The Diagnostic Value of Liver Biopsy for Unexplained Liver Dysfunction: A Retrospective Study

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Objective: To analyse clinical manifestations of unexplained abnormal liver function and perform hepatobiliary histopathology procedures on patients to evaluate the value of liver biopsy in diagnosing the aetiology of unexplained abnormal liver function.

Methods: A convenience sampling method was used to retrospectively collect the data of patients who were diagnosed with unexplained abnormal liver function and who received liver biopsy in the Pathology Department of Tianjin Second People’s Hospital, China, between March 2022 and July 2023 to analyse liver pathology and clinical manifestations.

Results: A total of 1302 patients were included in this study, which mainly included 11 diseases: autoimmune liver disease (74 cases, 5.68%), drug-induced liver injury (DILI) (204 cases, 15.67%), cancer (237 cases, 18.20%), non-alcoholic fatty liver disease (104 cases, 7.99%), non-alcoholic steatohepatitis (74 cases, 5.68%), viral hepatitis (490 cases, 37.63%), other types of hepatitis (30 cases, 2.30%), cholestatic liver disease (17 cases, 1.31%), alcoholic liver disease (15 cases, 1.15%), hepatic cyst (5 cases, 0.38%) and Gilbert syndrome (4 cases, 0.31%). The success rate of liver biopsy sampling was 100%, and (1.52 ± 0.130) tissue strips were sampled. The average operating time was 11.52 minutes. The percutaneous liver biopsy did not significantly increase short-term liver function index values (serum γ-glutamyl transpeptidase, total bilirubin, alanine transaminase, aspartate aminotransferase, alkaline phosphatase). Ninety-two patients had a small amount of liver subcapsular fluid, but there was no progress after medical treatment.

Conclusion: Ultrasound-guided percutaneous liver biopsy has value in the diagnosis of unexplained abnormal liver function. Viral hepatitis, cancer and DILI are the most common causes of unexplained abnormal liver function. Liver biopsy does not aggravate the organic and functional impairment of the liver.

Keywords: unexplained abnormal liver function, clinical manifestations, liver biopsy, pathology

Introduction

The liver, an important organ in the human body, has the primary functions of metabolism, biotransformation, detoxification and coagulation. Moreover, the liver has an extremely strong compensatory ability. Most people have no symptoms before being diagnosed with abnormal liver function. Thus, abnormal liver function has become the “largest invisible killer”. The main causes of abnormal liver function are viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), cholestasis, drug or poison damage, autoimmune liver disease, hepatobiliary malignancies and infections. Results show that alcohol is the most serious risk factor, and cirrhosis mortality is an effective indicator of the health consequences of alcohol abuse and is most severe in Europe. In addition to alcohol, infection with the hepatitis B (HBV) and C viruses (HBV and HCV, respectively) is a leading cause of cirrhosis. According to the World Health Organization’s 2017 hepatitis report, 96% of the 1.3 million global deaths from hepatitis viruses are due to HBV and HCV infections, and more than half of these cases occur in the cirrhosis stage. In Asia, the
prevalence of HBV infection is higher among patients with cirrhosis than the prevalence of HCV, while in Europe and the US, the prevalence of HCV is higher than that of HBV.\(^4\) China has the highest HBV infection burden in Asia, and although the prevalence of HBV in China has decreased in the past 40 years, the prevalence of hepatitis B surface antigen (HBsAg) in some subpopulations is still high in China.\(^5\) Furthermore, intravenous drug users are at relatively high risk of contracting hepatitis C, and those who have been previously infected with this disease but have not been cured account for the majority of existing patients.\(^6\) China is a high epidemic area for viral hepatitis. Viral hepatitis is the most common cause of clinically abnormal liver function, and the resulting mortality has remained high among China’s notifiable infectious diseases.

