Primary exploring the value of metagenomic next-generation sequencing in detecting pathogenic bacteria of cholangitis with biliary atresia after Kasai operation

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Abstract

Purpose To evaluate the value of metagenomic next-generation sequencing (mNGS) in detecting pathogenic bacteria of cholangitis for patients with biliary atresia after Kasai operation.

Methods This study retrospectively analyzed patients of biliary atresia with cholangitis after Kasai operation who were admitted to Xi’an Children’s Hospital from July 2019 to December 2021. Both blood culture and mNGS were carried out in all of these patients. We compared the detection rate of pathogenic bacteria, pathogenic bacteria spectrum, test time, inflammatory indicators and liver function. All the patients were followed up for 0.5–3 years to evaluate the onset of cholangitis and the survival status of autologous liver.

Results This study included total of 30 cholangitis occurred in 25 patients. There were significant differences in the detection rate of pathogenic bacteria [23.3 vs. 73.3%, \( P < 0.05 \)] and the test time [120 (114.5–120) vs. 16 (16–21) h, \( P < 0.001 \)] between the blood culture and mNGS. These two methods showed significant statistical differences in comparing inflammatory indicators (CRP, PCT) and liver function (TB, DB, GGT) before and after anti-infection. Four kinds of bacteria were detected by blood cultures and ten kinds of bacteria were detected by mNGS. Cholangitis occurred 3 times in one case (4%) and twice in three cases (12%). Autologous liver survived in 17 cases (68%).

Conclusion Comparing with traditional blood culture, mNGS is more efficient, convenient and accurate in the detection of pathogens. It provides a new method for accurately detecting pathogenic bacteria of cholangitis after Kasai operation.

Keywords Biliary atresia · Cholangitis · Blood culture · Metagenomic next · Generation sequencing · Pathogenic bacteria

Introduction

Biliary atresia (BA) is an obstructive jaundice disease characterized by intrahepatic and extrahepatic bile duct occlusion, which often occurs in the perinatal period, and its etiology is still unclear [1, 2]. It will eventually lead to death due to progressive aggravation of cirrhosis or liver failure without treatment. Kasai operation is the best way to restore bile flow and save autologous liver in biliary atresia [3]. Cholangitis is a serious complication after Kasai operation, with a morbidity of 40–93%, which has an important impact on the long-term survival and life quality of patients, and is also a risk factor for poor prognosis. Therefore, choosing antibiotics correctly is the key to the treatment of cholangitis [4, 5]. Cholangitis is mainly caused by bacterial infection, and detecting pathogenic bacteria is very important for the diagnosis and treatment of cholangitis. However, the results of traditional blood culture are affected by many factors, such as the time of blood
collection, the amount of blood collected, whether antibiotics are used before blood collection, the culture medium and the culture process. The probability of obtaining pathogenic bacteria is low, and the time required is long, thus it will affect the choice of antibiotics for the treatment of cholangitis. However, the emergence of metagenomic sequencing technology has brought dawn to the efficient detection of pathogenic bacteria, and some scholars have promoted metagenomic sequencing technology into the field of clinical practice of pathogen identification [6]. In recent years, metagenomics next-generation sequencing (mNGS) has been proved to be a successful diagnostic tool, which can be used to detect pathogenic bacteria accurately in a variety of infection sites, including the central nervous system, respiratory system, urinary system, etc. [7]. Therefore, this study aimed to compare the sensitivity of mNGS and blood culture, and explore the value of mNGS in detecting pathogenic bacteria of cholangitis after Kasai operation.

Materials and methods

Object

In this study, 25 biliary atresia patients with cholangitis after Kasai operation from July 2019 to December 2021 were collected, and all of them were compliant with the diagnostic criteria of cholangitis: (1) fever of unknown origin above 38 °C, (2) recurrence or aggravation of clinical jaundice, accompanied by elevated bilirubin levels, or from yellow stool to acholic stool. (3) Elevated C-reactive protein (CRP). Cholangitis can be diagnosed with at least a combination of the above two clinical manifestations [8].

Methods

Cholangitis occurred 30 times in 25 patients. Blood culture and mNGS were performed in all patients during the attack of cholangitis, and we compared the detection rate of pathogenic bacteria, pathogenic bacteria spectrum, detection time, inflammatory indicators and liver function before and after treatment. The patients were followed up for 0.5–3 years to assess the frequency of cholangitis and the survival status of the liver.

