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CASE REPORT

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First detection of HBV nucleic acid in the lung tissue of a patient with spontaneous recovery from HBV infection by tNGS: a case report

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Abstract

Background After recovery from hepatitis B virus (HBV) infection, the virus is likely to reactivate once the body is in an immunosuppressed state. Currently, routine liver biopsies are difficult to obtain, and there are few reports on HBV nucleic acid detection in biopsies of other human organs and tissues. Targeted Next-Generation Sequencing (tNGS) can precisely focus on specific gene regions. Through high-throughput sequencing techniques, it can efficiently obtain a large amount of target nucleic acid sequence information and is widely applied in fields such as disease gene detection and tumor molecular diagnosis. With the development of tNGS, a new path has been opened for HBV nucleic acid detection in different organs, tissues and body fluids, offering hope to break through the current predicament.

Case presentation This case is about a 78-year-old male patient. He was first hospitalized for pulmonary infection. During this hospitalization, tNGS of the bronchoalveolar lavage fluid sample detected a small amount of hepatitis B virus nucleic acid sequences. Two months later, he was re - hospitalized due to cough and expectoration. TNGS of his transthoracic lung fine needle aspiration sample showed a high number of hepatitis B virus nucleic acid sequences. The serological test results during hospitalization indicated that the patient was in a state of spontaneous recovery from hepatitis B virus infection, with HBsAg negative, Anti - HBs positive, Anti - HBc positive, and HBV nucleic acid negative. Fluorescent quantitative PCR detected HBV nucleic acid in the bronchoalveolar lavage fluid sample and the transthoracic lung fine needle aspiration sample at different times.

Conclusion This case study is the first to use tNGS to precisely analyze the bronchoalveolar lavage fluid sample and the transthoracic lung fine needle aspiration sample from different sampling periods. It accurately identifies HBV nucleic acid replication in the lungs of patients with spontaneous HBV - infection recovery, offering new ideas and evidence for HBV research in extra - hepatic tissues.

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Keywords Recovery from hepatitis B virus, TNGS, Transthoracic lung fine needle aspiration sample, Bronchoalveolar lavage fluid sample, Hepatitis B surface antigen, HBV DNA

Introduction

Background

The spontaneous recovery from hepatitis B virus (HBV) infection refers to the process in which the body, without human intervention such as antiviral treatment, successfully clears or effectively suppresses HBV relying on the powerful function of its own immune system [1]. It is specifically manifested as the negative conversion of hepatitis B surface antigen (HBsAg), the production of protective hepatitis B surface antibody (anti-HBs) in the body, the continuous positivity of hepatitis B core antibody (anti-HBc), the undetectability of Hepatitis B virus deoxyribonucleic acid (HBV DNA) in the blood as it is below the lower limit of detection, the restoration of liver function indicators to the normal range, the subsidence of liver inflammatory reaction, the repair of liver damage, and the disappearance of various clinical symptoms [2]. Similarly, the functional cure of hepatitis B requires that after the cessation of treatment, HBsAg remains at an undetectable level [2–3]. According to relevant studies, it is estimated that 2 billion people worldwide have been infected with the hepatitis B virus, and there are 1.5 million new cases of HBV infection each year [4]. Due to the difficulty in obtaining liver biopsy samples, only a few literatures have reported that HBV may reactivate in patients with serological recovery of hepatitis B under conditions such as immunosuppression. There are also literatures reporting that most patients with functional cure of hepatitis B are in the state of occult hepatitis B [5]. Until now, no literature has directly reported the situation in which HBV DNA is detected in lung tissues but remains undetectable in blood. In this case study, we have conducted a detailed analysis of the HBV DNA in the lung tissues of a patient who has spontaneously recovered from HBV infection.

TNGS is a targeted high-throughput sequencing technology that combines the advantages of "PCR" and "NGS" [6]. It improves the detection sensitivity by positively enriching the target pathogens and eliminating the interference of host nucleic acids [6]. The process includes links such as nucleic acid extraction and multiplex PCR amplification, and it can identify the core pathogens of different syndromes and patient types [6]. Through tNGS, we have accurately determined the presence of HBV nucleic acid in the patient's bronchoalveolar lavage fluid and lung puncture tissue. At the same time, the accuracy of the results has been verified by fluorescence quantitative PCR. This discovery may open up a new perspective for the research on HBV infection.

Case presentation

A 78-year-old male patient was admitted to our hospital. The patient has a past medical history of hypertension and coronary heart disease, but no history of diabetes. He has a history of trichiasis surgery, a history of undergoing surgery for a fracture of the left lower limb, and a history of prostate surgery. However, he has no history of trauma, nor any history of food or drug allergies.

The patient was admitted to the hospital due to "cough and expectoration for more than one month", and the CT results indicated pulmonary infection. During the hospitalization, the results of liver function tests and the determination of hepatitis B surface antigen (HBsAg) were both normal (see Table 1 for details). The bronchoalveolar lavage fluid sample was tested by tNGS, and a low number of HBV nucleic acid sequences (13 reads, see the first row of Table 2 for details) were detected.

