Modified stool color card with digital images was efficient and feasible for early detection of biliary atresia—a pilot study in Beijing, China

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Background: **The aim of this pilot study in Beijing, China, was to validate a screening system for early detection of biliary atresia (BA) by using a modified version of the stool color card (SCC).**

Methods: **From 2013 to 2014, a total of 29 799 live born infants were screened. SCC was distributed in maternal facilities. Guardians were asked to check their infants' stool colors daily using SCC up until four months after birth. The screening results among 92.5% of participants were reported. Cases deemed as high risk were referred to a surgical department immediately.**

Results: **Based on the results reported by the guardians, 24 infants showed pale-pigmented stools, of which two males without obvious signs of jaundice were diagnosed with BA at 52 and 55 days of age, respectively. The sensitivity was 100% and specificity was 99.9%. Four infants were confirmed as having other diseases. Two female patients failed to be screened by the SCC because they had severe jaundice and were referred to the Neonatal Intensive Care Unit after birth. They were diagnosed as BA at 14 and 17 days after birth, respectively. The overall prevalence of BA in this study was 1.3 in 10 000 live births.**

Conclusion: **The modified SCC was effective and feasible for early detection of BA, especially for patients with no apparent jaundice.**

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Key words: **early detection; jaundice; Kasai procedure; sensitivity; specificity**

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Original article

Introduction

Biliary atresia (BA) is the most frequent cause of death from hepatobiliary diseases in early childhood, and is characterized by a complete inability to excrete bile as a result of sclerosing inflammation of the extra, and possibly intra, hepatic bile ducts.[1] BA has three main clinical features: pale-pigmented stools, prolonged jaundice and dark urine. Pale-pigmented stools appear within the first four months after birth.[2,3] Prolonged jaundice, defined as jaundice in neonates beyond 14 days of age, is often misinterpreted as "physiologic". Dark urine is often overlooked.

BA is classified into three types according to the morphologic features: Type I, atresia of common bile duct; Type II, atresia of the hepatic duct; and Type III, atresia at the porta hepatis. The majority of BA patients belong to Type III, which has poor outcome when compared with the other types.[4,5] Kasai procedure (KP)[6] is commonly used as a first-line treatment for all Type III, II and most of the Type I BA. It is generally acknowledged that an early KP, especially before 60 days of age, can improve the long-term native liver survival and reduces likelihood of liver transplantation.[7]

Several methods for early detection of BA have been developed. The effectiveness of the stool color card (SCC), consisting of photographic images of stool colors, has been demonstrated previously by studies

in Japan and Taiwan.[3,8] Since April 2012, a modified version of the SCC (Fig. 1), which consists of digital images instead of photographic ones, has been used for nationwide BA screening in Japan.

The incidence rate of BA has been reported to be approximately 0.5 in 10 000 live births in the USA, UK and France.[9-11] In Asia, this figure is even higher, with approximately 1.0 in 10 000 live births in Japan and 1.5 in 10 000 live births in Taiwan.[3,8] The incidence rate of BA in mainland China, however, remains unclear. The estimated annual number of newborns with BA was between 1600 and 2400, against a backdrop of approximately 16 million live births. During the period of 2003 and 2010, the mean age at the time of KP was 82.4 days in Beijing and 84.5 days in Shanghai, which is much later than the recommended age of 60 days.[12,13] The main reason is that a system for the early detection of BA has yet to be implemented in mainland China.

In this pilot study, a system for early BA detection was established in Beijing. The aims were to assess the validity of the screening using the modified SCC, as well as to investigate the epidemiology of BA in the geographic area.

Methods

Modified SCC with digital images

The modified SCC (fifth edition, Fig. 1) used in this study consisted of seven digital color images of stool colors from healthy infants at around 30 days of age (images No. 4-7) and age-matched patients with BA (images No. 1-3). Image 4 represents a stool color in both healthy and BA infants. In such cases, the child was observed closely. Digital imaging ensures quality control and reproducibility. This pilot screening in Beijing was a collaborative study between the National

