# Effects of the Infant Stool Color Card Screening Program on Long-term Outcome of Biliary Atresia in Taiwan

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**Key words:** *biliary atresia, infant stool color card, Kasai operation, survival*

**Abbreviations:** BA, biliary atresia; OR, odds ratio; CI, confidence interval

**ABSTRACT**

***Objective:*** In Taiwan, a screening system using an infant stool color card to promote the early diagnosis of biliary atresia (BA) was established in 2002. This study aimed to investigate and compare the long-term outcome of BA before and after using the infant stool color card screening program.

***Methods:*** Biliary atresia patients were divided into three cohorts according to their birth dates. Cohort A (n=131) were born before the stool card screening program (1976-2000); cohort B (n=28) were screened by the stool card regional screening program (2002-2003); and cohort C (n=74) were screened by the stool card universal screening program (2004-2005). The relative odds ratios were computed by logistic regression model to compare the different factors affecting survival time.

***Results:*** The rate of the age at Kasai operation before 60 days old was 50.4% and 65.7% in cohorts A and B+C, respectively ($p$=0.019). The jaundice-free rate 3 months after surgery was 37.4% and 60.8%, respectively ($p$<0.001). The three-year jaundice-free survival rate with native liver was 35.9% in cohort A and 56.9% in cohort (B+C) ($p$=0.001), while the three-year overall survival rates were 64.9% and 89.2%, respectively ($p$<0.001). The five-year jaundice-free survival rate with native liver was 32.3% in cohort A and 64.3% in cohort B ($p$=0.002), and the five-year overall survival rates were 58.5% and 89.3%, respectively ($p$=0.002). Those who were jaundice-free at 3 months after surgery had significantly better survival rates than those who were not jaundice-free.

***Conclusions:*** The stool color card screening program for BA allows for early Kasai operation, which increases the jaundice-free rate at 3 months post-surgery. With higher surgical success rates, the long-term outcome of BA patients in Taiwan improves remarkably.

## INTRODUCTION

Biliary atresia (BA) is an inflammatory, progressive fibro-sclerosing cholangiopathy of infancy that variably affects both the extra- and intra-hepatic bile ducts,[1-3] resulting in the destruction and obstruction of the biliary tract.[3-5] If untreated, it progresses to cirrhosis with portal hypertension and liver failure leading to death within 2 to 3 years. Since the Kasai operation was first used for BA in 1959, there have been encouraging results in treating this disease such that it has become the first line treatment. Kasai operation can restore bile flow through a reconstructed hepatic portoenterostomy to a jejunal loop. Once the cholestasis progresses and/or complications of liver cirrhosis occur, liver transplantation remains the salvage way for BA. Although ongoing cholestasis, which further aggravates liver cirrhosis, is present in most BA children,[6] a successful Kasai operation may still delay or even decrease the need for liver transplantation.

It is generally accepted that the Kasai operation is more successful in children when performed earlier than 60 days of age.[7,8] The reported successful bile flow rate was 91%, 56%, 38%, and 17% in patients operated on before 60 days, in 61-70 days, in 71-90 days, and beyond 90 days, respectively.[9] In recent years, many studies directly show that patients with earlier Kasai operation have better survival. However, early identification and timely surgery, which are crucial for better prognosis, remain challenging.

In Taiwan, a pilot regional study using the infant stool color card to increase the efficacy of early identification of BA was started since 2002. Universal screening for BA using the infant stool color card was launched in 2004. This was the first nationwide screening program for BA using an infant stool color card in the world. The present study aimed to compare the outcome of the BA patients after Kasai

operation before versus after the launch of the infant stool color card screening program.

