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CASE REPORT

Successful surgical treatment of polybacterial gas gangrene confirmed by metagenomic next-generation sequencing detection: A case report

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Abstract

BACKGROUND

We report on a case of *Vibrio vulnificus (V. vulnificus)* detected by metagenomics next-generation sequencing (mNGS) in a 53-year-old male patient with polymicrobial gas gangrene and successful treatment by surgery. This report raises awareness among dermatologists that when a patient is clinically suspected of a special type of pathogenic infection, the mNGS method should be preferred to identify the patient's pathogen infection as soon as possible and then take effective treatment in time to save patients' lives.

CASE SUMMARY

A 53-year-old male who worked in the aquatic market complained of redness and swelling of the lower limbs, blisters and ulcers with fever for 3 d. We used mNGS to test the pathogens in ulcer secretions. The results were returned in 24 h and indicated: *V. vulnificus*, *Fusobacterium necrophorum*, *Staphylococcus haemolyticus*, *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Klebsiella aerogenes*. This patient was diagnosed with *V. vulnificus* infection. The emergency operation was performed immediately under combined lumbar and epidural anesthesia: Left leg expansion and exploration (August 10, 2021). After surgery, we continued to use piperacillin sodium tazobactam sodium 4.5 g every 8 h and levofloxacin 0.5 g for anti-infection treatment. The patient underwent further surgery under lumbar anesthesia on August 17, 2021 and August 31, 2021: Left leg deactivation and skin grafting, negative pressure closed drainage and right thigh skin removal. After treatment, the transplanted flap survived.

CONCLUSION
We could confirm the diagnosis of *Vibrio vulnificus* infection within 24 h through mNGS detection and then immediately performed emergency surgery.

Key Words: Metagenomics next-generation sequencing; Vibrio vulnificus; Polymicrobial gas gangrene; Surgery; Case report

Core Tip: We report on a case of *Vibrio vulnificus* detected by metagenomic next-generation sequencing (mNGS) in a 53-year-old male patient with polymicrobial gas gangrene and successful treatment by surgery. This report raises awareness among dermatologists that when a patient is clinically suspected of a special type of pathogenic infection, the mNGS method should be preferred to identify the patient's pathogen infection as soon as possible, and then take effective treatment in time to save patients' lives.

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INTRODUCTION

Vibrio vulnificus (V. vulnificus) is a halophilic, motile, gram-negative bacterium that is an important opportunistic pathogen. *V. vulnificus* can cause septicemia and necrotizing infections[1]. *V. vulnificus* necrotizing skin and soft tissue infections are a serious, highly fatal and disabling disease that can cause fulminant sepsis with a mortality rate of over 50%[2-4].

V. vulnificus usually causes infection *via* exposure to seawater or through the consumption of seafood, and its pathophysiology can be divided into three types: (1) Primary sepsis; (2) Gastrointestinal diseases; and (3) Wound infections. Men (90% of cases) and older patients (85% > 40 years) are susceptible, especially those with liver disease, diabetes and underlying conditions such as immunodeficiency and hemochromatosis[5].

Primary *V. vulnificus* sepsis is a serious disease with a high mortality rate. Approximately 1/3 of patients with primary sepsis develop shock or hypotension within 12 h of admission. Bullous lesions are characteristic in three quarters of patients. Thrombocytopenia is common and patients often have evidence of diffuse intravascular coagulation[6].

The diagnosis of *V. vulnificus* infection is usually verified by a nonculture method or by traditional culture. *V. vulnificus* grows easily on standard media. Isolation of this bacterium from feces usually requires the use of a specific selective medium, namely, thiosulfate-citrate-bile salts-sucrose medium[7].

Here, we report the case of a 53-year-old male with polymicrobial gas gangrene complicated with *V. vulnificus* infection. The patient was diagnosed with *V. vulnificus* infection by metagenomic next-generation sequencing (mNGS) within 24 h and underwent immediate emergency debridement, followed by effective antibiotics and further surgical treatment. The patient recovered 1 mo later.

CASE PRESENTATION

Chief complaints

Redness and swelling of lower limbs, blisters and ulcers with fever for 3 d.