Metagenomic next-generation sequencing and analysis

Sample collection and nucleic acid extraction

Enough whole Blood (children 2–4 ml) was collected in Cell-Free DNA BCT STRECK and then stored or shipped between 6 and 35 °C to Hugobiotech Co., Ltd., (Beijing, China) to perform mNGS detection immediately. The DNA was extracted and purified from 200 uL plasma according to the manufacturer’s instruction of QIAamp DNA Micro Kit (50) #56304. DNA concentration and quality were checked through Qubit and agarose gel electrophoresis.

Library generation and sequencing

The DNA libraries were constructed using QIAseq™ Ultralow Input Library Kit. The concentration and quality of libraries were checked using Qubit and agarose gel electrophoresis. Qualified libraries with different barcode labeling were pooled together, and then sequenced on an Illumina Nextseq platform.

Bio information pipeline

After obtaining the sequencing data, high-quality data were generated after filtering out adapter, low-quality, low-complexity and shorter reads. Next, remove human reads by mapping reads to the human reference genome using SNAP software. The remaining data were aligned to the microbial genome database using Burrows-Wheeler Alignment. The database collected microbial genomes from NCBI. It contains more than 20,000 microorganisms, including 11,910 bacteria, 7103 viruses, 1046 fungi, and 305 parasites. Finally, we get the microbial compositions of the sample.

Data collection and analysis

The clinical data of 25 patients with cholangitis after Kasai operation were analyzed retrospectively.

The positive detection rates of pathogenic bacteria and detection time between blood culture and mNGS in 30 episodes of cholangitis were compared, and the pathogenic bacteria spectrum was drawn according to the types and quantities of pathogenic bacteria detected.

The antibiotic therapy scheme and treatment time were selected according to the results of pathogenic bacteria, and the results of inflammatory indicators (CRP, PCT) and liver function before and after treatment were compared.

Twenty-five patients were followed up for 0.5–3 years, and the frequency of cholangitis and the survival status of autologous liver were recorded.

Statistical analysis

Software SPSS 26.0 was used for data analysis. The data following normal distribution were expressed as mean ± standard deviation (SD), and the data following non-normal distribution were expressed as median (P25, P75). Nonparametric test and χ² test were selected according to data distributions. P < 0.05 indicates statistical difference.
**Table 1** Demographics characteristics of patients

| Observation index                  | Value          |
|-----------------------------------|----------------|
| Age of Kasai operation (day)      | 57 (43.5–66)   |
| Sex (male/female)                 | 8/17           |
| Rate of jaundice regression (TB<34.2umol/L) | 84%           |
| Time of postoperative jaundice regression (day) | 82 (57–130)   |
| Age of the first cholangitis attack (day) | 133 (99.5–199.5) |
| Time of postoperative cholangitis (day) | 57 (40.5–159) |
| Temperature (°C)                  | 38.98 ± 0.43   |
| Stool color (pale/normal)         | 13/12          |

**Table 2** Pathogenic bacteria detected by two methods

|          | mNGS | Blood culture | Total |
|----------|------|---------------|-------|
|          | 7    | 23            | 30    |

**Result**

**Demographic characteristics of patients**

In this study, the basic information of 25 patients was analyzed (Table 1), including the age of operation, sex ratio, jaundice regression rate, jaundice regression time after the operation, age of first cholangitis attack, time of cholangitis after the operation, highest body temperature, stool color, etc.

**The detection rate of pathogenic bacteria by two methods**

According to the $\chi^2$ test of paired fourfold table data (Table 2), there was significant statistical difference in positive detection rates between the two methods ($\chi^2 = 11.53$, $P < 0.05$). In 30 episodes of cholangitis, the detection rate of pathogenic bacteria in blood cultures was 23.3%, which was 73.3% in mNGS. The detection rate of pathogenic bacteria in mNGS was significantly higher than that in the blood culture.

**Pathogenic spectrum**

In 30 episodes of cholangitis, 11 types of pathogenic bacteria were detected in this study, including four types of pathogenic bacteria detected in blood culture and ten types of pathogenic bacteria detected in mNGS. The pathogenic bacteria detected by blood culture was *Klebsiella pneumoniae* in four cases, *Stenotrophomonas maltophilia*, *Klebsiella oxytoca* and *Enterococcus faecalis* in one case, respectively. *Klebsiella pneumoniae* was detected 11 person-times by mNGS, *Stenotrophomonas maltophilia*, *Escherichia coli* were detected twice by mNGS, *Klebsiella oxytoca*, *Acinetobacter baumannii*, *Enterococcus faecium*, *Enterobacter cloacae* complex, *Biliphilus wolfmanii*, *Candida tropicalis* and *Fusarium oxysporum* were detected only once by mNGS. The number and types of pathogenic bacteria detected by mNGS were much more than those detected by blood culture, including fungi (Fig. 1).