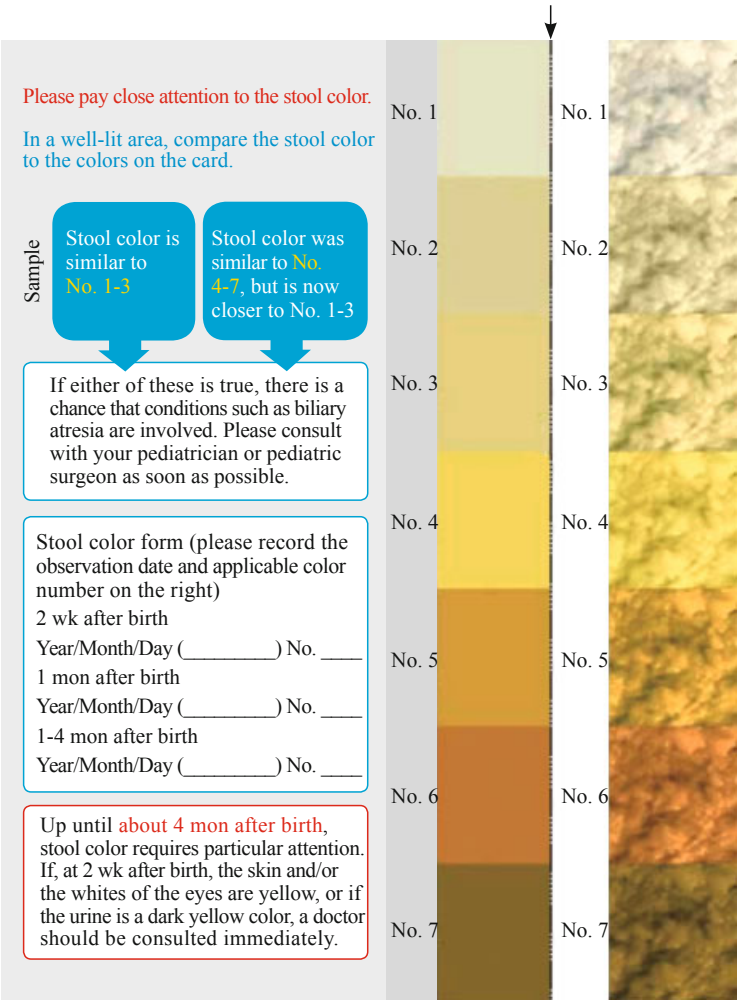

Fig. 1. The stool color card used in the Beijing pilot study consisted of seven digitally created images (fifth edition, translated in English for this paper). Images No. 1-3 show abnormal stool color, while No. 4-7 show normal stool colors. Image No. 4 represents a stool color in both healthy and biliary atresia infants. The panel of stool colors is reproducible with quality control easy to perform. It has been included in the Maternal and Child Health Handbook and distributed for free nationwide in Japan since April 2012. The arrow represents the cutoff line.

Center for Child Health and Development, Tokyo, Japan, and the Beijing Obstetrics and Gynecology Hospital of China. In September 2013, a material transfer agreement with a non-profit clause was signed and 30 000 SCCs were shipped to Beijing in October the same year. The SCC was designed and developed by Akira Matsui. The explanation text on the SCC was translated to Chinese from Japanese by Yan-Hong Gu.

A screening system based on the usage of the SCC by maternal facilities and guardians

The study protocol was reviewed and approved by the Ethics Board of the National Center for Child Health and Development and the Beijing Obstetrics and Gynecology Hospital of China. This pilot study was performed from December 2013 to October 2014 in 25 maternal facilities located in the Chaoyang district of Beijing. This particular district, which covers the urban areas of northern and eastern Beijing, is the largest district in the city with 48 802 live births in 2012. Participants of this study were all live births born between December 4, 2013 and July 30, 2014, and whose guardians had signed an informed consent. A total of 29 799 live births were enrolled (Fig. 2).