## MATERIALS AND METHODS

### *Infant Stool Color Card*

An infant stool color card was designed with 6 photographs of different colored stool samples from Taiwanese infants. Three colors on this card were labeled abnormal (clay-colored, pale yellowish, and light yellowish), whereas the other 3 were labeled normal (yellowish, brown, and greenish). Telephone and fax numbers for consultation were also printed on this card, and parents, guardians, and medical personnel were instructed to inform the stool card registry center if abnormal stools were noticed. In 2002, 47180 newborns from 49 hospitals and clinics in Northern and Central Taiwan were enrolled. In 2003, the range of cooperation extended to Southern and Eastern Taiwan, and 72793 newborns from 96 hospitals were enrolled. In 2004, the universal stool color screening program was launched and the stool color card was integrated into the child health booklet. All neonates born in Taiwan participated in the screening program since then.

### *Participants*

All of the participants had a diagnosis of BA made by clinical, biochemical, imaging data, surgical findings, and liver histology. The children were divided into 3 cohorts by their birth date. The historical control cohort was derived from the 185 cases diagnosed as BA in the National Taiwan University Hospital from January 1976 to December 2000. Twenty-two patients who did not receive Kasai operation and 32 patients who underwent Kasai operation but were not followed-up for at least 3 years post-operatively were excluded. The remaining 131 patients became cohort A. All of

them were followed-up for at least 5 years except one who was followed-up for only 3 years post-operatively. Cohort A represented the group of patients born in the era before the stool card screening program.

There were 29 BA children born between 2002 and 2003. One who did not receive Kasai operation was excluded. The other 28 patients who were followed-up for at least 5 years post-operatively were enrolled to cohort B, which represented the era of the regional pilot study of the stool card screening program in Taiwan.

Seventy-five BA children were born between 2004 and 2005. After excluding one patient without Kasai operation, the 74 who were followed-up for at least 3 years post-operatively were enrolled to cohort C, which represented the nationwide screening data in Taiwan. Cohort (B+C) was the merged data of cohorts B and C and represented the era of the stool color card screening program.

***Statistical Analysis***

Statistical analyses were performed using the STATA package software (StataCorp LP, Texas, USA). Chi-square test was used to compare categorical variables, including age at Kasai operation before 60 days old, jaundice-free rates at 3 months after Kasai operation, 3- and 5-year survival rates with native liver, 3- and 5-year jaundice-free survival rates with native liver, and 3-year and 5-year overall survival rates between different cohorts. Overall survival included those survived with either native liver or transplanted liver. Jaundice-free was defined as total serum bilirubin <2.0 mg/dL (34 μmol/L).

Quality outcome was defined as jaundice-free survival with native liver. All survival time was calculated after the date of the Kasai operation. Relative odds ratios were computed by logistic regression models to compare the different factors

affecting survival time. Kaplan-Meier method and log rank test were also used to assess factors affecting survival. A *p* value <0.05 was considered statistically significant while a *p* value of 0.05-0.1 implied a trend of difference.

## RESULTS

### *Comparisons of Cohort A versus Cohort (B+C)(Table 1)*

There was no difference in the gender between cohort A and cohort (B+C). The rates of Kasai operation performed before 60 days old were 50.4% in cohort A and 65.7% in cohort (B+C) ($p$=0.019). At 3 months after Kasai operation, the jaundice-free rate was significantly higher in cohort (B+C) than in cohort A (60.8% vs. 37.4%, $p$<0.001). The three-year survival rate with native liver in cohort A and cohorts (B+C) was 55.7% and 61.8%, respectively. The three-year jaundice-free survival rate with native liver was significantly higher in cohort (B+C) than in cohort A (56.9% vs. 35.9%, $p$=0.001). The three-year overall survival rate was 64.9% in cohort A and 89.2% in cohort (B+C) ($p$<0.001).

The five-year follow-up was not yet finished in Cohort C so only cohort B was compared with cohort A in the analyses of five-year survival time. The five-year survival rate with native liver in cohorts A and B was 45.4% and 64.3%, respectively ($p$=0.07). The five-year jaundice-free survival rate with native liver was significantly higher in cohort B than in cohort A (64.3% vs. 32.3%, $p$=0.002) and the five-year overall survival rate was 89.3% and 58.5%, respectively ($p$=0.002).