History of present illness

Three months prior, erythema and blisters had appeared in both lower extremities. He was diagnosed with a "skin infection" at a local hospital and improved after treatment. Three days prior, erythema, blisters, ulcers and fever had suddenly appeared in the left lower extremities. The local hospital administered cefoperazone for anti-infection, mannitol for dehydration, a magnesium sulfate wet compress, and other treatments without significant improvement.

History of past illness

The patient denied a history of medical illness. System physical examination showed no obvious abnormality.

Personal and family history

The patient had no relevant personal or family history.

Physical examination

Obvious ulcerations and a large blister area were observed on the left calf; the blister walls were thin, and the blister fluid was pale yellow or bloody; the left inguinal lymph nodes were palpable and swollen (Figure 1); the patient also had a fever, with a maximum temperature of 38.9 ℃.

Laboratory examinations

Infection index C-reactive protein (CRP) 134.30 mg/L (0.00-6.00 mg/L) (August 6, 2021), liver function: Alanine aminotransferase 47 U/L (9-50 U/L), Aspartate aminotransferase (AST) 40 U/L (15-40 U/L). The culture results showed that *V. vulnificus*, *Staphylococcus haemolyticus* and *Proteus vulgaris/penneri* were positive, and no fungus grew. The antimicrobial sensitivity of *S. haemolyticus* shows that it was sensitive to Linezolid, Nitrofurantoin, Rifampin, Trimethoprim-Sulfamethoxazole, Teicoplanin, Trimethoprim, and Vancomycin. The antimicrobial sensitivity of *P. vulgaris/penneri* shows that it was sensitive to Amoxicillin/Clavulanic, Amikacin, Aztreonam, Ceftazidime, Chloramphenicol, Ciprofloxacin, Cefotaxime, Cefepime, Gentamicin, Imipenem, Levofloxacin, Meropenem, Piperacillin, Trimethoprim-Sulfamethoxazole, and Piperacillin-Tazobactam. The results of AST showed that the cultured bacteria were sensitive to Piperacillin-Tazobactam and Levofloxacin. Detailed antimicrobial susceptibility testing results are shown in Table 1 and 2.

Imaging examinations

Lung radiographs and computed tomography showed exudative changes in both lungs. The left lower limb ultrasound showed a dark fluid area. Abdominal ultrasonography revealed chronic liver disease.

MULTIDISCIPLINARY EXPERT CONSULTATION

The patient did not undergo a multidisciplinary consultation.

FINAL DIAGNOSIS

Polybacterial gas gangrene.

TREATMENT

The emergency operation was performed immediately under combined lumbar and epidural anesthesia; a left leg expansion and exploration (August 10, 2021). After surgery, we continued to use piperacillin sodium tazobactam sodium 4.5 g every 8 h and levofloxacin 0.5 g for anti-infection treatment. The patient underwent further surgery under lumbar anesthesia on August 17, 2021, and again on August 31, 2021, including left leg deactivation and skin grafting, negative pressure closed drainage and right thigh skin removal.

OUTCOME AND FOLLOW-UP

After treatment, the transplanted flap survived (Figure 2). The patients' infection indicators, CRP and Procalcitonin, decreased gradually and returned to normal on September 1, 2021 (Figure 3 and 4).

DISCUSSION

Although *V. vulnificus* infections are rare, *V. vulnificus* is responsible for the largest number of vibrio deaths[8,9]. Recent surveillance by the Centers for Disease Control and Prevention shows that the geographic area affected by *V. vulnificus* is expanding and that infection rates are rising worldwide due to global warming and rising ocean temperatures[10,11].

Table 1 List of antimicrobial susceptibility testing results of *Proteus vulgaris/penneri*

