed by protease inhibitor typing and liver biopsy. Diagnosis of cystic fibrosis was established by genetic testing. Hypothyroidism-related cholestasis was diagnosed with low serum levels of thyroid hormone in a jaundiced neonate. The presence of a choledochal cyst was confirmed at surgery. Total parenteral nutrition cholestasis was diagnosed in cholestatic infants who had received prolonged parenteral nutrition in the absence of other known causes.

Hepatobiliary scans available on the PACS (2005–2011) were reviewed with specific attention to hepatic uptake and biliary excretion. A single pediatric nuclear medicine physician with more than 40 years of experience who was blinded to the final diagnoses reviewed all the scans stored on PACS (total 59). His interpretation was concordant with the original official report in all except two, for which his interpretation was used in the tabulation of the results. In one case the hepatic uptake was recorded as decreased in the report, while on review of images it was interpreted to be normal. Excretion was absent in this case in both the final report and in the interpretation by the blinded reviewer. In the second case, the original report recorded no excretion, while on review of images minimal excretion was found to be present. For the studies from 1990 to 2005 (not stored on PACS), the existing reports were used to record the hepatic uptake, biliary excretion and the scan diagnosis.

Hepatobiliary scintigraphy: technique and interpretation

Our routine protocol for hepatobiliary scintigraphy in neonatal cholestasis has been pretreatment with phenobarbital (5 mg/kg/day for 5 days in 2 divided doses) and to achieve a serum phenobarbital level of ≥15 mcg/ml. Per our protocol, the infants were not fed from 1 h before until 2 h after injection of the tracer to prevent possible dilution of the excreted tracer in the bowel and also to minimize gallbladder contraction.

The radiopharmaceutical used was 99m-technetium trimethyl bromo-iminodiacetic acid (mebrofenin). In earlier studies (before 2004) 99mTc diisopropyl iminodiacetic acid (disofenin) was used. After intravenous administration of 1 mCi of radiopharmaceutical, sequential 1-min anterior images of the abdomen were obtained for 1 h. Thereafter, static anterior and right lateral images of the abdomen were obtained at 2 h, 4 h, 6 h and 8 h till biliary excretion was demonstrated or up to a maximum of 24 h post-injection.

Our interpretation of the scans is based on two criteria:

1. Presence or absence of biliary excretion (visualization of tracer activity in the intestinal tract or gallbladder)
2. Quality of hepatic uptake (normal or decreased). The evaluation of the quality of hepatic uptake is done by visual inspection. If the intensity of tracer activity in the liver is less than the cardiac blood pool tracer activity at about 5 min after injection of the tracer, it is considered decreased.

These criteria form the basis for the following three scan patterns:

1. Presence of biliary excretion, *regardless of the quality of hepatic uptake*, is interpreted as evidence for cholestasis from causes other than biliary atresia (Figs. 1 and 2).
2. Absence of biliary excretion up to 24 h after injection but *with decreased hepatic uptake*. This pattern is also interpreted as cholestasis from causes other than biliary atresia in infants up to 3 months of age. In infants older than 3 months, this pattern can be seen in children with biliary atresia in whom the liver function is already compromised (Fig. 3).
3. Absence of biliary excretion up to 24 h after injection but with *normal hepatic uptake* is interpreted as biliary atresia unless proved otherwise (Figs. 4 and 5).

Statistical analysis

We calculated sensitivity, specificity and accuracy of hepatobiliary scintigraphy for the diagnosis of extrahepatic biliary atresia. Three children who showed scan pattern 2 and were older than 90 days of age were excluded because they were not classifiable by our criteria. All statistical analyses were performed running SPSS Statistical Package 17 for Windows (SPSS, Chicago, IL).

Results

A total of 186 children (210 hepatobiliary scans) composed the study group. All except three children had received phenobarbital pretreatment. This included a repeat scan in 24 children for varying reasons. The age range was 9–130 days (median age 48 days, mean age 52.7 days). There were 111 term infants (gestational age ≥37 weeks, median age 40 days, mean age 46.9 days), 68 preterm infants (gestational age <37 weeks, median age 57, mean age 61.2 days) and seven infants in whom the gestational age was not known. There were 108 boys and 78 girls (ratio 1.4:1). The distribution of race was 90 African-Americans, 39 Caucasians, 27 Hispanics, five Asians and nine others. The race was not known for 16 children.

Forty-three children (23%) had a final diagnosis of biliary atresia, one with coexistent choledochal cyst. Other diagnoses included neonatal hepatitis (82), total parenteral nutrition cholestasis (37), Alagille syndrome (8), cystic fibrosis (3), isolated choledochal cyst (2), congenital hypothyroidism (2), alpha-1 antitrypsin deficiency (1) and persistent cholestasis of unknown etiology (8).

