Increased conjugated bilirubin is sufficient to initiate screening for biliary atresia

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ABSTRACT

INTRODUCTION: Biliary atresia is the leading cause of liver transplantation in children. It affects 1:15,000 in Denmark. With a national birth rate of 60,000, four children are born every year with biliary atresia. Early correction of biliary obstruction is essential to prevent fatal biliary cirrhosis. The Danish Health and Medicines Authority (DHMA) demands diagnostic evaluation of children with elevated level of serum bilirubin after two weeks of age. Biliary atresia has to be excluded if conjugated bilirubin level is above than 20 µmol/ l, and/or more than 20% of total bilirubin. This percentage value has caused diagnostic trouble over the years. The objective of the present study was to investigate the possibility of changing the recommendations.

METHODS: This was a retrospective analysis of the medical records of children operated for biliary atresia in the 1993-2012 period.

RESULTS: During the period, 73 patients where operated with a portoenterostomy ad modum Kasai. Patients older than 84 days at the time of operation were excluded, 54 patients were available for analysis. Conjugated bilirubin in µmol/l and the percentage value were significantly above the DHMA threshold limit: mean 129.7 µmol/l (42-334) and 73% (28-97%), respectively.

CONCLUSION: The total amount of conjugated bilirubin above 20 µmol/l is sufficient to require further evaluation for biliary atresia. The percentage value is unnecessary and may cause confusion.

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Biliary artresia (BA) is the most common surgical cause of neonatal cholestasis and the most frequent indication for liver transplantation in the paediatric population [1]. The disorder is a fatal obliterative cholangiopathy with an uncertain pathogenesis. Histologically, small bile ducts at the porta hepatis are found and these are, to some extent, connected to the intrahepatic biliary tree. This explains why bile drainage can be restored by the Kasai portoenterostomy (KP) [2]. Subsequently, liver transplantation is the only surgical option.

Before the introduction of the KP, biliary atresia survival rates were below 5% at 12 months [3]. This likely reflects the ongoing cirrhosis that occurs with this disease; by 90 or 120 days of age, no ductal tissues are left, and only fibrotic tissue remains.

Early diagnosis is crucial since KP performed after 56 days of life (eight weeks) might lead to an impaired prognosis [4]. Therefore, the Danish Health and Medicines Authority (DHMA) recommends diagnostic evaluation no later than three weeks after birth [5]. DHMA recommends diagnostic evaluation if a child has conjugated hyperbilirubinaemia after two weeks of age, defined as a conjugated bilirubin value > 20 µmol/l and/or a conjugated bilirubin value of total bilirubin > 20%. Children complying with the criteria should receive a ^{99m}Tc-hepatobiliary iminodiacetic scintigraphy. If no tracer is visualised in the gut 24 hours post-injection, the child shall be referred to a centre with expertise in infant KP within 24 hours [5].

Our study is based on recommendations from the DHMA for diagnostic evaluation concerning BA [5]. The aim of this study was to evaluate the validity of the current recommendations for children with conjugated hyperbilirubinaemia and to investigate the possibility of simplifying the recommendations by excluding the estimation of conjugated bilirubin value of total bilirubin and focusing on the value of conjugated bilirubin only.

METHODS

The preoperative values of conjugated and total bilirubin were registered retrospectively through medical record review. Furthermore, the age at operation was registered. The preoperative serum values were used and assumed analogous to the prognostic values since the KP was performed shortly after hospitalisation. In Denmark, 73 patients proceeded to the KP at Rigshospitalet from 1993 to 2012. Many of these patients had the operation performed when they were older than the recommended eight weeks, and it was originally intended to exclude these patients. However, the exclusion would have left a relatively small population for evaluation and therefore only patients who were more than 12 weeks old at surgery were excluded (population ≤ 84 days: 60 patients. Population ≤ 56 days: 35 patients). Of the remaining 60 patients, serum values were available for 54 patients (90%). The patient files were extracted from Rigshospitalet's archives. Furthermore, some serum values were found in a database at the Department of Clinical Biochemistry. In total, 19 patients were excluded for one of the following causes: 1) In two cases the medical records were lost; 2) in seven cases the values of

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conjugated bilirubin were never registered; and 3) in ten cases the operation was performed more than 84 days after birth (all with increased values of conjugated and total bilirubin).