Code	Antimicrobial	Result	MIC
AMC	Amoxicillin/Clavulanic	Sensitivity	≤ 4/2
AMK	Amikacin	Sensitivity	≤ 8
AMP	Ampicillin	Resistance	8
ATM	Aztreonam	Sensitivity	≤ 2
CAZ	Ceftazidime	Sensitivity	≤ 1
CDR	Cefdinir	-	-
CEC	Cefaclor	-	-
CEP	Cephalothin	-	-
CFM	Cefixime	-	-
CFP	Cefoperazone	-	-
CHL	Chloramphenicol	Sensitivity	≤ 4
CID	Cefonicid	-	-
CIN	Cinoxacin	-	-
CIP	Ciprofloxacin	Sensitivity	0.25
CL	Colistin	Resistance	> 2
CMZ	Cefmetazole	-	-
CPR	Cefprozil	-	-
CPX	Cefpodoxime proxetil	-	-
CRB	Carbenicillin	-	-
CRO	Ceftriaxone	-	-
CSL	Cefoperazone/Sulbactam	-	-
CTT	Cefotetan	-	-
CTX	Cefotaxime	Sensitivity	≤ 1
CXM	Cefuroxime	-	-
CZO	Cefazolin	Resistance	> 16
CZX	Ceftizoxime	-	-
DOX	Doxycycline	-	-
ETP	Ertapenem	-	-
FEP	Cefepime	Sensitivity	≤ 2
FLE	Fleroxacin	-	-
FOS	Fosfomycin	-	-
FOX	Cefoxitin	-	-
GAT	Gatifloxacin	-	-
GEN	Gentamicin	Sensitivity	≤ 2
IPM	Imipenem	Sensitivity	≤ 1
KAN	Kanamycin	-	-
LOM	Lomefloxaccin	-	-
LOR	Loracarbef	-	-
LVX	Levofloxacin	Sensitivity	0.5
MAN	Cefamandole	-	-
MEC	Mecillinam	-	-

MEM	Meropenem	Sensitivity	≤ 1
MEZ	Mezlocillin	-	-
MFX	Moxifloxacin	Mediation	4
MNO	Minocycline	-	-
MOX	Moxalactam	-	-
NET	Netilmicin	-	-
NIT	Nitrofurantoin	-	-
NOR	Norfloxacin	-	-
OFX	Ofloxacin	-	-
PIP	Piperacillin	Sensitivity	≤ 4
SAM	Ampicillin-Sulbactam	Sensitivity	≤ 4/2
SSS	Sulfonamides	-	-
STR	Streptomycin	-	-
SXT	Trimethoprim-Sulfamethoxazole	Sensitivity	≤ 0.5/9.5
TCC	Ticarcillin/Clavulanic	-	-
TCY	Tetracycline	Resistance	≤ 2
TIC	Ticarcillin	-	-
TMP	Trimethoprim	-	-
TOB	Tobramycin	-	-
TZP	Piperacillin-Tazobactam	Sensitivity	≤ 4/4

MIC: Minimum inhibitory concentration.

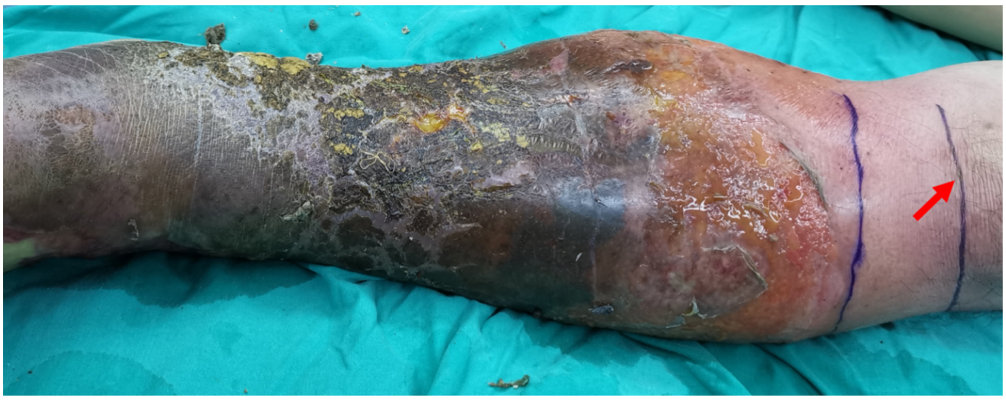

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Figure 1 Skin lesions of the left lower limb on admission.

Infections caused by *V. vulnificus* were first reported by Hollis *et al*[12] in 1976. *V. vulnificus* has the highest case fatality rate among all foodborne pathogens. Infections spread extremely rapidly, with an average incubation period of only 16 h for wound infection and 26 h for septicemia[13]. A case series study from South Korea reported that the incubation period for sepsis ranged from 3 h to 6 d[14].