Thirty-nine of the 43 children with biliary atresia (90.7%) were term infants and 2 (4.7%) were preterm (gestational ages 31 weeks and 36.5 weeks). Gestational ages in the other two children were not known. Also, of the 111 term infants, 39 (35.1%) had biliary atresia, while of the 68 preterm infants only 2 (2.9%) had biliary atresia. Eight of 43 (18.6%) children with biliary atresia had coexistent congenital anomalies (Fig. 5) including heterotaxy syndrome (5), isolated cardiac anomalies (2) and

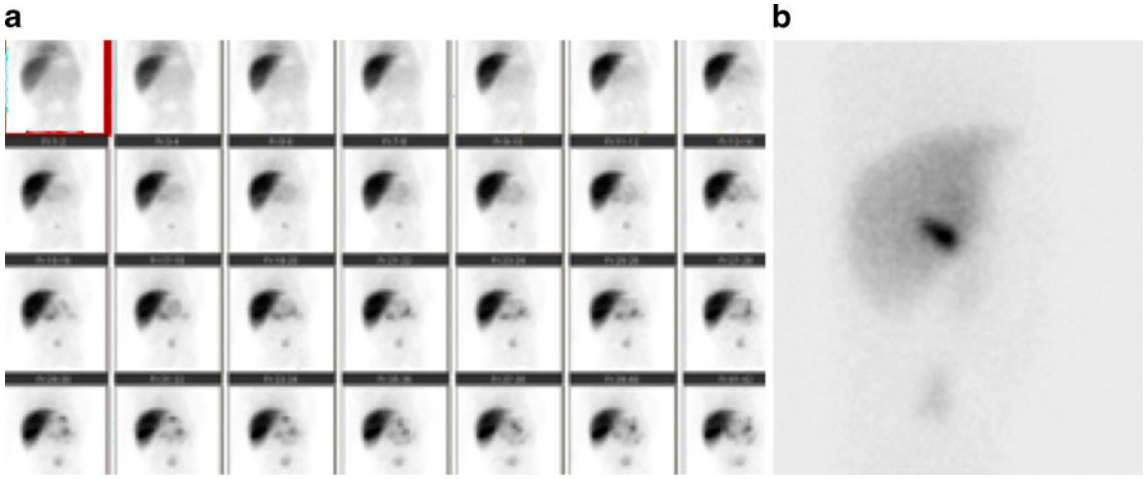

Fig. 1 Cholestasis in a boy born at 31 weeks' gestation who had been on total parenteral nutrition for more than a month. **a** Sequential anterior abdominal scintigraphic images demonstrate prompt hepatic extraction of the tracer. There is progressive biliary excretion and accumulation of the tracer in the intestinal tract and the gallbladder. **b** Lateral image at 1 h depicts the gallbladder. Scintigraphic pattern is excretion with normal uptake. Final diagnosis: total parenteral nutrition cholestasis

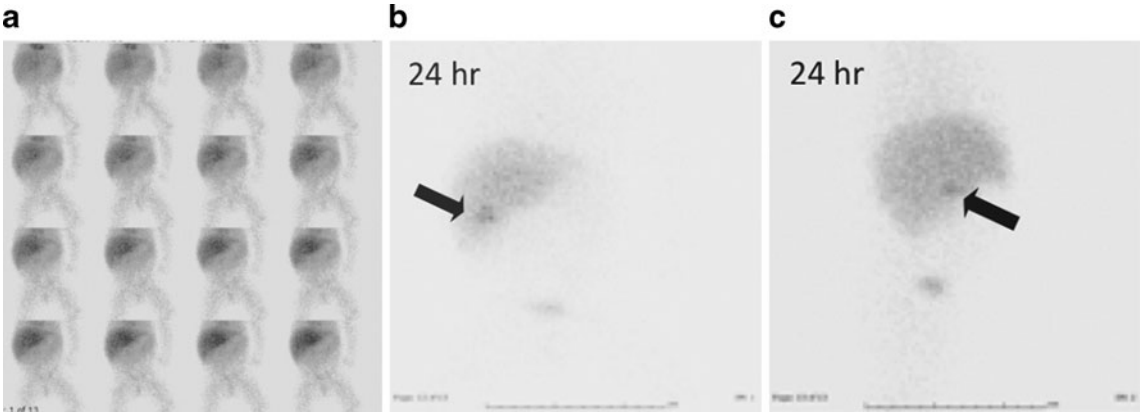

Fig. 2 Cholestasis in a 3-month-old girl on long-term total parenteral nutrition. **a** Sequential anterior abdominal scintigraphic images demonstrate poor hepatic uptake and no biliary excretion in the first hour of imaging. **b, c** The gallbladder is well-visualized on the anterior and lateral images obtained 24 h after injection but without any demonstrable tracer accumulation in the intestinal tract. Scintigraphic pattern is excretion with decreased hepatic uptake. Final diagnosis: total parenteral nutrition cholestasis