**Test time of pathogenic bacteria by two methods**

In 30 episodes of cholangitis, blood culture and mNGS pathogenic bacteria test time [120 (114.5–120) vs. 16 (16–21) h, $P < 0.001$] were statistically different significantly. The detection of pathogenic bacteria by mNGS is superior to blood culture in timeliness.

**Bacteria and treatment schemes**

Pathogenic bacteria were detected in 23 of 30 episodes of cholangitis, including 21 cases of bacteria and two cases of fungi. For 21 cases with bacterial infections, antibiotics were selected according to the results of drug sensitivity of blood culture, and if the blood culture was negative, antibiotics were selected according to the results of mNGS and drug resistance genes. For carbapenem-resistant and multi-drug resistant bacteria, if the conventional treatment regimen is ineffective, with the discussion and agreement by the clinical expert group, approval by the ethics committee, and informed consent of the patient’s parents, the clinical pharmacist guided the use of tigecycline-based combined anti-infective treatment regimen according to the patient’s condition, the results of drug sensitivity in blood culture or resistance genes in mNGS and reference [9, 10] (Table 3).

**Inflammatory indicators**

The inflammatory indicators (CRP and PCT) of 30 cholangitis were compared before and after anti-infection treatment, the results are: CRP [61.29 (24.43–119.27) vs. 2.41 (16–21) h, $P < 0.001$] were statistically different significantly.
(1.13–4.34) mg/L, \( P < 0.001 \) and PCT [0.94 (0.34–7.41) vs. 0.16 (0.07–0.36) ng/dl, \( P < 0.001 \)] (Table 4). There were statistically significant differences in CRP and PCT. After treatment, the inflammatory indicators were significantly decreased compared with that before treatment.
Serum liver function

The liver function (ALT, AST, TB, DB, GGT) of 30 persons-times (with 25 patients) were compared before and after anti-infection. ALT [125.5 (37.5–197.75) vs. 64.5 (40.75–129.5) U/L, \( P > 0.05 \]), AST [125.5 (72.25–182.25) vs. 108 (80.25–160.5) U/L, \( P > 0.05 \)], ALT and AST had no significant changes before and after treatment, TB [61.85 (36.55–118.03) vs. 50.75 (25.93–102.68) \( \mu \)mol/L, \( P < 0.05 \)], DB [45.65 (22.98–75.55) vs. 31.55 (13.48–66.9) \( \mu \)mol/L, \( P < 0.05 \)], TB and DB decreased significantly after treatment compared with the data before treatment. GGT [531.10 (282.28–1024.13) vs. 718.65 (251.63–1723.45 \( \mu \)mol/L, \( P < 0.05 \)), GGT after treatment was significantly higher than that before treatment (Table 5). The increase in GGT is considered to be related to the edema and obstruction of intrahepatic bile capillaries caused by cholangitis, and the decline in GGT lags behind TB and DB.

Follow-up

Twenty-five patients were followed up for 0.5–3 years, including the frequency of cholangitis and the survival of autologous liver.

1. Cholangitis attack frequency. Among the 25 cases, one case had three cholangitis attacks and three cases had two cholangitis attacks (Table 6). The results of pathogenic bacteria in different attack periods of cholangitis in these four patients indicated that each attack of cholangitis in the same patient was independent, and there is no connection between cholangitis (Table 7).

2. Survival status of the autologous liver: 17 cases of autologous liver survived, six cases had liver transplantation, and two cases died (Table 8).

Discussion

Cholangitis is a common complication of biliary atresia after Kasai operation. The morbidity of bacterial cholangitis is 70–90%, and most cases may recur [10, 11]. Cholangitis recurrence will affect the prognosis, for bacteria and inflammation in the bile canaliculi can impair bile drainage, cause cholestasis, lead to further liver injury and fibrosis [12]. Routine use of antibiotics after Kasai procedure can effectively prevent the occurrence of cholangitis [13, 14]. In recent years, although the popular understanding of BA mechanism has been improved, the morbidity of cholangitis is still high. Most cholangitis can be controlled by direct intravenous infusion of sufficient antibiotics, but some cholangitis does not respond to conventional treatment [15]. It may be related to the diversity of pathogenic microorganisms and the gradual increase of drug-resistant pathogenic bacteria. Therefore, the acquisition of pathogenic bacteria plays a key role in the treatment of cholangitis. In this study, the inflammation was quickly and effectively controlled after choosing the treatment plan according to the pathogenic bacteria, which avoided antibiotic resistance and uncontrolled infection that may be caused by empirical treatment.