Primary *V. vulnificus* sepsis is a serious disease with a high mortality rate. *V. vulnificus* has the highest case fatality rate (39%) of all reported foodborne infections in the United States, with a fatality rate of more than 90% in cases with existing hypotension at the time of presentation[15,16]. In Japan, the majority of cases of *V. vulnificus* infection manifest as primary septicemia, with a mortality rate of up to 70%, and more than one-half of the patients die within 3 d[17]. Researchers have found that the longer the interval between the onset and the initiation of antimicrobial therapy, the higher the mortality rate of *V. vulnificus* sepsis and severe wound infection[18]. Although patients with *V. vulnificus* infection can fully recover, complications associated with multiple organ system failure may persist. Therefore, it is

Table 2 List of antimicrobial susceptibility testing results of *Staphylococcus haemolyticus*

Code	Antimicrobial	Result	MIC
AMC	Amoxicillin/Clavulanic	Resistance	> 4/2
AMP	Ampicillin	Resistance	> 8
AZM	Azithromycin	-	-
CHL	Chloramphenicol	-	-
CIP	Ciprofloxacin	Resistance	> 4
CLI	Clindamycin	-	-
CLR	Clarithromycin	-	-
DAP	Daptomycin	-	-
DOX	Doxycycline	-	-
ERY	Erythromycin	Resistance	> 4
GEN	Gentamicin	Resistance	> 8
LNZ	Linezolid	Sensitivity	1
LVX	Levofloxacin	-	-
MFX	Moxifloxacin	-	-
MUH	Mupirocin	Resistance	> 256
NIT	Nitrofurantoin	Sensitivity	≤ 16
OFX	Ofloxacin	-	-
OXA	Oxacillin	Resistance	> 2
PEN	Penicillin	Resistance	> 0.25
QDA	Quinupristin-Dalfopristin	-	-
RA	Rifampin	Sensitivity	≤ 0.5
RIF	Rifampin		
SXT	Trimethoprim-Sulfamethoxazole	Sensitivity	≤ 1/19
TCY	Tetracycline	Resistance	> 8
TEC	Teicoplanin	Sensitivity	4
TLT	Telithromycin	-	-
TMP	Trimethoprim	Sensitivity	2
VAN	Vancomycin	Sensitivity	2

MIC: Minimum inhibitory concentration.

very important to recognize this disease and diagnose it correctly as soon as possible.

However, the identification of microorganisms by culturing methods usually takes 7-10 d or longer [19]. In addition, many microorganisms require specific growth conditions that are difficult to simulate in a laboratory environment, and microbial culture methods may not always detect pathogenic agents [20,21]. Using blood samples from patients with skin and soft tissue infections, C-polymerase chain reaction (PCR) and N-PCR were found to be 45% and 86% sensitive to *V. vulnificus* target gene toxicity R gene, respectively. Previous studies have reported that Q-PCR detection of *V. vulnificus*-specific genes is the most sensitive and specific technology and the fastest diagnostic method at present[5]. In this study, we propose that mNGS is a promising new diagnostic technique that can theoretically identify all known microbial genomes from clinical specimens. This analysis is usually performed in a short period of time (24-36 h)[22].

The traditional medical treatment for *V. vulnificus* infection is third-generation cephalosporins combined with tetracycline or fluoroquinolones. However, as a result of the excessive use of antibiotics in human, agriculture, and aquaculture systems, antibiotic resistance has emerged and evolved in many bacterial genera, including Vibrio, during the past few decades[23,24]. A case-series study of 121 Taiwanese patients presenting with necrotizing fasciitis found that surgical treatment within 12 h of