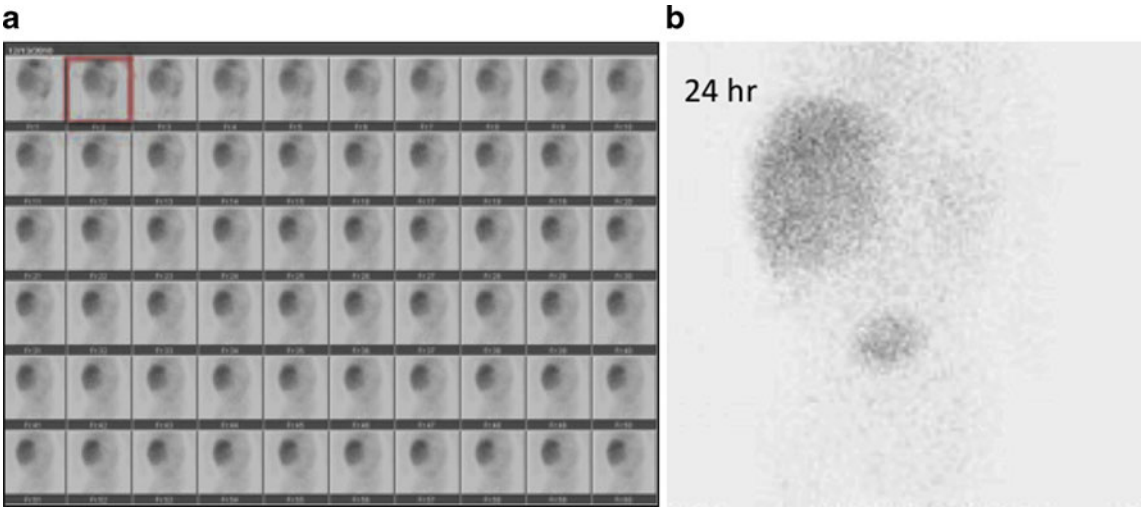

Fig. 3 Conjugated hyperbilirubinemia in an 8-week-old boy. **a** Sequential anterior abdominal scintigraphy images demonstrate poor hepatic uptake and no biliary excretion during the initial hour of imaging. **b** Anterior image at 24 h post-injection shows no evidence of biliary excretion. Scintigraphic pattern: no excretion, decreased uptake. Final diagnosis: total parenteral nutrition cholestasis

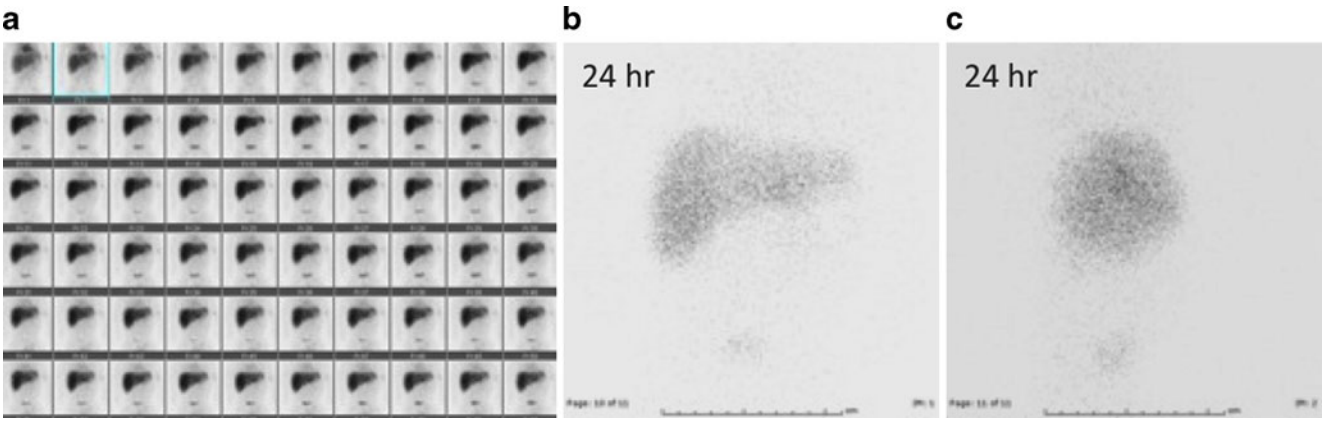

Fig. 4 Cholestatic jaundice in a 2-month-old boy. **a** Sequential anterior abdominal images demonstrate normal hepatic uptake and no biliary excretion during the first hour of imaging. **b, c** Anterior and lateral images at 24 h post-injection do not demonstrate excretion. Scintigraphic pattern: no excretion, normal uptake. Final diagnosis: biliary atresia