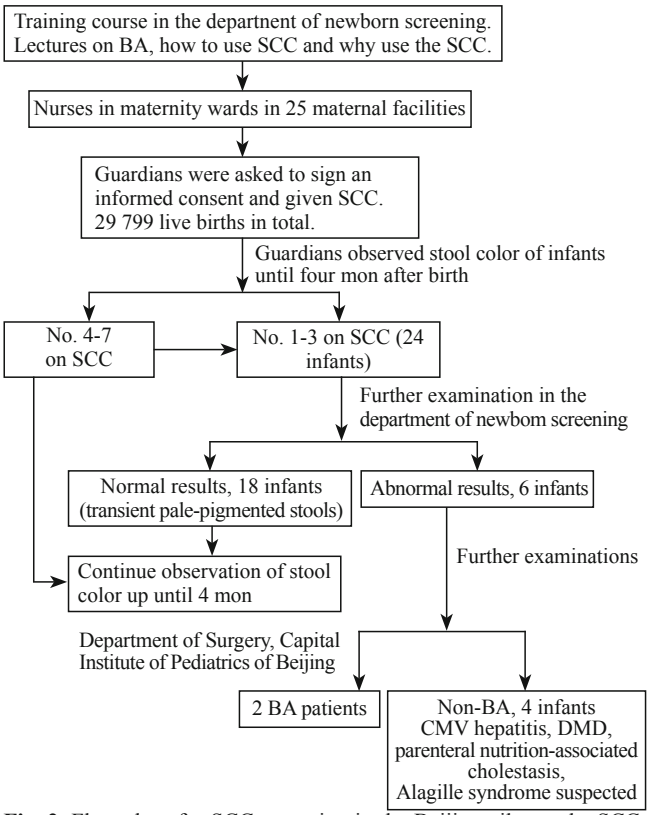

Fig. 2. Flow chart for SCC screening in the Beijing pilot study. SCC: stool color card; BA: biliary atresia; CMV: cytomegalovirus; DMD: Duchenne muscular dystrophy.

SCCs were distributed to the guardians by trained nurses in maternal facilities during information sessions on neonatal screening for phenylketonuria and congenital hypothyroidism. The guardians were advised to observe the stool color of their infant until four months after birth, and fill in the corresponding number of the image on SCC which most resembled the color of her infant's stool on day 14, one month, and during one to four months after birth. In addition, the SCC office checked the stool color in the babies at the health check-up on day 42, and follow-up with telephone calls and mobile text messages during one to four months after birth.

When pale-pigmented stool corresponding to images No. 1-3 was observed, the guardians must report the event to the medical staff along with confirmation and further examinations, and visit the SCC office at the Department of Newborn Screening, Beijing Obstetrics and Gynecology Hospital. For those infants with apparent positive signs and symptoms compatible with BA, laboratory examinations including standard tests of liver function and tests for viral and bacterial infections were performed. When serum direct/total bilirubin ratio was higher than 20%, an infant would be categorized as high-risk and referred accordingly. Alagille syndrome, congenital bile duct dilatation and neonatal intrahepatic cholestasis caused by citrin deficiency were clinically excluded. Two high-risk male infants were transferred to the Department of Surgery, Capital Institute of Pediatrics of Beijing, where the most numbers of KP were performed in Beijing. Further examinations including abdominal ultrasonography were performed. When a diagnosis of BA was confirmed, the parents would be informed and asked to provide consent for the infant to undergo KP. The associated monetary implications were also revealed to them.

Reference data

A registration record of congenital abnormalities in Chaoyang district was used as reference data for confirmed BA patients in the district during the period of the study. After comparing our results with the registration data, those who were not registered on this list were considered as negative.

Statistical analysis

Calculation of sample size was based on the Poisson distribution. The probability distribution of a Poisson random variable X representing the number of BA patients occurring during the period of this pilot study in Chaoyang district was given by the formula:

$$P(X;\mu)=(e^{-\mu}\mu^{x})/x!, \text{ where } x=0, e=2.71828.$$

μ=3 was assumed as the mean number of BA patients in the time of pilot study in 30 000 live births

in Chaoyang district because we supposed that the incidence was similar to that of Japan (1 in 10 000 live births). Therefore, P (0; 3)=0.0498. The probability of finding no BA patients (n=0) in 30 000 live births was less than 0.05. To quantify uncertainty, 95% confidence interval (CI) was used.

Results

Early detection of BA by guardians using SCC

In total, 29 799 SCC were distributed and 27 561 (92.5%) of them were returned until four months after births (Fig. 2). Those who did not return their results of SCC were considered as negative, because we compared our list of BA patients to the record of registration of congenital abnormalities in Chaoyang district. Based on the guardians' results, 24 infants showed pale-colored stools corresponding to images No. 1-3 (Figs. 1 and 2). Among them, two male infants were diagnosed with BA at 52 days and 55 days of age, respectively. One of them was born at 40 gestational weeks. Slight jaundice was observed from the third day and lasted for more than two weeks. At 23 days of age, his stool color corresponded to image 2; ALT, TBIL, DBIL, TBA and GGT was 174.0 IU/L, 227.5 μmol/L, 119.6 μmol/L, 160.8 μmol/L and 93.0 IU/L, respectively. Laparoscopic surgery was performed at 52 days of age. The other infant was born at 37 gestational weeks without obvious jaundice. At 48 days of age, his stool color corresponded to image 3; ALT, TBIL, DBIL, TBA and GGT was 70.0 IU/L, 121.4 μmol/L, 65.4 μmol/L, 97.0 μmol/L and 128.0 IU/L, respectively. Laparoscopic surgery was performed at 55 days of age. The guardians of both patients refused KP although the diagnosis was confirmed as BA type III. The sensitivity and specificity for BA was 100.0% and 99.9% (95% CI: 99.9-99.9), respectively. The positive predictive value was 8.3% (95% CI: 2.7-19.4) by guardian using SCC.