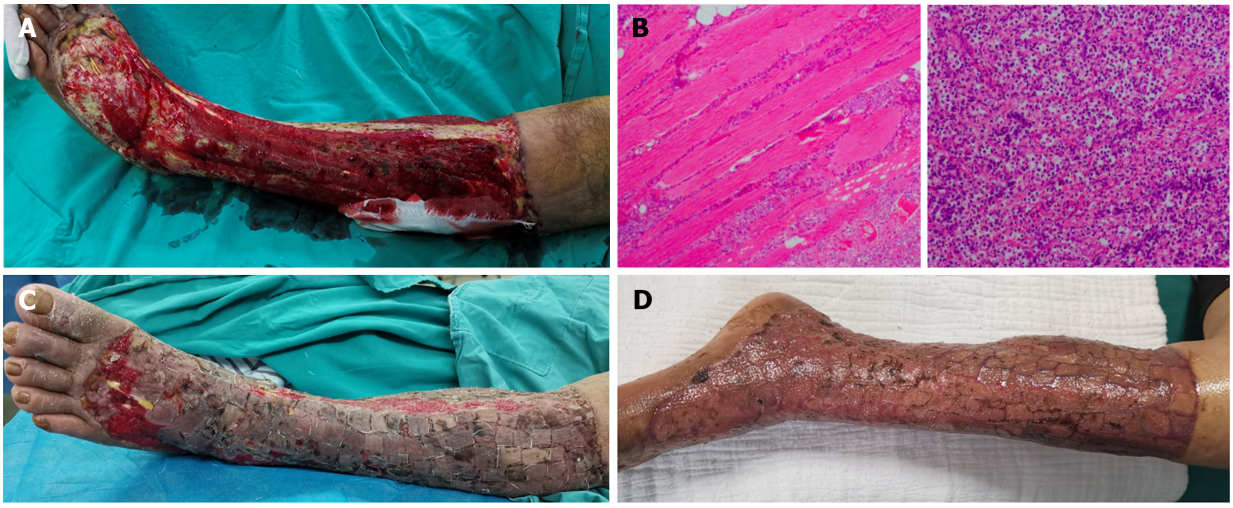

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Figure 2 Left lower extremity skin lesions at different stages of treatment and postoperative pathological examination results. A: Image of left lower limb after enlarged debridement; B: The pathological findings of surgical specimens suggested the formation of soft tissue abscess; C: Image of left lower limb flap after transplantation; D: Left lower extremity flap transplantation.

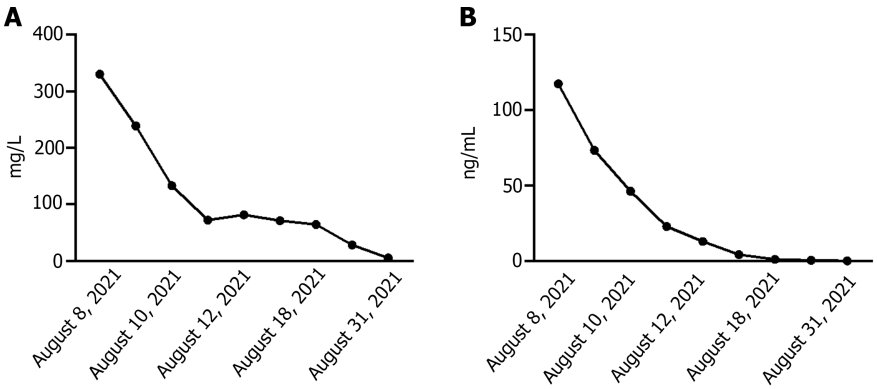

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Figure 3 Patient's infection index. A: C-reactive protein; B: Procalcitonin.

admission resulted in significantly improved survival[25]. Of the 423 *V. vulnificus* wound infections reported in the United States, 10% required some type of amputation. However, some patients still die after undergoing surgical treatment[26]. In 1992, Chuang *et al*[27] reviewed 28 cases of *V. vulnificus* infection in 27 patients in Taiwan. They argued that clearing the infected area within the first 24 h was crucial because most patients died within 48 h of being hospitalized.

In this study, the patient developed a skin infection of the left lower limb 3 mo prior to admission, and a hemorrhagic blister with fever had suddenly appeared at the site of the original skin infection 3 d prior. The infection progressed rapidly with a tendency toward multiple organ failure. We found *V. vulnificus*, a highly lethal pathogen of skin infection, in time through the mNGS method. Meanwhile, *Staphylococcus hemolyticus*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Fusobacterium necrophorum* and Klebsiella were also detected through mNGS (Table 3). This result suggested that the patient had a mixed infection with multiple pathogenic bacteria. Therefore, we quickly chose antibiotic treatment covering *V. vulnificus* and other pathogens, followed by immediate surgical debridement and treatment resulting in effective control of the patient's infection and avoiding amputation and even death.