Currently, the number of patients with liver injury is increasing annually. Many causes of liver dysfunction are observed in clinical practice. Hepatic pathology is an important method in diagnosis, disease severity assessment and efficacy monitoring, and it is known as the “gold standard” for the diagnosis and treatment of liver diseases.\(^7\)–\(^11\) Liver biopsy is an important method for identifying liver pathology. Pathological examination can identify the nature and extent of lesions, providing an objective and accurate basis for the diagnosis of liver diseases, and it has become a key tool for the diagnosis and research of liver diseases.\(^7\) Among the methods used for liver biopsy, ultrasound-guided liver biopsy is simple to operate. Under ultrasound guidance, the puncture point can be accurately located to avoid vital organs such as large blood vessels in the liver, gallbladder and lungs. This approach is a simple and convenient operation with few complications, and it is widely used.\(^9\)–\(^12\),\(^13\)

Clinically, liver injury has a variety of causes. Some patients lack specificity in terms of medical history, laboratory examinations and imaging. Liver injury with a cause that cannot be identified through routine examinations is diagnosed as unexplained liver injury. Specific manifestations of hepatic pathology are observed under the microscope, which provides an important basis for the diagnosis of unexplained liver injury and plays a crucial role in the diagnosis and treatment of liver diseases. Therefore, in this study, a retrospective analysis is conducted to summarise the data of patients that met the inclusion and exclusion criteria and who underwent liver biopsy to evaluate the value of liver biopsy in the diagnosis of liver diseases. In addition, possible complications during liver biopsy and the procedure’s safety are analysed.

**Participants and Methods**

**Participants**

A convenience sampling method was used to retrospectively collect the data of 1302 patients who were diagnosed with unexplained abnormal liver function and who underwent liver biopsy in the Pathology Department of Tianjin Second People’s Hospital, China, between March 2022 and July 2023. The inclusion criteria were as follows: (1) the patient’s case data are available; (2) biochemical indicators related to liver disease (eg total bilirubin [TBIL], alanine transaminase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP] and serum \(\gamma\)-glutamyl transpeptidase [GGT]) are persistent or repeatedly abnormal; and (3) the aetiology cannot be determined through existing technical means or by improving non-invasive examinations such as aetiology (eg bacteria, viruses and parasites), immunology (eg autoantibody spectrum) or liver imaging. The exclusion criteria included: (1) patients with liver function abnormalities caused by viral hepatitis, cirrhosis, alcoholic liver disease, autoimmune liver disease, space-occupying lesions of the liver, calculi and biliary obstruction that have been diagnosed through routine examinations; and (2) patients with liver function abnormalities caused by major medical diseases of the heart, spleen, lungs, kidneys, stomach and so on. This study was approved by the Ethics Committee of Tianjin Second People’s Hospital, and all participants gave informed consent and signed written consent forms.

**Methods**

Primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) were diagnosed based on the *Guidelines on the Diagnosis and Management of Autoimmune Hepatitis* (2011), revised by the Chinese Society of Hepatology of the Chinese Medical Association.\(^14\) Primary sclerosing cholangitis and cholestatic liver disease were diagnosed according to the *Expert Consensus on the Diagnosis and Management of Primary Sclerosing Cholangitis and Cholestatic Liver*
Disease, revised by the Chinese Society of Hepatology of the Chinese Society of Gastroenterology and Chinese Society of Infectious Diseases of the Chinese Medical Association in 2015.\textsuperscript{15,16} The diagnosis of drug-induced liver injury (DILI) was based on the Guidelines for Diagnosis and Management of Drug-Induced Liver Injury (2015) of the Drug-Induced Liver Disease Group under the Chinese Society of Hepatology of the Chinese Medical Association.\textsuperscript{17} Alcoholic hepatitis and NAFLD were diagnosed based on the 2018 Revision of China’s Guidelines for the Prevention and Treatment of Non-Alcoholic Fatty Liver Disease and Alcoholic Liver Disease.\textsuperscript{18,19}

The laboratory examinations and related auxiliary examinations were as follows. Prior to liver biopsy, the three routine examinations (blood, urine, stool) and examinations to determine coagulation, biochemistry, tumours, inflammation indicators, hepatitis B (qualitative and/or quantitative), antinuclear antibody test, AIH antibodies, electrocardiogram, B-mode ultrasound, abdominal computed tomography (CT) and other related examinations were completed. All patients were diagnosed using a combination of clinical and pathological examinations.