Blood culture is widely used as an important means to obtain pathogenic bacteria, but there are still some shortcomings. The positive rate of blood culture is low, only 8.9–25.8% [16, 17], and the results can be affected by many factors, such as the time of blood collection and blood collection technology. Intravenous use of antibiotics before blood collection will lead to false negative blood culture results and improper operation during blood collection will lead to specimen contamination, which may lead to false

| Liver function | Pre-treatment | After-treatment | \( P \) |
|----------------|---------------|----------------|------|
| ALT (U/L)      | 125.5 (37.5–197.75) | 64.5 (40.75–129.5) | >0.05 |
| AST (U/L)      | 125.5 (72.25–182.25) | 108 (80.25–160.5) | >0.05 |
| TB (\( \mu \)mol/L) | 61.85 (36.55–118.03) | 50.75 (25.93–102.68) | <0.05 |
| DB (\( \mu \)mol/L) | 45.65 (22.98–75.55) | 31.55 (13.48–66.9) | <0.05 |
| GGT (U/L)      | 531.10 (282.28–1024.13) | 718.65 (251.63–1723.45) | <0.05 |
positive blood culture results. The antibiotic susceptibility test is the main method to detect the drug resistance of pathogenic bacteria in the clinic, it is also the “gold standard” for the diagnosis of drug-resistant bacterial infections, but there are also shortcomings. The antibiotic susceptibility test usually needs to be carried out on isolated pathogens, which is not only time-consuming but also further ignores some unculturable bacteria.

In this study, the detection rate of pathogenic bacteria, test time, pathogenic bacteria spectrum, and inflammatory indicators were compared with the two methods, and it was found that the detection rate of pathogenic bacteria by mNGS was higher and the test time was shorter. In terms of pathogen detection, the number of pathogens detected by mNGS is not only large, but also more abundant. mNGS is a clinical laboratory diagnostic technology for gene testing of pathogenic bacteria based on high-throughput sequencing technology and bioinformatics analysis technology [18, 19]. mNGS has both the ability to simultaneously detect DNA and RNA viruses, bacteria, fungi, and parasites present in one sample, and the ability to exclude infections [6]. This technology has gradually been widely used in the field of pediatric infections, including the respiratory system, nervous system, blood system, bone and joint, and parasitic infections [20–24]. Therefore, we used mNGS to detect the pathogenic bacteria of cholangitis after Kasai operation in biliary atresia patients in this study, and obtained satisfactory results.

### Table 6 Cholangitis episodes

| Cholangitis attack frequency (Times) | No.of cases | Ratio (%) |
|-------------------------------------|-------------|-----------|
| 3                                   | 1           | 4         |
| 2                                   | 3           | 12        |
| 1                                   | 21          | 84        |

### Table 7 Pathogenic bacteria of cholangitis (Cholangitis episodes ≥ 2times)

| Case | Cholangitis attack sequence | Pathogenic bacteria         |
|------|-----------------------------|-----------------------------|
| 1    | 1                           | Klebsiella pneumoniae       |
| 2    |                             | Klebsiella oxytoca          |
| 3    |                             | Klebsiella pneumoniae       |
| 2    | 1                           | Klebsiella pneumoniae       |
| 3    | 1                           | Undetected                  |
| 2    |                             | Undetected                  |
| 4    | 1                           | Undetected                  |
| 2    |                             | Undetected                  |

In recent years, mNGS is in the exploratory research stage of detecting drug resistance genes, and has clinical application potential and broad application prospects [25]. Simultaneously, some problems should be solved, such as the inability to determine the source of drug resistance genes, the existence of incomplete gene-phenotype matching, and the qualitative but not quantitative bacterial resistance [26].

The mNGS can efficiently and quickly detect the pathogenic bacteria of cholangitis after Kasai operation. However, this technology has limitations in the detection and interpretation of drug resistance genes of pathogenic bacteria, and the drug resistance genes of mNGS can not completely replace the traditional blood culture. [27, 28] Thus, the two methods can complement each other and provide a guidance for clinical precise treatment.

### Author contributions

Pu Yu participated in the experimental design and wrote the main manuscript text, Mengdi Li participated in data collection, collation and article revision. Rongjuan Sun participated in the content review and language revision of the article. Jianghua Zhan guided the research and revised the article. YongKang Pan participated in the experimental design, data collection and article revision. All authors reviewed the manuscript.

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### Declarations

**Conflict of interest** The authors declare that they have no conflicts of interest.

**Ethical approval** This study was approved by the Xi’an Children’s hospital’s ethical board (20220076).

**Human and animal rights** This article does not contain any studies with animals performed by any of the authors.

**Informed consent** The individual written informed consent was waived because of the retrospective design.

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