### *Jaundice-Free at 3 Months after Kasai Operation (Table 2)*

In univariate and multivariate logistic regression analyses of jaundice-free rate at 3 months after the Kasai operation, those who underwent surgery before 60 days old had odds ratios of 2.67 and 2.45, respectively, compared to those who underwent

surgery after 60 days old ($p$<0.001 and $p$=0.001, respectively). For every day of delay of the Kasai operation, jaundice-free rate at 3 months post-operatively declined by 2.37% (OR: 0.977, 95% CI: 0.965-0.989, $p$<0.001).

Cohort (B+C) had significantly higher jaundice-free rate at 3 months post-surgery than cohort A by univariate and multivariate logistic regression analyses (OR 2.59, $p$<0.001 and OR 2.34, $p$=0.002, respectively). These implied that the age at Kasai operation and the intervention by stool card screening program strongly correlated to the jaundice-free rate at 3 months post-surgery.

***Survival with Native Liver***

The three-year survival rates with native liver in those who received Kasai operation before 60 days old and after 60 days old were 65.4% and 49.0%, respectively (OR: 1.97, 95%CI: 1.16-3.35, $p$=0.012). The five-year survival rates with native liver in those who underwent surgery before 60 days old and after 60 days old were 57.3% and 39.5%, respectively (OR: 2.06, 95%CI: 1.09-3.88, p=0.026). The three-year survival rates with native liver in those who were and those who were not jaundice-free at 3 months after Kasai operation were 86.5% and 32.8%, respectively (OR: 13.12, 95%CI: 6.76-25.45, $p$<0.001). The five-year survival rates with native liver in those who were and those who were not jaundice-free at 3 months post-surgery were 82.1% and 24.2%, respectively (OR: 14.38, 95%CI: 6.54-31.60, $p$<0.001).

***Jaundice-Free Survival with Native Liver (Table 3)***

Jaundice-free survival with native liver was considered as quality outcome. Cohort (B+C) had higher rates of 3- and 5-year jaundice-free survival with native liver than cohort A (OR 2.36, $p$=0.002, and OR 3.77, $p$=0.002, respectively). Those who received Kasai operation before 60 days old had better 3- and 5-year

jaundice-free survival with native liver than those operated on after 60 days of age (OR 2.79, $p<0.001$, and OR 2.12, p=0.025, respectively). Moreover, those who were jaundice-free at 3 months post-surgery had better 3- and 5-year jaundice-free survival with native liver than those who were not jaundice-free (OR 40.60, $p<0.001$, and OR 29.04, $p<0.001$, respectively). Gender did not affect outcome. Intervention by the stool card screening program, Kasai operation before 60 days of age, and jaundice-free at 3 months post-surgery were the predictors of quality outcome for BA patients.

***Overall Survival (Table 4 and Figures 1 and 2)***

Those who were jaundice-free at 3 months after Kasai operation had better 3- and 5-year overall survival rates than those who were not jaundice-free (OR 12.57, $p<0.001$ and OR 12.96, $p<0.001$, respectively). Cohort (B+C) had better 3- and 5-year overall survival rates than cohort A (OR 4.48, $p<0.001$ and OR 5.92, $p=0.005$, respectively).

## DISCUSSION

Biliary atresia (BA) is an obliterative cholangiopathy of unknown etiology. It is the most common cause of end-stage liver disease in children, with an incidence of 0.51 per 10000 in France,[10] 0.60 per 10000 in the U.K.,[11] 0.70 per 10000 in Sweden,[12] and 0.85 per 10000 in North America.[12] There is a higher incidence in Asia, including 1.04 per 10000 in Japan[13,14] and 1.78 per 10000 in Taiwan[8]. Taiwan is one of the areas with highest incidence in the world.

Kasai operation is the primary surgical therapy for BA even in the era of liver transplantation.[15] Survival of BA patients with their native liver relies mainly on the success of the Kasai operation,[16] which is correlated with the age at surgery.[13] In the

Swiss national study,[12] four-year survival with native liver is 75% in patients who underwent Kasai operation before 46 days, 33% in those operated on between 46 and 75 days, and 11% in patients operated on after 75 days ($p$=0.02). In long-term follow-up, the 20-year survival with native liver is significantly better in children operated on before the age of 90 days than those operated on after 90 days (28% vs. 13%, $p$=0.006).[6] In the current study, those who underwent Kasai operation before the age of 60 days have significantly better survival with native liver than those operated on after 60 days.