For the liver biopsy, after eliminating relevant contraindications to liver biopsy, the patients (or families) were informed of the complications and precautions before and after the procedure. After receiving consent, the patients were placed in a supine position. With the assistance of B-mode ultrasound positioning, routine disinfection was performed, an aseptic hole towel was placed on the patient, the eighth rib in the right midaxillary line was selected as the puncture point and 2% lidocaine was injected for local anaesthesia. A Bard biopsy gun (Becton, Dickinson & Company, NJ, USA) was used to insert a matched biopsy needle vertically to a depth of 1.9 cm. The trigger was then pulled, and a piece of pale red liver tissue with a length of approximately 1.5 cm was removed. The removed liver tissue was placed in formalin and sent to the pathology department for uniform X-ray reading, and the patient, with no complaints of discomfort during or after liver biopsy, was sent to the ward, where their blood pressure and vital signs were closely monitored.

### Data Collection

Patient data were collected, including sex, age, medical history, physical examination results, prehospitalisation routine examination results, viral indicators associated with hepatitis (A, B, C and E), autoantibodies and liver disease-related antibodies. Routine examinations including B-mode ultrasound, CT, magnetic resonance imaging and magnetic resonance cholangiopancreatography were performed when necessary to exclude liver function abnormalities caused by historical major diseases of the heart, spleen, lungs, kidneys, stomach and so on, as well as liver function abnormalities caused by confirmed viral hepatitis, cirrhosis, alcoholic liver disease, autoimmune liver disease, hepatic space-occupying lesions, calculi and biliary obstruction.

### Statistical Analysis

The data analysis was conducted using SPSS 26.00 software, and measurement data were expressed as mean ± standard deviation. The comparison between groups was conducted using the t-test, with the statistical data expressed as a proportion (%). A chi-squared ($\chi^2$) test was conducted to compare the proportion when the conditions were satisfied, and Fisher’s exact probability test was used when the conditions were not satisfied. A statistical difference was indicated by $P < 0.05$.

### Results

#### Patient’s Liver Biopsy Status

A total of 1302 patients with unexplained abnormal liver function were included in this study, all of whom received liver biopsy. All patients successfully completed the liver biopsy, with a tissue sampling success rate of 100% and tissue strip length of 1.5–2 cm. Naked-eye observation determined that these samples met the requirements for histological diagnosis. One tissue strip was sampled from 51.77% (674/1302) of the patients, and two (1.52 ± 0.130) tissue strips were taken from 48.23% (628/1302) of the patients, with an average liver biopsy time of 11.52 minutes.
Liver Biopsy Complications
Here, 746 patients experienced mild to moderate discomfort at the puncture site, with pain as the main manifestation, all of which subsided within 24 hours. Only 92 patients had a small amount of liver subcapsular fluid, and no patients had symptoms of an immediate or delayed build-up of peritoneal or perihepatic fluid or corresponding clinical symptoms. No serious complications, such as thoracic haemorrhage, pneumothorax or bile peritonitis, were observed.

Pathological Examination results of Patient Biopsy Tissue
A total of 1302 patients were included in this study, mainly with one of the following 11 diseases: autoimmune liver disease (74 cases, 5.68%), DILI (204 cases, 15.67%), cancer (237 cases, 18.20%), NAFLD (104 cases, 7.99%), non-alcoholic steatohepatitis (NASH) (74 cases, 5.68%), viral hepatitis (490 cases, 37.63%), other types of hepatitis (30 cases, 2.30%), cholestatic liver disease (17 cases, 1.31%), alcoholic liver disease (15 cases, 1.15%), hepatic cyst (5 cases, 0.38%) and Gilbert syndrome (4 cases, 0.31%). Primary biliary cirrhosis (58 cases, 4.45%) accounted for the largest proportion of autoimmune diseases, and hepatitis B (467 cases, 35.87%) accounted for the largest proportion of viral hepatitis. In addition, 17 patients still could not be diagnosed clearly after liver biopsy. The sex and age distribution of patients with unexplained liver dysfunction were statistically tested; AIH, PBC and autoimmune liver disease were not classified, and the differences were statistically significant ($P < 0.05$), as shown in Table 1.