Therefore, this report raises awareness among dermatologists that when a patient is clinically suspected of a special type of pathogenic infection, the mNGS method can be preferred to identify the patient's pathogen infection as soon as possible, and then take effective treatment measures in time, which can benefit the patient to a greater extent and even save the patient's life.

Table 3 List of bacteria detected by metagenomics next-generation sequencing

Type	Genera	Number of sequences	Species	Number of sequences
G+	Staphylococcus	7166	*Staphylococcus haemolyticus*	6493
			Staphylococcus aureus	88
G-	Vibrio	7026	*Vibrio vulnificus*	6491
G+	Streptococcus	973	*Streptococcus dysgalactiae*	664
G-	Fusobacterium	43	*Fusobacterium necrophorum*	40
G-	Klebsiella	11	*Klebsiella aerogenes*	10

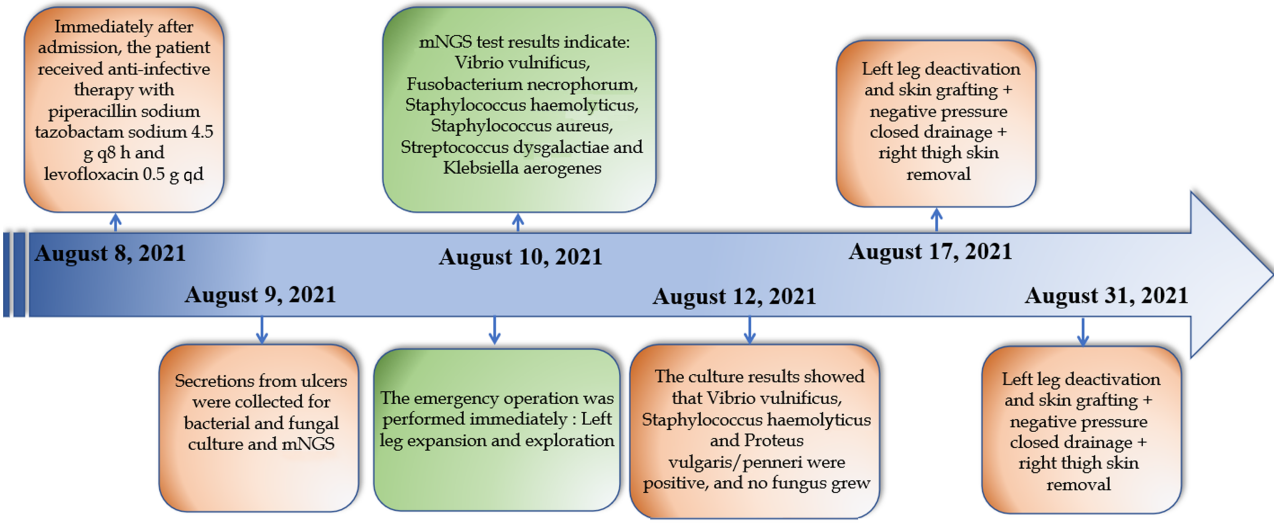

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Figure 4 Flow charts of diagnosis and treatment. mNGS: Metagenomics next-generation sequencing.

CONCLUSION

In conclusion, we reported a case of *V. vulnificus* detected by mNGS in a 53-year-old male patient with polymicrobial gas gangrene and successful treatment by surgery. This patient was successfully treated with surgery, while amputation or even death was avoided. The main benefit was that we were able to confirm the diagnosis of *V. vulnificus* infection within 24 h through mNGS detection. We immediately performed emergency surgery which helped gain precious time to save the patient's life. Conversely, the traditional method of bacterial and fungal culture has a low positive rate, a small detection range and is time-consuming.

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FOOTNOTES

Author contributions: Lu HY performed sequencing and was a major contributor in writing the manuscript; Gao YB and Qiu XW performed the surgery; Wang Q and Huang XW provided samples, clinical data, contributed to project design and operation, data interpretation, and manuscript preparation; Liu CM and Chen HY contributed to project design and operation; Li CX and Zeng K contributed to project design and operation, data interpretation, and was a major contributor in writing the manuscript; All authors read and approved the final manuscript.

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