The statistical analysis included calculation of the mean, range and 95% confidence interval (CI) for both conjugated and conjugated bilirubin fraction as a percentage of total bilirubin. To quantify the difference between the mean value in our study and the limits of the DHMA, a calculation of probability was accomplished by using the t-distribution. The difference between the compared factors was considered significant at a p-value of < 0.05.

Furthermore, a graphic correlation between the age at operation and the value of the conjugated bilirubin was illustrated. The correlation was quantified using the Pearson Product Moment correlation.

Trial registration: not relevant.

RESULTS

The median patient age at the operation was 48 days. Using medical record review, the following values were calculated: conjugated bilirubin mean 129.13 µmol/l (range: 42-334, 95% CI: 113-145) and conjugated bilirubin fraction as a percentage of total bilirubin 73% (range: 28-97%; 95% CI: 69-77%). The mean values of conjugated and conjugated bilirubin fraction as a percentage of total bilirubin were both significantly higher than the DHMA limits (**Table 1**). Furthermore, the lower value of range for both conjugated and conjugated bilirubin fraction as a percentage of total bilirubin was above the DHMA limit.

Based on the registered data, the correlation between age at operation and value of conjugated bilirubin is presented in **Figure 1**. A correlation coefficient of –0.29 was calculated. The correlation showed a decreasing concentration of conjugated bilirubin with increasing age. Furthermore, the correlation was not strong.

TABLE 1

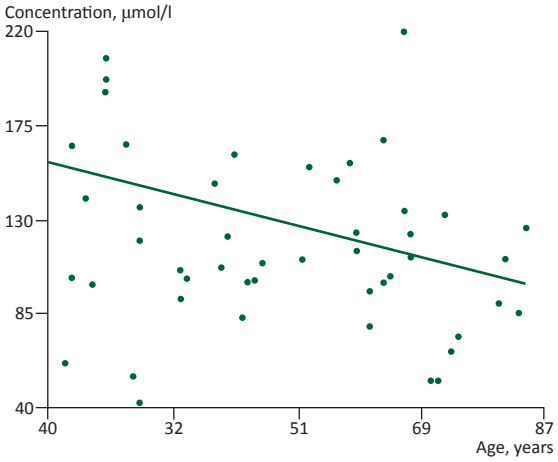
Conjugated bilirubin concentration and conjugated bilirubin in % of total bilirubin of 54 children with biliary atresia and the lower limits for diagnostic evaluation recommended by the Health and Medicines Authority.

Compared factor	DHMA limit	This study, mean (range)	p-value
Conjugated bilirubin concentration, µmol/l	20.00	129.13 (42-334)	< 0.001[a]
Conjugated bilirubin,% of total bilirubin	20	73 (28-97)	< 0.001[a]

DHMA = Danish Health and Medicines Authority.
a) With a p-value < 0.001, our study reports significantly higher values for both conjugated and fraction of conjugated bilirubin in percentage of total bilirubin compared with the limits of the Danish Health and Medicines Authority. Furthermore, the lower value of range for both conjugated and fractionated bilirubin is above the limit of the Danish Health and Medicines Authority.

FIGURE 1

Correlation between the age at operation and concentration of absolute conjugated bilirubin. A correlation coefficient of –0.29 was calculated. The correlation shows a decreasing concentration of conjugated bilirubin with increasing age. Furthermore, the correlation is not strong.