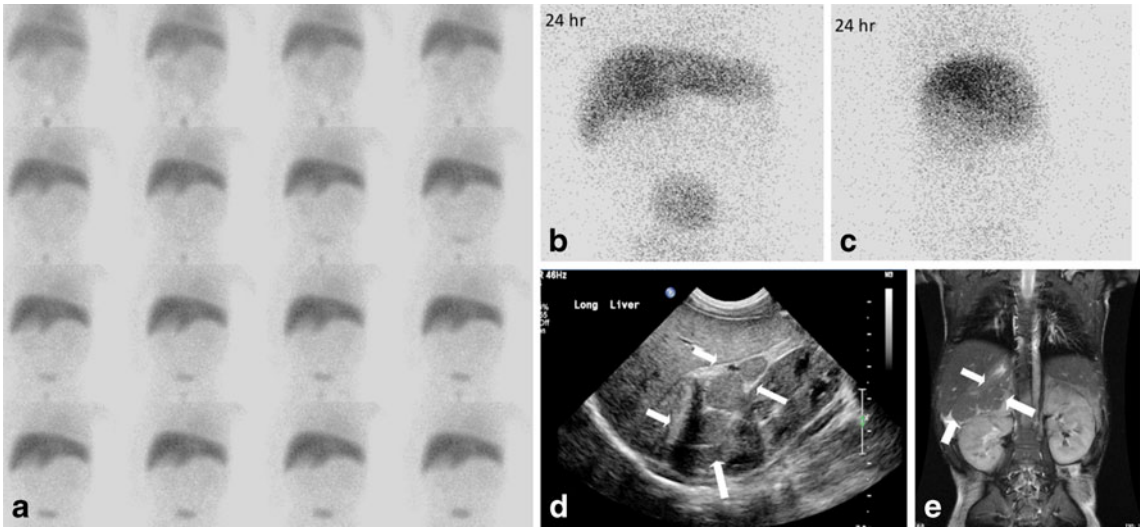

Fig. 5 Conjugated hyperbilirubinemia in a 3-month-old full-term boy. **a** Sequential anterior abdominal images demonstrate normal hepatic uptake and no biliary excretion during the first hour of scanning. Note the transverse orientation of the liver. **b, c** Anterior and lateral scintigraphy images at 24 h post-injection do not demonstrate excretion. **d** Longitudinal US image of the right upper quadrant in the same boy shows polysplenia (*arrows*). **e** T2-W coronal MR image also depicts polysplenia in the right upper abdomen (*arrows*). Also notice the liver extending into the left upper quadrant on the MR image. Scintigraphic pattern: no excretion, normal uptake. Final diagnosis: biliary atresia, associated heterotaxy/polysplenia

imperforate anus with rectovaginal fistula, supernumerary digits and 2-vessel cord (1).

Repeat scans

Twenty-four children had repeat scans. Of these, 17 had non-excretion with normal hepatic uptake on the initial scans. They underwent repeat study because of no or suboptimal phenobarbital pretreatment (9), prematurity (2), low clinical suspicion for biliary atresia (4), discordant/inconclusive biopsies (4) or persistent cholestasis (2), some for multiple reasons. Seven of the 17 showed excretion on the repeat study and did not have biliary atresia, thus changing the category of scan interpretation in those children. Three of these seven had no or inadequate phenobarbital premedication on their initial scans. Of the remaining ten with no excretion even on repeat scans, eight had biliary atresia, one had persistent cholestasis of unknown etiology and one had neonatal hepatitis. Six of these ten had no or inadequate phenobarbital premedication.

The other seven of the 24 repeat scans were done because of persistent cholestasis. One of these seven had decreased uptake on the initial scan with no excretion, a pattern not compatible with biliary atresia in a young patient (<3 months) and had been interpreted as such prospectively per the original report. The remaining six children had normal uptake with presence of excretion on the initial scan. All demonstrated excretion even on the repeat scan, thereby excluding biliary atresia. In this small subgroup of children the theory of "biliary atresia in evolution" was not supported.

Scintigraphic scan patterns on the initial and repeat scans:

1. As seen in Table 1, the majority of the patients (125=117+8) had biliary excretion with normal or decreased hepatic uptake. None of these patients had biliary atresia.
2. The initial studies in nine children showed no biliary excretion but with decreased hepatic uptake; only one of these proved to have biliary atresia. This child, however, was 107 days old at the time of hepatobiliary scintigraphy and the original report stated that biliary atresia in this child could not be excluded with certainty because the child was older than 3 months. One of the nine children underwent a repeat scan and again did not demonstrate excretion, with the final diagnosis being persistent cholestasis of unknown etiology. The diagnoses in the remaining seven children were total parenteral nutrition cholestasis (5), cystic fibrosis (1) and persistent cholestasis of unknown etiology (1).
3. The initial studies in 59 children showed normal hepatic uptake and no evidence of biliary excretion. Of these, 42 had the final diagnosis of biliary atresia. Of the 17 children without biliary atresia, eight were preterm infants and eight had no or inadequate phenobarbital premedication, possible reasons for false-positivity. The final diagnosis included eight children with neonatal hepatitis, four with total parenteral nutrition cholestasis, three with Alagille syndrome, one with choledochal cyst and one with persistent cholestasis of unknown etiology. Nine of these 17 children

Table 1 Final diagnoses in children with the three scintigraphic patterns

Scintigraphic patterns	Total (initial)	Total (after repeat scan)	Biliary atresia	Neonatal hepatitis	Alagille	TPN	Others
1. Excretion, normal uptake	111	117	0	75	5	26	11
Excretion, decreased uptake	7	8	0	3	0	4	1
2. No excretion, decreased uptake	9	9	1 (>3 month old)	0	0	5	3
3. No excretion, normal uptake	59	52	42	4	3	2	1

TPN total parenteral nutrition

underwent repeat scans. Taking the repeat scans into consideration, 52 children continued to demonstrate this pattern. The results of the repeat studies as well as the final diagnoses of these 17 children are detailed in Table 2.