In terms of gender, the patient group consisted of 671 men and 631 women. The three most common diseases in the male patients were hepatitis B (277 patients, 41.28%), hepatocellular carcinoma (HCC) (163 patients, 24.29%) and DILI (60 patients, 8.94%). The three most common diseases in the female patients were hepatitis B (190 patients, 30.11%), DILI (144 patients, 22.82%) and HCC (62 patients, 9.83%). In terms of age distribution, most of the patients were middle-aged or elderly, but some diseases exhibited a younger trend, including DILI, hepatitis B and NASH (Table 2).

<table>
<thead>
<tr>
<th>Etiological Classification</th>
<th>Cases</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoimmune liver disease</td>
<td>74</td>
<td>5.68%</td>
</tr>
<tr>
<td>AIH</td>
<td>12</td>
<td>0.92%</td>
</tr>
<tr>
<td>PBC</td>
<td>58</td>
<td>4.45%</td>
</tr>
<tr>
<td>AIH+PBC</td>
<td>4</td>
<td>0.31%</td>
</tr>
<tr>
<td>DILI</td>
<td>204</td>
<td>15.67%</td>
</tr>
<tr>
<td>Cancer</td>
<td>237</td>
<td>18.20%</td>
</tr>
<tr>
<td>HCC</td>
<td>225</td>
<td>17.28%</td>
</tr>
<tr>
<td>HCC + bile duct cancer</td>
<td>12</td>
<td>0.92%</td>
</tr>
<tr>
<td>NAFLD</td>
<td>104</td>
<td>7.99%</td>
</tr>
<tr>
<td>NASH</td>
<td>74</td>
<td>5.68%</td>
</tr>
<tr>
<td>Viral hepatitis</td>
<td>490</td>
<td>37.63%</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>467</td>
<td>35.87%</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>10</td>
<td>0.77%</td>
</tr>
<tr>
<td>Hepatitis E</td>
<td>8</td>
<td>0.61%</td>
</tr>
<tr>
<td>Unclassified hepatitis</td>
<td>5</td>
<td>0.38%</td>
</tr>
<tr>
<td>Other types of hepatitis</td>
<td>30</td>
<td>2.30%</td>
</tr>
<tr>
<td>Cholestatic liver disease</td>
<td>17</td>
<td>1.31%</td>
</tr>
<tr>
<td>Alcoholic liver disease</td>
<td>15</td>
<td>1.15%</td>
</tr>
<tr>
<td>Hepatic cyst</td>
<td>5</td>
<td>0.38%</td>
</tr>
<tr>
<td>Gilbert syndrome</td>
<td>4</td>
<td>0.31%</td>
</tr>
<tr>
<td>Other</td>
<td>31</td>
<td>2.38%</td>
</tr>
<tr>
<td>Unknown disease</td>
<td>17</td>
<td>1.31%</td>
</tr>
</tbody>
</table>

Abbreviations: AIH, Autoimmune hepatitis; PBC, Primary biliary cirrhosis; DILI, Drug-induced liver injury; HCC, Hepatocellular carcinoma; NAFLD, Non-alcoholic fatty liver disease; NASH, Non-alcoholic steatohepatitis.
Part of the hepatic histopathology is shown in Figure 1. Figure 1A presents the cases of hepatitis B (HBsAg: [++++], mainly plasma type, sporadic inclusion body type and oligo-membrane type; hepatitis B core antigen [HBcAg]: [+++] karyotype and plasma type). Figure 1B shows chemically toxic liver injury (microscopic findings: clear intralobular necrotic lesions, central periphlebitis is easily observed and mixed inflammatory cell infiltration found in the portal area). Figure 1C shows PBC (microscopic findings: mild intralobular lesions, infiltration of lymphocytes and plasma cells mixed with inflammatory cells in the portal area, ductopenia and proliferation of bile ducts in some portal areas). Figure 1D shows AIH (microscopic findings: mixed inflammatory cell infiltration [mainly lymphocyte and plasmacyte] around the portal area and necrotic zone and obvious interface inflammation). Figure 1E shows NASH (microscopic findings: centrilobular macrovesicular steatosis, peri-sinusoidal fibrosis frequently seen, a small amount of mononuclear cell infiltrated and dense mononuclear cells in only a small portal area). Figure 1F shows Gilbert syndrome (microscopic findings: clear liver lobule structure, no obvious inflammation, mild lipofuscinosis in hepatocytes around the central vein, macrovesicular steatosis seen in a few hepatocytes and small necrotic lesions occasionally seen).