DISCUSSION

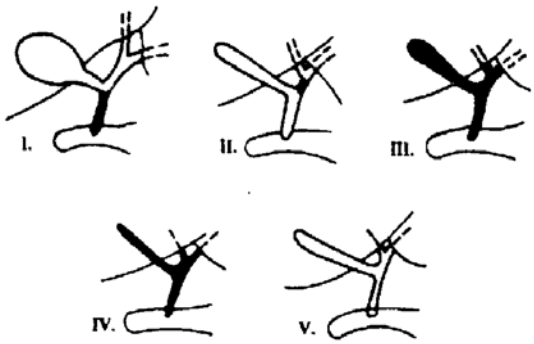
The literature contained only few studies on conjugated and conjugated bilirubin fraction as a percentage of total bilirubin for BA.

Firstly, Wang et al explored the possibility that serum markers may be employed to distinguish BA from other causes of neonatal cholestasis [6]. Secondly, Harpavat et al explored how early the concentration of conjugated bilirubin can distinguish patients with BA from healthy patients [7]. Since Harpavat et al registered the bilirubin values shortly after birth [7], a direct comparison was only possible with Wang et al.

Focusing on the results concerning conjugated bilirubin, our study was consistent with the results of Wang et al who indicated a value of 6.2 ± 0.03 mg/dl (mean 106 ± standard error (SE) 0.51 µmol/l) [6]. Furthermore, the mean value for the conjugated bilirubin fraction as a percentage of total bilirubin was consistent with Wang et al who indicated a mean value of 80% ± SE 3% at diagnosis [6].

This present study showed that the lower value of range for both conjugated and conjugated bilirubin fraction as a percentage of total bilirubin were above the DHMA limit. Therefore, it seemed recommendable to exclude the limit for conjugated bilirubin fraction as a percentage of total bilirubin from the DHMA's recommendations since all of the children would have been found by employing the value for conjugated bilirubin only.

The importance of maintaining a limit for conjugated bilirubin that is independent to total bilirubin



Classification of biliary atresia into five types. Original drawing from Ugeskrift for Læger [12].

should be emphasised. This was supported by Harpavat et al who pointed out that 53% of the patients with increased conjugated bilirubin were not evaluated in the American healthcare system [7]. The diagnostic evaluation was omitted as the American recommendations lacked a limit for conjugated bilirubin that was independent of total bilirubin [8].

Concerning the correlation between age at operation and conjugated bilirubin, a significantly higher value for conjugated bilirubin was observed in the younger group. An explanation for this observation may have been that an earlier diagnosis was made for patients with a particularly aggressive form of BA causing more severe liver injury, as proposed by Volpert et al in a study with similar findings [9].

It may, of course, be discussed whether other aspects of the DHMA's recommendations should be changed. Harpavat et al suggested screening of all new born children for conjugated hyperbilirubinaemia to achieve an earlier diagnosis [7]. We cannot support this recommendation as operation shortly after birth had no evident beneficial impact on the prognosis compared with surgery performed at 2-4 weeks of age [2].

One weakness of the study is that the bilirubin serum values were unavailable in 10% of the relevant cases.

To achieve an early diagnosis, it is not only necessary to optimise the recommendations to physicians. It is also important to encourage awareness among parents of certain observations related to the newborn child. A limited number of countries have countered this problem by introducing a *stool colour card*, first introduced by Matsui & Dodoriki [10]. The card was applied in Taiwan where it increased the number of patients operated before 60 days of life and yielded an improvement in prognosis [11].

CONCLUSION

BA is a fatal disease that requires early diagnosis to ensure optimal prognosis. The most evaluated method for diagnosis is serum values for conjugated and conjugated bilirubin fraction as a percentage of total bilirubin, which explains why the DHMA's recommendations for diagnostic evaluation of BA are primarily based on bilirubin values. Compared with the recommended limits, our study shows significantly higher values for both conjugated and conjugated bilirubin fraction as percentage of total bilirubin. The values are consistent with a previous study and there seems to be no evidence to support lowering the limits.

A total amount of serum conjugated bilirubin above 20 µmol/l in a 2-3-week old neonate is sufficient to require further evaluation for biliary atresia. The currently recommended percentage value is unnecessary and may cause confusion.

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