For calculation purposes two older children (>90 days) with decreased hepatic uptake and no excretion were excluded because they did not meet our interpretation criteria; one was diagnosed with biliary atresia (107 days) and the second total parenteral nutrition cholestasis (128 days). Taking into account the results of the repeat scans, the sensitivity for hepatobiliary scintigraphy was 100%, specificity was 93% and accuracy was 94.6%.

Discussion

In our series of 186 children, hepatobiliary scintigraphy proved to be highly accurate (94.6%) in the diagnosis of biliary atresia (100% sensitive and 93% specific). Our specificity is higher than that reported in the majority of the studies in the literature (Table 3) [10–20]. We compared a total of 11 studies from the last three decades with regard to their methodology, interpretation and results. Their reported specificities range from 35% to 90%. Sensitivity is close to 100% in most of the studies; in 2 it is ≤90%.

Gerhold et al. [10] reported a high specificity value in 1983 (82–90%). In 1997, Lin et al. [16] also reported a high specificity (87.5%) and accuracy (90.5%) of hepatobiliary scintigraphy in differentiating biliary atresia from other

Table 2 Children with a scan pattern of normal hepatic uptake and no excretion on initial study with final diagnoses other than biliary atresia. Potential reasons for false positivity are in bold

No.	Gestation	Phenobarbital level (adequate ≥15 mcg/ml)	Days of phenobarbital (adequate ≥5 days)	Repeat study (result)	Final diagnosis
1	**Preterm**	Not known	**3**	No	Alagille syndrome
2	Term	25	11	No	Neonatal hepatitis
3	**Preterm**	20	5	No	TPN cholestasis
4	**Preterm**	Not known	**4**	No	Neonatal hepatitis
5	Term	**12**	5	No	Neonatal hepatitis
6	**Preterm**	24	**3**	No	Alagille syndrome
7	**Preterm**	18	17	No	TPN cholestasis
8	**Preterm**	**14**	11	No	Alagille syndrome
9	Term	27	7	Yes (excretion present)[a]	TPN cholestasis
10	Unknown	**No premedication**		Yes (excretion present)[a]	Neonatal hepatitis
11	**Preterm**	19	7	Yes (excretion present)[a]	Neonatal hepatitis
12	Term	Not known	**1**	Yes (excretion present)[a]	Choledochal cyst
13	Term	15	5	Yes (no excretion)	Persistent cholestasis of unknown etiology
14	Term	**13**	20	Yes (excretion present)[a]	Neonatal hepatitis
10	Term	26	8	Yes (excretion present)[a]	Neonatal hepatitis
11	Term	18	6	Yes (excretion present)[a]	TPN cholestasis
17	**Preterm**	32	5	Yes (no excretion)	Neonatal hepatitis

[a] Scan pattern changed on repeat study

TPN total parenteral nutrition

Table 3 Review of literature [10–20]

Year	Author	Sensitivity	Specificity	No. of patients/ scans	Radiopharmaceutical	Phenobarb prep (dose)	Phenobarb prep (days)	Serum phenobarb level measured (yes/no)	Delayed imaging	Hepatic uptake used for interpretation (yes/no)
1983	Gerhold et al. [10]	97–100%	82–90%	27/27	Tc-99 m-diethyl-IDA or ^{99m}Tc-DISIDA	None	NA	No	Up to 24 h	Yes, hepatocyte clearance grade
1987	Spivak et al. [11]	100%	55%	28/38	^{99m}Tc-DISIDA	5 mg/kg	≥3	No	Up to 24 h	Yes (cardiac blood pool activity similar to above)
1987	Cox et al. [12]	100%	67%	33/?	^{99m}Tc-DISIDA	5 mg/kg	5	No	2–4, 24 h	Unclear
1995	Ben-Haim et al. [13]	100%	92.8%	36/39	^{99m}Tc-mebrofenin	5 mg/kg (22 patients)	2–28	No	2–3, 4–5, 6–8, 20–24 h	No
1997	Gilmour et al. [14]	100%	74%	86/86	Tc-99 m IDA agent	5 mg/kg	3–5	No	2, 4, 6, 24 h	No
1997	Park et al. [15]	96%	35%	71/71	^{99m}Tc-DISIDA	5 mg/kg	3–5	No	2,4, additional imaging as necessary up to 24 h	No
1997	Lin et al. [16]	100%	87.5%	66/66	^{99m}Tc-DISIDA	5 mg/kg and cholestyr-amine 8 g	≥7	No	2,4, additional imaging as necessary up to 24 h	Unclear
1998	El-Desouki et al. [17]	100%	88.9%	77/77	^{99m}Tc-DISIDA	5 mg/kg	5	No	2–3, 4–8, 20–24 h	No
1998	Johnson et al. [18]	100%	67%	58/68	^{99m}Tc-mebrofenin	5 mg/kg	≥5	No	2, 3, 24 h	No
2007	Esmaili et al. [19]	90%	80%	70/70	^{99m}Tc-mebrofenin	5 mg/kg	3	No	1, 2, 6, 24 h	No
2009	Yang et al. [20]	88%	46%	69	Tc-99 m IDA agent	None	None	No	6, 24 h	No

forms of neonatal jaundice, very similar to our current study results. They concluded that hepatobiliary scintigraphy after premedication with phenobarbital and cholestyramine is a convenient and reliable method of differentiating biliary atresia from neonatal hepatitis [16]. Another recent study, by Esmaili et al. [19], also found hepatobiliary scintigraphy to be a reliable and convenient method to distinguish biliary atresia and neonatal hepatitis, although less accurate than histology.