Comparison of Liver Function Indicators Before and After Liver Biopsy
After comparing the patients’ liver function indicators before and after liver biopsy, the results showed that the differences in the patients’ GGT, TBIL, ALT, AST and ALP before and after liver biopsy were not statistically significant (all \( P > 0.05 \)), as shown in Table 3.

Discussion
The causes of abnormal liver function include viral hepatitis, autoimmune dysfunction, biliary obstruction, tumours, genetic metabolic diseases, damage from drugs, alcohol or poison, hemodynamic disorders, malnutrition and obesity. With the continuous development of modern molecular biology, immunology, imaging and other technologies, most...
patients with abnormal liver function can be clearly diagnosed through viral detection, biochemical tests and imaging examinations, but the aetiology of a small number of patients still needs to be identified using liver biopsy. Among the causes of unexplained abnormal liver function, viral hepatitis (type B) ranks first, and its mechanism may be related to factors such as genetic variation, low-level replication, low expression or host factors. (1) S gene: an encoded surface antigen that causes HBsAg expression deficiency when mutated, resulting in HBsAg negativity; (2) X gene: an encoded X protein of 154 amino acids, which can activate the replication process of the HBV gene and is

![Figure 1](https://doi.org/10.2147/JMDH.S460338)

**Table 3** Comparison of Liver Function Indicators Before and After Liver Biopsy ($\bar{x} \pm s$)

<table>
<thead>
<tr>
<th>Liver function indicators</th>
<th>GGT/(IU/L)</th>
<th>TBIL/(μmol/L)</th>
<th>ALT/(IU/L)</th>
<th>AST/(IU/L)</th>
<th>ALP/(IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before liver biopsy</td>
<td>191.4±192.4</td>
<td>55.7±55.4</td>
<td>113.2±122.1</td>
<td>102.4±123.2</td>
<td>195.7±256.7</td>
</tr>
<tr>
<td>After liver biopsy</td>
<td>164.3±171.1</td>
<td>46.7±45.3</td>
<td>93.4±124.1</td>
<td>92.3±114.6</td>
<td>208.1±272.5</td>
</tr>
<tr>
<td>t value</td>
<td>0.893</td>
<td>1.158</td>
<td>0.766</td>
<td>0.457</td>
<td>0.030</td>
</tr>
<tr>
<td>P value</td>
<td>0.374</td>
<td>0.249</td>
<td>0.445</td>
<td>0.649</td>
<td>0.976</td>
</tr>
</tbody>
</table>

**Abbreviations:** GGT, serum $\gamma$-glutamyl transpeptidase; TBIL, Total bilirubin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase.
the zone with the most obvious overlap of structure and function in the HBV genome, with the replication of the HBV reduced when mutated; (3) anterior C gene variation: the synthesis of the two antigenic proteins of the HBeAg and HBeAg viruses is mainly controlled by some initiation codons in the anterior C gene in the C region, also known as the C gene promoter, which plays a key regulatory role in the expression of HBeAg and HBeAg and HBV replication. (4) Hepatitis B virus maintains an extremely low level of replication, antigen expression is low and the HBsAg level is lower than the lowest detection value. (5) Abnormal host immune response: the body’s immune function is low and low-level viruses may not be cleared, resulting in latent HBV infection.

Cancer, primarily HCC, is the second leading cause of unexplained liver dysfunction. In the diagnosis and treatment of primary HCC, it is noted that liver puncture can determine the degree of differentiation and depth of HCC invasion and clarify preliminary staging of HCC. Most patients with liver cancer can be diagnosed by imaging examination, but for those who lack characteristic imaging findings, the diagnosis of liver cancer should be confirmed by liver biopsy. At the same time, the liver puncture technique can be used as an ideal treatment for liver tumours, and some malignant liver tumours can be treated by interventional therapy through liver puncture. Therefore, liver puncture plays an irreplaceable role in the diagnosis and treatment of liver malignant tumours.