More than two months later, the patient was admitted to the hospital again due to "cough and expectoration for more than four months". During the hospitalization, the results of enhanced chest CT examination indicated that there was a mass-like shadow in the posterior segment of the right upper lobe of the lung, with a cavity inside, and punctate, patchy and strip-like edema shadows around the lesion. Since the effect of anti-infection treatment was not satisfactory, after consulting the chief physician and obtaining the consent of the family members, the transthoracic lung fine needle aspiration sample was performed, and the sample was sent for tNGS testing. The results showed a high number of HBV nucleic acid sequences (4,392 reads, see the second row of Table 2 for details). In order to rule out sample contamination, a second tNGS test was conducted on the tissue sample, and a high number of HBV nucleic acid sequences were still detected (4,615 reads, see the third row of Table 2 for details). At the same time, a quantitative test of the hepatitis B triple serology and a test for hepatitis B virus nucleic acid were performed on the blood samples collected at the same time. The results showed that hepatitis B surface antigen (HBsAg) was negative, hepatitis B core antibody (Anti-HBc) was positive, and hepatitis B virus nucleic acid (HBV-DNA) was not detected (the specific results are shown in Table 3).

To verify the accuracy of the results, our laboratory used the fluorescence quantitative PCR method to conduct a highly sensitive hepatitis B virus nucleic acid test on the nucleic acids of the patient's blood, bronchoalveolar lavage fluid samples, and tissue samples. The results showed that there was no amplification curve for hs-HBV DNA in the serum; there was an amplification curve for

Table 1 Results of the detection of hepatitis B surface antigen in the patient's blood during the first hospital admission examination

Sample Type	Test Item	Test Result	Unit	Reference Value	Detection Method
Serum	HBsAg	0.00	IU/mL	0.0-0.05	Chemiluminescence Method

Table 2 The tNGS results of the patient

NO.	Sample Type	English Name of Pathogen	Number of Reads	Pathogen Copy Number	Signal Intensity
A	The bronchoalveolar lavage fluid sample	Hepatitis B virus	13	10	Low
B	The transthoracic lung fine needle aspiration sample	Hepatitis B virus	4615	2400	high
C	The transthoracic lung fine needle aspiration sample	Hepatitis B virus	4392	2560	high

A: The tNGS results of the bronchoalveolar lavage fluid specimen of the patient during the first hospital admission. B: The tNGS results of the lung puncture tissue specimen of the patient during the second hospital admission. C: The rechecked tNGS results of the lung puncture tissue specimen of the patient during the second hospital admission

hs-HBV DNA in the bronchoalveolar lavage fluid, but the concentration was less than 20 IU/mL; and the hs-HBV DNA in the nucleic acid of the lung puncture tissue was 5328 IU/mL (see Table 4 for details).

More than one month after discharge, the patient returned to the outpatient department for follow-up. Blood was re-drawn for reexamination of liver and kidney functions, quantitative determination of the hepatitis B triple serology, and HBV-DNA. The results showed that the liver and kidney functions were normal, HBsAg was negative, Anti-HBc was positive, and hepatitis B virus nucleic acid was not detected (the specific results are shown in Table 5).

Discussion and conclusions

Until now, no literature has directly reported the situation in which HBV DNA is detected in lung tissues but remains undetectable in blood.However, there are relevant studies on similar situations in the liver. From a theoretical perspective, it may be due to the following reasons: Firstly, in the field of hepatitis B virus research, most previous studies have focused on the detection of the virus in liver tissue, with a serious lack of attention paid to lung tissue. Secondly, the replication level of the virus in lung tissue is relatively low, making it difficult to be detected by conventional testing methods. Thirdly, existing detection technologies have a lower limit of sensitivity in blood testing, and it is difficult to accurately identify low-level viral nucleic acids [7]. Fourthly, there are differences in immune control among different tissues of the human body [8–9], which may affect the distribution and detection results of the hepatitis B virus in lung tissue and blood. Finally, HBV has a certain tropism for lung tissue, which makes it easier for the virus to remain in lung tissue [10], thus resulting in the situation where the virus can be detected in lung tissue but not in the blood.