Two female BA patients were found in the congenital abnormalities registration of Chaoyang district. They showed severe jaundice from the first two days after birth, and were referred to a neonatal intensive care unit (NICU) immediately. One of them was born at 38 gestational weeks. On the first day after birth, ALT, TBIL, DBIL and GGT was 49.1 IU/L, 283.5 μmol/L, 118.1 μmol/L, and 671.8 IU/L, respectively. The nurse who provided the SCC to the parents confirmed that the infant's stool color corresponded to image No. 2 on the eighth day after birth. A diagnosis of BA type III was confirmed by laparoscopic surgery and KP was performed at 17 days of age. The other female infant was born at 39 gestational weeks and 2 days. At the second day after birth, ALT, TBIL, DBIL and TBA were 20.7 IU/L, 157.0 μmol/L, 24.5 μmol/L, and 28.5 μmol/L, respectively. A diagnosis of BA type III was confirmed at 14 days of age by laparoscopic surgery and KP was performed at 30 days of age in another city. Overall, the prevalence of BA in Chaoyang district was 1.3 in 10,000 live births (4/29 799, 95% CI: 0.03-2.7).

Diseases other than BA detected by the SCC

Among the 24 positive cases detected by the guardians, four infants were subsequently diagnosed with cytomegalovirus hepatitis, Duchenne muscular dystrophy (DMD), parenteral nutrition-associated cholestasis, and suspected Alagille syndrome, respectively (Table).

Table. Clinical features of patients with diseases other than biliary atresia detected by the SCC

Number of cases	gender	Gestational weeks	Age at presentation of pale-pigmented stool checked by SCC	Image number on SCC	Biochemical examinations of liver function at the time of presentation of pale-pigmented stools					Other examinations	Diagnosis
					ALT (IU/L)	TBIL (μmol/L)	DBIL (μmol/L)	TBA (μmol/L)	GGT (IU/L)		
1	Female	32	55	2	13.0	39.6	16.7	102.9	89.0	Ultrasound scan of liver and biliary tract was normal.	Parenteral nutrition associated cholestasis based on history of parenteral nutrition
2*	Male	35	47	3	73.0	16.5	7.5	47.5	36.0	CK, 33 960 U/L. Partial deletion of the DMD gene (exons 46 and 47)	DMD
3	Female	37	75	3	139.0	18.3	5.2	30.2	51.0	CMV IgM was positive	CMV hepatitis
4	Female	39	12	3	49.0	87.1	49.0	84.9	122.0	Distinctive facial features, butterfly shape of the bones of the spinal column (vertebrae) that can be seen in an X-ray	Alagille syndrome suspected

*: This patient was first diagnosed with hepatitis. During follow-up, CK were found obviously elevated. SCC: stool color card; CK: creatine kinase; DMD: Duchenne muscular dystrophy; CMV: cytomegalovirus; ALT: alanine aminotransferase, normal range: 5-40 IU/L; DBIL: direct bilirubin, normal range: 0-3.4 μmol/L; GGT: gamma-glutamyl transferase, normal range: 11-50 IU/L; TBA: total bile acid, normal range: 0-12 μmol/L; TBIL: total bilirubin, normal range: 3.4-17.1 μmol/L.

Discussion

We have demonstrated a high prevalence of BA in Chaoyang, the largest district in Beijing with the largest number of live births. BA occurs between the prenatal period and four months of age. An early detection of BA is crucial for improvement of native liver survival.[3]

To date, BA screening has been adopted in Japan and Taiwan as part of the general neonatal screening system. However, due to the difference in methodology used to create the photographic images and for assessment, Japan and Taiwan have different regiments. The reported sensitivity of the SCC was 72.5% and 76.5% in the studies in Taiwan and Japan, respectively.[3,8] In those studies, however, SCC had been collected only at the first-month checkup or at vaccination until 60 days of age. Therefore, there is a potential risk of false negative results presented beyond the first-month checkup or at vaccination, and this risk might elevate if the medical staff did not response promptly after reporting by the guardians. To minimize the chances of that risk, we expanded period of observation after birth up until four months and introduced three inspection points to record stool colors at 14 days, one month and 1-4 months after birth. By extending the test phase, the performance of SCC was markedly improved (sensitivity, 100%; specificity, 99.9%) in comparison with the previous reports.[3,8] Notably, there were no false negative cases in this study. By having a longer observation period using the modified SCC, which allows easier identification, the performance of SCC was markedly improved.