The results showed that DILI was the third cause of unexplained liver dysfunction, consistent with the findings obtained by Khalifa et al. Drug-induced liver injury is defined as direct or indirect liver damage of different degrees caused by the drug itself or its metabolites during the course of drug use within the range of normal treatment or clinical trial dose. There are two main mechanisms by which drugs cause liver damage: (1) the direct toxic effects of drugs and their intermediate metabolites on the liver; (2) the specific reactions of the body to drugs, including allergic (immune-specific) and metabolic (metabolic-specific). Drug-induced liver injury can occur in healthy individuals with no prior history of liver disease or in patients with pre-existing severe disease; it can occur when the drug is over dosed, and it can occur at normal doses. Andrade et al noted that the severity of DILI varies from patient to patient and that an accurate diagnosis in some cases requires a liver biopsy.

In recent years, with the continuous improvement in living standards, changes in dietary structure and reduction in physical exercise, the morbidity of fatty liver in China has increased, especially in relation to NASH. In Europe and the United States, 42–90% of the continued increase in serum transaminase for unknown reasons is related to NASH, which has become the primary cause of liver function abnormalities in patients. The number of patients with fatty liver disease is increasing annually, and NASH is another common cause of abnormal liver function. Non-alcoholic steatohepatitis is a clinicopathological syndrome with similar histological changes to alcoholic steatohepatitis but without excessive drinking, and it is mainly caused by excessive fat deposition in the liver as the result of obesity, nutritional imbalance and dyslipidaemia.

Liver disease caused by long-term heavy drinking that leads to hepatocyte damage is called alcoholic liver disease. It can progress from early-stage fat to liver fibrosis and cirrhosis. Studies have found that ethanol or alcohol type, sex, heredity, race and individual differences are all factors that affect alcoholic liver disease. Mortality in alcoholic liver disease is increased by malnutrition, vitamin A deficiency and reduced vitamin E levels. The clinical symptoms are non-specific and may not appear, but there may also be symptoms such as right upper quadrant discomfort (hidden pain), loss of appetite, fatigue and jaundice. At present, there is no specific method for the clinical diagnosis of patients with alcoholic liver disease. Following routine clinical examination, clinical misdiagnosis still occurs. Based on the patient’s condition, a liver biopsy should be performed to clarify the pathology when necessary and rule out other liver diseases.

The study by Hunyady et al suggested that liver biopsy is essential when extrahepatic diseases are suspected of affecting the liver. Ultrasound-guided percutaneous liver biopsy is currently one of the internationally recognised liver biopsy methods and overcomes the shortcomings of blind puncture, which has numerous complications and high risks. In the present study, the tissues were successfully sampled from all the patients under ultrasound, and high-quality biopsy samples were taken at the required sampling accuracy. The most common complications of percutaneous liver biopsy is pain, with an incidence rate of more than 30%. In this study, 746 out of 1302 patients experienced mild pain, a higher proportion than Androutsakos et al’s study, where only 10 out of 261 patients experienced pain. However, no obvious complications were found in the patients in the present study.

In this study, liver samples were pathologically diagnosed under ultrasound, which played a key role in guiding clinicians in formulating treatment plans. Through the comparison of preoperative and postoperative liver functions, it
was found that percutaneous liver biopsy did not aggravate the organic and functional damage of the liver, and percutaneous liver biopsy can be performed on patients who cannot be clearly diagnosed.

This study has a number of limitations. First, it only provides a pathological analysis of unexplained abnormal liver function and does not analyse or explore the common causes of abnormal liver function on an individual basis; this will be the focus of future studies. Second, due to time constraints, this study only provides a retrospective analysis, and more prospective studies will be conducted in the future. Since general data collected from medical records comes from clinical physicians, omissions or deviations are inevitable.

In conclusion, this study summarised and analysed the case data of patients with unexplained liver dysfunction and found that ultrasound-guided percutaneous liver biopsy has value in the diagnosis of unexplained abnormal liver function. Viral hepatitis, cancer and DILI are the most common causes of unexplained abnormal liver function. By summarising the complications and liver function of patients following liver biopsy, it was found that the procedure does not exacerbate organic and functional damage to the liver.

Data Sharing Statement
All data generated or analyzed during this study are included in this published article.

Ethics Approval and Consent to Participate
This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Tianjin Second People’s Hospital.

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