Several studies, however, did not find hepatobiliary scintigraphy to be a very specific test. Cox et al. [12] in 1987 found hepatobiliary scintigraphy to be less specific than US or biopsy. They recommended that hepatobiliary scintigraphy not be routinely used in evaluating neonatal cholestasis, especially if it delayed surgical intervention [12]. Similarly in 1996 Gilmour et al. [14] reported that hepatobiliary scanning required cautious interpretation because "non-draining" scans could also be seen in severe neonatal hepatitis or with interlobular bile duct paucity. Their reported specificity of hepatobiliary scanning in neonatal cholestasis was 74%. They also found that good liver extraction does not exclude neonatal hepatitis [14]. Park et al. [15] also did not find hepatobiliary scintigraphy to be very specific (35%) for diagnosis of biliary atresia. In 1998 Johnson et al. [18] reported both hepatobiliary scintigraphy and histology to be highly sensitive (100%) but not accurate individually. In their study hepatobiliary scintigraphy was only 67% specific for diagnosing biliary atresia [18]. They concluded that the evaluation of prolonged neonatal jaundice needed a multi-modality strategy [18]. Results from a study by Yang et al. [20] in 2009 also reported a low diagnostic accuracy of 67% for hepatobiliary scintigraphy.

The majority of these studies did not utilize hepatic uptake as a parameter in interpretation and calculation of results, although some of them did record the uptake. The variability in results could also be related to differences in the patient group selection in some of these studies. For example, Gilmour et al. [14] chose to include only patients with hepatobiliary scans who also had liver biopsies. Although in the majority of these studies phenobarbital premedication was used, duration of treatment was less than 5 days in some. More important, none of these studies measured serum phenobarbital levels to test for adequacy of premedication.

Role of phenobarbital

In 1981, Majd et al. [9] concluded that hepatobiliary scintigraphy with ^{99m}Tc-PIPIDA after phenobarbital premedication was a very accurate test to distinguish biliary atresia from neonatal hepatitis. The exact mechanism by which phenobarbital enhances excretion of IDA compounds is not well understood. Phenobarbital induces microsomal enzymes, increases bilirubin conjugation and excretion and enhances uptake and excretion of certain compounds. The choleretic effect of phenobarbital is thought to be independent of enzyme induction. It appears that phenobarbital acts on the whole hepatic transport system of organic anions. This ensures the best possible excretion of hepatobiliary radiopharmaceutical and visualization of the biliary tree [9, 24]. Since then with the advent of newer IDA analogues, the role of phenobarbital has been controversial, some studies reporting increased diagnostic accuracy and others indicating that it is not necessary [13]. In a retrospective review in 2003, Charearnrad et al. [21] concluded that phenobarbital therapy might not be necessary prior to ^{99m}Tc-DISIDA scan examination in the evaluation of cholestatic infants and thus a delay in diagnosis and surgical therapy of biliary atresia could be avoided.

We believe that routine use of adequate phenobarbital premedication is a very important reason for higher accuracy of hepatobiliary scintigraphy at our center. Our routine protocol for hepatobiliary scintigraphy in neonatal cholestasis is pretreatment with phenobarbital (5 mg/kg/day for 5 days in two divided doses) to achieve a phenobarbital level of ≥15 mcg/ml. In the current study only three children did not receive phenobarbital premedication for their initial scan. It is our practice to document serum phenobarbital level on the day of the study or 1 day prior. The prescribed dosing does not always ensure adequate treatment, as can occur in cases of non-compliance or loss of dose because of vomiting or spitting. This is especially important in an outpatient setting, where the dosing cannot be controlled.

Occasionally if the serum level is not available or not therapeutic, the study is still performed for clinical reasons (older patient and urgency to exclude biliary atresia). In such a scenario, if the study demonstrates excretion, biliary atresia is excluded. However, if the scan demonstrates normal hepatic uptake with no excretion, the results are considered unreliable for diagnosis of biliary atresia and a repeat study with adequate phenobarbital premedication is recommended.

In our study, eight of the 17 children with no excretion on the initial scan (and final diagnosis other than biliary atresia) had no or inadequate phenobarbital premedication. Of these eight, three underwent repeat scans with phenobarbital and all demonstrated excretion (Figs. 6 and 7).