Unlike screening of other newborn diseases such as phenylketonuria and congenital hypothyroidism, where diagnosis is determined by the reading output of medical instruments, the screening of BA was done with the naked eyes of the guardians rather than by medical professionals. There are several advantages to this. First, it is very cost-effective, and is particularly useful for mainland China where there is a shortage of medical resources against a backdrop of a population of 1.3 billion with 16 million live births annually. Notably, the estimated printing cost of each SCC is approximately US$0.07 per card. Moreover, there are two recent reports in Canada and the US, respectively, that demonstrated the cost-effectiveness of SCC screening for BA.[14,15] Hence, we believe that the SCC is feasible to be applied nationwide for early BA detection. Going forward, we will be examining the costs of implementing the SCC across Beijing as well as nationwide.

Secondly, stool samples are not as easily collected by medical professionals compared to other samples such as blood. The consistency of the stool samples can also be affected by the other factors such as diarrhea. In this study, as the guardians checked the stool colors every day, the likelihood of having reliable readings is higher. Thirdly, the modified SCC, with its digital images, ensures quality control and consistency. Finally, in a country like China where comprehensive healthcare insurance is not provided, it is more beneficial to have the guardians observe the stool colors of their infants rather than have the medical staff do it.

In this study, two patients did not receive the KP due to refusal by their guardians. Apart from the concern over financial implications, it was noted that the guardians were not well informed of the prognosis of BA, especially when detected at a very early stage of life. During this study, we recognized how crucial it is to have patients' family understand the importance of early detection of BA and its impact on prognosis. This is also an important result of this pilot study. Since then we have done some measures to raise the literacy. In April of 2015, we reported that a 19-year cohort study using SCC in Japan resulted in a long-term native liver survival.[3] This report provided evidence for us to educate the people concerned with BA. We believe that through our action for raising literacy and improvement of medical aid for children diseases by the Chinese government, early detection and early treatment for BA will be improved, as will neonate screening of phenylketonuria.

We showed that BA screening using SCC has excellent sensitivity and specificity. As other diseases other than BA were detected by SCC, we believe that it will be advantageous to extend SCC screening to all NICUs for the detection of diseases such as DMD and cytomegalovirus infection. Here, we reported that an infant with DMD accompanied by pale-pigmented stools and direct hyperbilirubinemia although the mechanism was not clear.

In conclusion, this pilot study in Beijing, China, further demonstrated that SCC is a very efficient and feasible tool to screen BA in newborns, and revealed the prevalence of BA in this geographic area for the first time. In view of the high prevalence of BA in this study, we propose the SCC be applied nationwide in China for the early detection of BA to improve outcome of BA patients. It is necessary to extend SCC screening up until four months after birth in order to achieve higher sensitivity and a satisfactory diagnosis rate. It is also crucial to ensure that parents of BA patients understand the importance of early detection of this disease and its impact on prognosis.

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Ethical approval: This study was approved by the National Center for Child Health and Development of Japan (No. 758) and Beijing Obstetrics and Gynecology Hospital of China (No. 2013-ky-010).

Competing interest: There is no conflict of interest among the authors of this manuscript. There is no potential conflict of interest, real or perceived in the role of the study sponsor, study design, the collection, analysis, interpretation of data, the writing of the report, and the decision to submit the paper for publication.

Contributors: Kong YY designed and established the system of screening, followed high-risk cases, and wrote the first draft of this manuscript. Zhao JQ implemented screening, and did the follow-up of results of SCC and partly epidemiological analysis. Wang J implemented screening, summarized results of SCC and did the follow-up high risk cases. Qiu L and Yang HH prepared SCC delivery and did the follow-up of high risk cases. Diao M and Li L did diagnosis of BA patients and surgery. Gu YH advised this and designed this pilot research, analyzed the partly data, translated the explanation on the SCC and revised this manuscript. Matsui A designed and provided the SCC and planned this collaboration study.

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