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CLINICAL CHALLENGE

Diagnosis of local hepatic tuberculosis through next-generation sequencing: Smarter, faster and better

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Summary
Background: A 45-year-old man who complained of continuous fever and multiple hepatic masses was admitted to our hospital. Repeated MRI manifestations were similar while each radiological report suggested contradictory diagnosis pointing to infections or malignances respectively. Pathologic examination of the liver tissue showed no direct evidence of either infections or tumor. We performed next-generation sequencing on the liver tissue and peripheral blood to further investigate the possible etiology.
Methods: High throughput sequencing was performed on the liver lesion tissues using BGISEQ-100 platform, and data was mapped to the Microbial Genome Databases after filtering low quality data and human reads.
Results: We identified a total of 299 sequencing reads of *Mycobacterium tuberculosis* (*M. tuberculosis*) complex sequences from the liver tissue, including 8, 229 of 4,424,435 of the *M. tuberculosis* nucleotide sequences, and *Mycobacterium africanum, Mycobacterium bovis*, and *Mycobacterium canettii* were also detected due to the 99.9% identical rate among these strains. No specific *Mycobacterial tuberculosis* nucleotide sequence was detected in the sample of peripheral blood. Patient's symptom quickly recovered after anti-tuberculosis treatment and repeated Ziehl—Neelsen staining of the liver tissue finally identified small numbers of positive bacillus.

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Conclusions: The diagnosis of this patient was difficult to establish before the next-generation sequencing because of contradictive radiological results and negative pathological findings. More sensitive diagnostic methods are urgently needed. This is the first case reporting hepatic tuberculosis confirmed by the next-generation sequencing, and marks the promising potential of the application of the next-generation sequencing in the diagnosis of hepatic lesions with unknown etiology.

Tuberculosis (TB) is a globally pandemic infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*). The bacilli typically affect the lungs, but can also exist in any organ of one's body, which is known as extrapulmonary TB. Hepatic tuberculosis (hepatic TB) is a rare manifestation of the extrapulmonary TB and most hepatic TB cases were concurrent with extrahepatic TB presentation like miliary TB or tuberculous meningitis. To this date, local TB infection of the liver, known as primary or isolated hepatic TB, has only been reported in few countries [1]. Typical TB clues or evidences tend to be absent in the clinical progression of local hepatic TB, thus resulting in confusion and difficulty in diagnosis [2]. Here we report a case of a middle-aged man presenting with continuous fever and liver masses observed in various imagine tests. In this particular case, next-generation sequencing (NGS) played a key role in the expeditious identification of *M. tuberculosis* as the causative agent from the sequencing of the liver tissue while laboratory, various imagine examinations and pathological assessments reported negative or even contradictive results. This is the first case of NGS assisting in the diagnosis of local hepatic TB, and highlighting the potential of such technique in the rapid etiological diagnosis of liver lesions in the future.

On May 25th, 2017, a 45-year-old man was admitted to our hospital due to three weeks of continuous fever and hepatic masses. Computed tomography (CT) of the chest and brain in local hospital showed no abnormalities. Magnetic Resonance Imaging (MRI) of the abdomen was then conducted and reported multifocal ill-defined lesions dispersed throughout the liver (the largest measuring 60.5×52.5 mm), and further contrasted images demonstrated rim and septal enhancement around the masses as well as the enlargement of the lymph nodes within the porta hepatis and the retroperitoneal space. Hence, the radiologists suspected the high possibility of malignant tumor. Liver biopsy and pathologic examination showed lymphocytes cells infiltration and large necrotic areas, suggesting inflammation. Immunohistochemical analyses were negative for methenamine silver, Periodic acid-Schiff, and Ziehl—Neelsen staining. The next day the patient developed progressive fever, along with chills and hyperhidrosis. His febrile illness followed a pattern of nightly fevers with morning defervescence. Empirical anti-infective therapy with moxalactam, ceftazidime, ornidazole and meropenem failed to relieve the patient's symptoms. The patient was thus transferred to our hospital for further evaluation.

On admission, laboratory tests showed T-SPOT.*TB* positive (Panel A: > 30; Panel B: > 50), an elevated erythrocyte sedimentation rate (114 mm/h), C-reactive protein (74.9 mg/L) and ferritin level (1850 ng/mL). Other laboratory tests including repeated blood cultures and physical examination showed no abnormality. Abdomen MRI and contrasted CT were performed again in our hospital and the former favored the diagnosis of multiple pyogenic liver abscess while the latter suggested malignancies (Fig. 1A and B). A positron emission tomography (PET)/CT scan found multiple hepatic nodular hypermetabolic lesions with increased standard uptake value, a finding suggestive of cholangiocarcinoma with liver metastases (Fig. 1C). Besides, abdomen ultrasound was also conducted and did not provide a definite opinion (Fig. 1D).

To this point, there was still no clear indication whether the nature of the liver lesions was infectious or malignant. Therefore, an ultrasound-guided percutaneous liver biopsy was performed again and samples of liver tissue and peripheral blood were sent for NGS and histological test after the patient gave written informed consent. Full details about sample processing and NGS analysis were provided in the Supplementary Materials. Three days later, the NGS reported detection of a total of 299 sequencing reads of *M. tuberculosis* complex including 8, 229 of 4,424,435 of *M. tuberculosis* nucleotide sequences from the liver tissue. 7709 of 4,389,314 of *Mycobacterium africanum* nucleotide sequences, 7757 of 4,376,711 of *Mycobacterium bovis* nucleotide sequences, and 1247 of 4,525,948 of *Mycobacterium canettii* nucleotide sequences were also detected due to that all these strains are composed of genetically smiliar16S rRNA and have 99.9% identical nucleotides [3]. No specific *Mycobacterial tuberculosis* complex nucleotide sequences were detected in the sample of blood (Supplementary Figure S1). Therefore, the physicians diagnosed the patient with local hepatic tuberculosis and immediately initiated the anti-tuberculosis drugs therapy including rifampicin, isoniazid, ethambutol, pyrazinamide and levofloxacin. On the next day, the patient's temperature decreased sharply to 36.8 °C without recurrence (Supplementary Figure S1). One week later, histological results of the liver biopsy reported no suspicious findings. Two weeks later, the pathological sections of liver tissue from the first liver biopsy were borrowed to our hospital for consultation. Ziehl—Neelsen staining was performed again and pathologists found small numbers of positive bacillus