Technique and interpretation of hepatobiliary scintigraphy

We also follow a consistent technique whereby images are obtained at 2, 4, 6, 8 and 24 h after the initial 1 h of

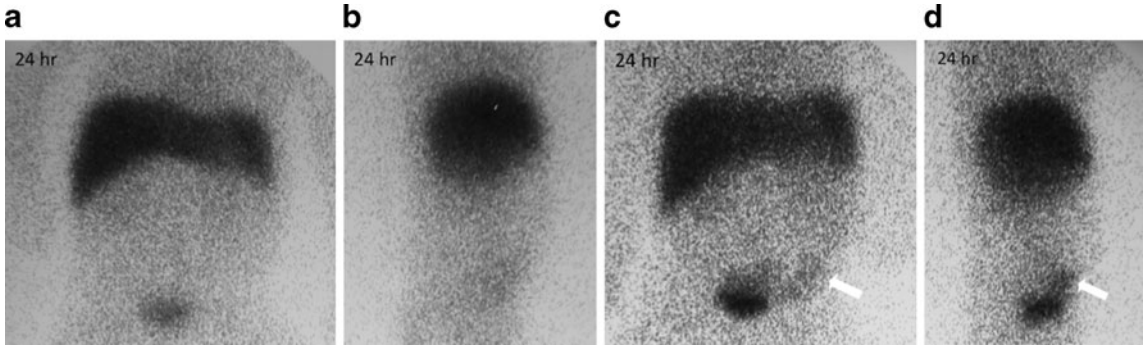

Fig. 6 Direct hyperbilirubinemia in a 3-month-old girl. **a, b** Anterior and lateral 24-h delayed images of the initial hepatobiliary scan demonstrate normal hepatic uptake with no excretion. No phenobarbital premedication was given. **c, d** Anterior and lateral 24-h delayed images of the hepatobiliary scan performed 6 days later with adequate phenobarbital premedication demonstrate excretion (*arrows*). Scintigraphic pattern: normal uptake, no excretion on the initial scan changed to normal uptake, excretion after adequate phenobarbital premedication. Final diagnosis: neonatal hepatitis

sequential 1-min imaging. Biliary excretion of the tracer as evidenced by visualization of the tracer in the gallbladder or intestine indicates patency of the extrahepatic biliary ducts, thus excluding biliary atresia. In only one of our patients, the gallbladder was visualized without demonstrable tracer activity in the intestinal tract. This was interpreted as evidence of patent extrahepatic ducts, which was confirmed by subsequent improvement in clinical course with final diagnosis of total parenteral nutrition cholestasis. Lee et al. [25] found that visualization of the gallbladder even in the absence of tracer activity in the bowel rules out biliary atresia. They found that in case of cholestasis or hepatic dysfunction, if the radiotracer was concentrated in a relatively small volume in the gallbladder it could easily be visualized. However if the tracer was ejected into a relatively large volume of the intestine it could become too diluted to be detectable [25].

In infants younger than 3 months, decreased hepatic uptake essentially excludes biliary atresia even though biliary excretion might not be demonstrated. After 3 months of age, hepatic uptake is not as reliable to distinguish neonatal hepatitis and biliary atresia because cirrhosis can set in by then in advanced cases of biliary atresia [3]. There were a total of nine children in our series with reduced hepatic uptake and no biliary excretion, only one of whom had biliary atresia. However, this child was 107 days old and subsequently underwent a primary liver transplant rather than a Kasai procedure.

Repeat scans

In our study 24 children had repeat scans for various reasons. It is noteworthy that of the 17 with normal uptake and no excretion on the initial scan, 9 were repeated for no or inadequate phenobarbital premedication. Of these, six continued to demonstrate no excretion on the repeat scans and were later proved to have biliary atresia. The remaining

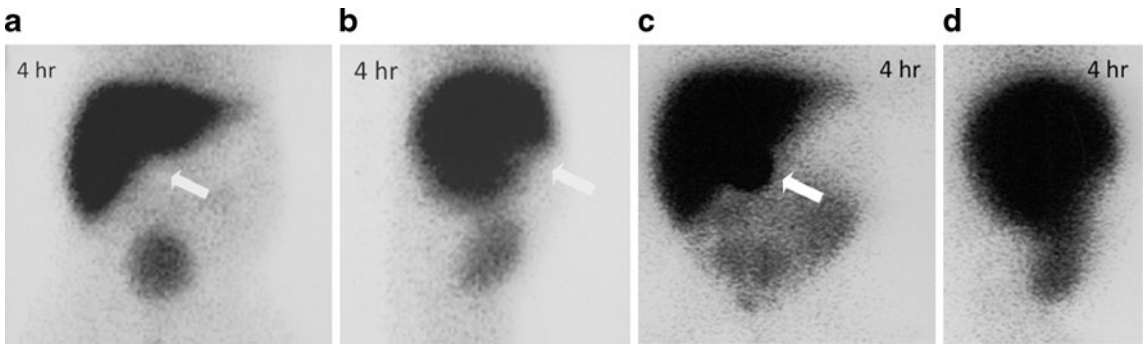

Fig. 7 Neonatal cholestasis in a 2-month-old girl with sonographic diagnosis of choledochal cyst; hepatobiliary scan was requested to rule out associated biliary atresia. **a, b** Anterior and lateral 4-h delayed images of the initial hepatobiliary scan obtained after only 1 day of phenobarbital premedication demonstrate normal hepatic uptake with no excretion. Note the photopenic defect along the inferior aspect of the liver consistent with the known choledochal cyst (*arrow*). **c, d** Anterior and lateral 4-h delayed images of the hepatobiliary scan performed 4 days later with adequate phenobarbital premedication demonstrate tracer accumulation in the choledochal cyst (*arrow*) and in the intestinal tract. Scintigraphic pattern: normal uptake, no excretion on the initial scan changed to normal uptake, excretion after adequate phenobarbital premedication. Final diagnosis: choledochal cyst without biliary atresia