The earlier age at Kasai operation is indeed an important predictive factor of better long-term survival with native liver. For early diagnosis of BA, the stool card screening program was started in Taiwan in the regional areas in 2002 and extended nationwide in 2004. The rate of the children with Kasai operation before 60 days old significantly improved in the stool card screening era.

The three-year survival rate with native liver in the era before the stool card screening program was 55.7%, which increased to 61.8% in the stool card screening era. Why is this improvement not as evident as expected? Persistent and/or progressive jaundice is usually the first alarm of impaired bile flow and progressive liver cirrhosis. In the early years before the stool card screening program, the skills and care of liver transplantation were not fully mature as they are nowadays. Moreover, the concept of living-related donor had not yet been well accepted by the general population. The requirement and timing of liver transplantation thus tended to be more conservative and delayed. Some patients though lived with their native liver despite severe jaundice-related complications.

In the era of the stool card screening program, liver transplantation have become more polished and have gained more social acceptance. Pediatricians and surgeons in

recent years have preferred to choose an appropriate but earlier timed liver transplantation for those patients with persistent jaundice, before many complications occur. Hence, the three-year survival rate with native liver in the stool card screening era is only slightly better than that of the era without screening. As time goes by, fewer and fewer patients can survive without transplantation if their jaundice is persistent. So in the analyses of five-year survival with native liver, those born in the stool card screening era already show a trend of better results.

We believe that jaundice-free survival rate with native liver can reflect the true outcome of BA without the interference of the timing change of liver transplantation. This study defined those who were of jaundice-free survival with native liver as quality outcome. Data shows that with intervention by the stool card screening program, there are significantly more BA children with quality outcome than before.

In the study by Shneider et al.,[17] jaundice-free (total bilirubin <2 mg/dL) at three months after Kasai operation is an excellent predictor of two-year survival with native liver. In the current study, patients who are jaundice-free at three months post-surgery have significantly higher survival rates with native liver and overall survival rates, as well as more quality outcome in both the three- and five-year analyses. Jaundice-free at three months after Kasai operation can be an indicator for success of the surgery and a valuable predictor of five-year outcome. In the analyses here, jaundice-free at three months after surgery is significantly correlated to the implementation of the stool card screening program and earlier age at surgery.

Overall survival is significantly better in the era of the stool card screening program. Other studies show that the better the results of the Kasai operation, the better overall survival.[16,18] Though more developed transplantation techniques in the stool card screening era partly contribute to survival, the need for liver transplantation

still adds the risk to impair the prognosis. Successful Kasai operation still provides children with the best chances of survival and every effort should be made to improve its results.[16] The stool card screening program is a step in this direction as it efficiently increases the success rate of Kasai operation and contributes to better overall survival.

The five-year survival rate with native liver and five-year overall survival rate in other studies range from 30.1% to 59.7% and from 75.5% to 85%, respectively.[13,19,20] In Taiwan, these are 64.3% and 89.3%, respectively. This corroborates the promising results of intervention using the stool card screening program.

## CONCLUSION

The stool card screening program for biliary atresia enhances early Kasai operation and increases the jaundice-free rate at three months post-surgery, which is a valuable predictor of long-term outcome. In Taiwan, the infant stool color card screening program has remarkably improved the long-term outcome of BA patients.

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## FIGURE LEGENDS

**Figure 1.** The 5-year overall survival rate in cohort B (dotted line), which represented the era of stool color card screening program, and in cohort A (actual line), which represented the era before the stool color card screening program, was 89.3% and 58.5%, respectively ($p=0.003$).

**Figure 2.** The 5-year overall survival rate in those who were jaundice-free (dotted line) versus those who were not jaundice-free (actual line) at 3 months after Kasai operation was 91.0% and 44.0%, respectively ($p<0.0001$).

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