In conclusion, In the fields of medical research and clinical practice, this case has a pioneering significance. It is the first special case directly reporting that HBV DNA can be detected in lung tissue, while remaining undetectable in blood.The clinical manifestations of spontaneous recovery from HBV infection do not have typical characteristics, which makes pathogen detection a crucial link in clinical diagnosis and adjustment of treatment plans. In this case, the researchers for the first time used tNGS

Table 3 Results of quantitative hepatitis B serological markers and HBV-DNA in the patient's serum during the second hospital admission

Sample Type	Test Item	Test Result	Unit	Reference Value	Detection Method
Serum	HBsAg	0.00	IU/mL	0.0~0.05	Chemiluminescence Method
Serum	Anti-HBs	60.95	mIU/mL	0.0~10.00	Chemiluminescence Method
Serum	HBeAg	0.01	S/CO	0.0~1.0	Chemiluminescence Method
Serum	Anti-HBe	0.54	S/CO	> 1.0	Chemiluminescence Method
Serum	Anti-HBc	16.77	S/CO	0.0~1.0	Chemiluminescence Method
Serum	HBV-DNA	Not detected	IU/mL	"Not detected" indicates that the sample does not contain HBV DNA, or the content is extremely low and has not reached the detection lower limit of this method.	Fluorescent Probe PCR Method

Table 4 Results of hs-HBV DNA of the patient

NO.	Sample Type	Test Item	Ct value	Result	Unit	Reference Value	Detection Method
A	Serum	Hs-HBV DNA	NO Ct	0	IU/mL	Minimum detection limit: 20	Fluorescent Probe PCR Method
B	The bronchoalveolar lavage fluid sample	Hs-HBV DNA	37.16	4.45	IU/mL	Minimum detection limit: 20	Fluorescent Probe PCR Method
C	Nucleic acid of the transthoracic lung fine needle aspiration sample	Hs-HBV DNA	27.23	5328	IU/mL	Minimum detection limit: 20	Fluorescent Probe PCR Method

A: The results of the hypersensitive hepatitis B virus nucleic acid test of the patient's serum during the second hospitalization. B: The results of the hypersensitive hepatitis B virus nucleic acid test of the patient's bronchoalveolar lavage fluid sample during the first hospitalization. C: The results of the hypersensitive hepatitis B virus nucleic acid test of the nucleic acid in the transthoracic lung fine needle aspiration sample of the patient during the second hospitalization.Ct (Cycle Threshold): Ct value represents the number of amplification cycles required for the fluorescent signal to cross a pre-determined threshold. It is a crucial parameter for quantifying the amount of target nucleic acid in a sample

Table 5 The test results of the blood specimensresults of quantitative hepatitis B serological markers and HBV-DNA in the patient's serum duringoutpatient follow-up

Sample Type	Test Item	Test Result	Unit	Reference Value	Detection Method
Serum	HBsAg	0.00	IU/mL	0.0~0.05	Chemiluminescence Method
Serum	Anti-HBs	51.65	mIU/mL	0.0~10.00	Chemiluminescence Method
Serum	HBeAg	0.01	S/CO	0.0~1.0	Chemiluminescence Method
Serum	Anti-HBe	0.48	S/CO	> 1.0	Chemiluminescence Method
Serum	Anti-HBc	20.05	S/CO	0.0~1.0	Chemiluminescence Method
Serum	HBV-DNA	Not detected	IU/mL	"Not detected" indicates that the sample does not contain HBV DNA, or the content is extremely low and has not reached the detection lower limit of this method.	Fluorescent Probe PCR Method

to track and detect the content of HBV nucleic acid in the bronchoalveolar lavage fluid sample and transthoracic lung fine needle aspiration sample of patients with spontaneous recovery from HBV infection. This detection method has important clinical significance. When patients cannot obtain serological test results as a basis for diagnosis, or are unable to undergo liver puncture examination due to physical conditions, psychological factors, or other reasons, the detection of HBV nucleic acid in other tissue fluids can provide powerful auxiliary information for clinical diagnosis. In addition, this detection method can also be applied to the regular monitoring of hepatitis B patients after achieving functional cure, providing key data support for evaluating the treatment effect and preventing the recurrence of the disease.

Abbreviations

HBsAg	Hepatitis B Virus Surface Antigen
Anti-HBs	Hepatitis B Virus Surface Antibody
HBeAg	Hepatitis B Virus e Antigen
Anti-HBe	Hepatitis B Virus e Antibody
Anti-HBc	Hepatitis B Virus Core Antibody
HBV DNA	Hepatitis B Virus Nucleic Acid
Hs-HBV DNA	Highly Sensitive Hepatitis B Virus Nucleic Acid
tNGS	Targeted Next Generation Sequencing

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-10997-1.

Supplementary Material 1

Supplementary Material 2

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Author contributions

Li Zhenzhen collected, analyzed and interpreted the clinical data, and also drafted the initial version of the manuscript. Cheng Zhengjiang performed the supervision work. Dai Xiaoqing, Yang Xiaoxia and Zhou Lan-Ting revised the manuscript. Huang Shaojun reviewed the manuscript and finally approved the version to be published. All the authors reviewed this manuscript.

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Data availability
Data is provided within this publication and the supplementary tables.

Declarations

Ethics approval and consent to participate
This was a retrospective study based on the clinical tNGS result. Approval for this study was obtained from the research ethics board of Xiangyang Central Hospital, Affiliated Hospital of Hubei University of Arts and Science. The patient provided informed consent for inclusion prior to participation in the study.

Consent for publication
The patients were informed to the use of their clinical data and personal information in this study. And all participants provided written informed consent for publication of their data.

Competing interests
The authors declare no competing interests.

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