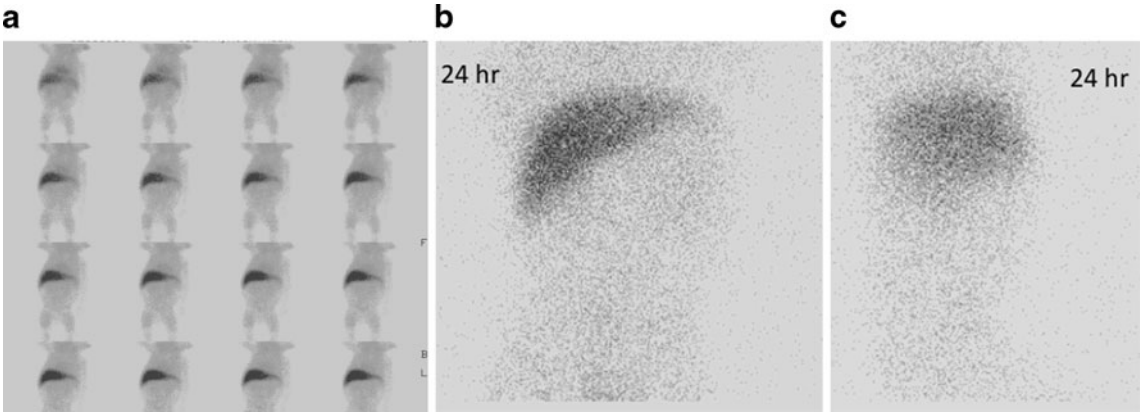

Fig. 8 Cholestasis in a girl born at 29 weeks' gestation. **a** Sequential anterior abdominal images show with normal hepatic uptake and no biliary excretion. **b, c** Delayed anterior and lateral images at 24 h did not show any excretion. Scintigraphic pattern: no excretion, normal uptake. Final diagnosis: Alagille syndrome

three children demonstrated excretion on the repeat scans and none had biliary atresia.

The other reasons for repeating studies included clinical improvement, premature status or inconclusive biopsies, persistent cholestasis or a combination of these reasons. Spivak et al. [11] recommend repeating hepatobiliary scintigraphy in infants with no excretion on the first study when the diagnosis of biliary atresia is not clear. Their study, however, had considerably lower specificity of 43% based on the first scan compared to ours [11]. Although the majority of their patients (25 of 28) had received phenobarbital, they received fewer days of phenobarbital (minimum 3) as compared to our standard of minimum 5 days, and the serum phenobarbital levels were not documented in any of their cases [11].

Biliary atresia *in premature infants*

In our study there were 111 term infants, of whom 39 (35.1%) had biliary atresia, and 68 preterm infants, of whom only two (2.9%, gestational ages 31 and 36.5 weeks) had biliary atresia. This suggests that biliary atresia is rare in the premature population. Similar findings were reported by Mowat et al. [26] in 1976; they had only one premature infant in their population of 32 infants with biliary atresia. The clinical observation that biliary atresia is rarely encountered in premature infants supports the concept that some unknown perinatal or postnatal event triggers progressive injury and fibrosis of a normally developed biliary tree. This might explain why biliary atresia is rare in the premature population, where the pathological obliterative process might not have begun. On the other hand a Swedish study in 2002 reported prematurity to be an independent risk factor for biliary atresia, a finding supported by an epidemiological study from New York state published in 2004 [27, 28]. The reasons for disparate findings in these studies are unclear.

Alagille syndrome

Alagille syndrome is a rare genetic disorder associated with abnormalities of the liver, heart, eye, skeleton and characteristic facies [29]. Diagnosis is made by a combination of phenotypic features, genetic tests and paucity of intrahepatic bile duct on liver biopsy. In our study, three of eight (37.5%) children with Alagille syndrome had normal hepatic uptake with no excretion, a scintigraphic pattern that mimics biliary atresia (Fig. 8). In such cases, liver biopsy might prove useful after other mimickers including cystic fibrosis and alpha-1 antitrypsin deficiency have been excluded by biochemical tests [24]. Hepatobiliary scan and liver biopsy might prove to be complementary in making the diagnosis.

Conclusion

Phenobarbital-enhanced hepatobiliary scintigraphy is highly accurate in differentiating biliary atresia from other causes of neonatal cholestasis provided that a serum phenobarbital level of 15 mcg/ml is achieved and both biliary excretion and quality of hepatic uptake are considered in interpretation of the scans. Alagille syndrome is a mimicker of biliary atresia on hepatobiliary scintigraphy. Biliary atresia is rare in premature infants.

Conflicts of interest None.

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