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Case Report

Metagenomic Next-Generation Sequencing for the Diagnosis of Epstein-Barr virus Pleurisy: A Case Report

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Abstract

Chronic active Epstein-Barr virus infection (CAEBV) progresses rapidly in the later stage and has a poor prognosis. The treatments of CAEBV have no unified standard and a bad effect. Only few person could cure. It is important to diagnosis early. Metagenomic next-generation sequencing (mNGS) offers an effective means for the diagnosis of difficult, critical and rare pathogenic microbial infections. Here, we report a case of viral pleurisy caused by CAEBV identified by mNGS in an 88-year-old Chinese male patient.

Keywords: Metagenomic next-generation sequencing; Chronic active Epstein-Barr virus infection; Viral pleurisy

Introduction

Hospitalization of patients with infectious diseases often leads to inadequate or delayed treatment, prolonged stays, high cost, and increased mortality. It is often more likely to occur to patients with advanced age, chronic disease, decreased immunity, cancer, long-term bedridden and other susceptible reasons. The 2016 revised WHO classification recognizes chronic active Epstein-Barr virus infection (CAEBV) as a chronic active Epstein-Barr virus (EBV) infection of T- and natural killer (NK)-cell types, systemic and cutaneous forms, among EBV-positive T- and NK-cell lymphoproliferative diseases of childhood (1). The mechanism is still unknown. However, similar clinical reports of adults have existed (2,3). CAEBV often develops into malignant diseases, such as multiple organ failure, difused intravascular coagulaton, and malignant tumors (4).

Metagenomic next-generation sequencing (mNGS) provides a possibility for early identification of pathogens by detecting pathogens deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) in infections. mNGS can detect a broad range of pathogenic microorganisms — viruses, bacteria, fungi and parasites — from culture or direct from clinical samples based on uniquely identifiable DNA and RNA sequences. Cases of meningitis and encephalitis have identified bacteria (5), viruses (6-10), fungi and parasites (7,11) from cerebrospinal fluid and brain tissue by mNGS. mNGS can identify the number of pneumonia pathogens (12,13), and potential pathogens in patients with acute cholecystitis (14). mNGS can detect the pathogen DNA or RNA in bloodstream infection to offer a possibility for diagnosis of infection in patients with culture-negative sepsis (14-17). mNGS can identify

more pathogens in a limited number of eye samples in the ocular infections (8, 18).
Here, we report a case of viral pleurisy caused by CAEBV identified by mNGS.

Case Report

An 88-year-old Chinese male patient was in a second-class hospital because of repeated chest tightness for 3 years and aggravating for 2 months. Previous diseases included coronary atherosclerotic heart disease, hypertension, old cerebral infarction, chronic obstructive pulmonary disease and persistent atrial fibrillation. During hospitalization, a dull pain in the upper right chest and recurrent pleural effusion happened. Sometimes chest pain could self-relieve. Write the day of the first chest pain down as 'Day1' in the following tables, and so on. The blood tests showed no obvious abnormality, as described in Table 1. The results of pleural effusion tests are shown in Table 2.
With the consent of the patient and his families, we used mNGS to detect pathogens in pleural effusion. Steps of mNGS included nucleic acid extraction, DNA and RNA enrichment, library preparation, polymerase chain reaction amplification, and sequencing on Illumina HiSeq and bioinformatics analysis. The bioinformatics pipeline consisted of a series of analysis steps from the original file of FASTQ format, including quality and low complexity filtering, joint pruning, human host subtraction, microbial identification, optional sequence assembly, and classification of individual read and consecutive sequences.
We compared identified microorganisms from the sample with the reference database to get the pathogen. The database includes over 3000 species of bacteria, over 4000 species of viruses, over 200 species of fungi and 140 species of parasites.
On the 80th day after the first chest pain, EBV found in the detectation sequence. The patient was given famciclovir orally as an antiviral treatment, with a dosage of 0.25 gr each time, 3 times a day. While it did not work well, and had to stop because of the side effect of the drug. On the 108th day after the first chest pain, the patient worsened sharply and transferred to a higher-level hospital for further treatments, but still died of septic shock 2 days later.
Because of diseased and personal reasons, such as changes of hospitals, limitations of examination equipment in the second-class hospital, and poor cooperation of the patient, there were only two imaging examinations of chest, namely the frontal X-ray and computed tomography (CT), as shown in Fig. 1.

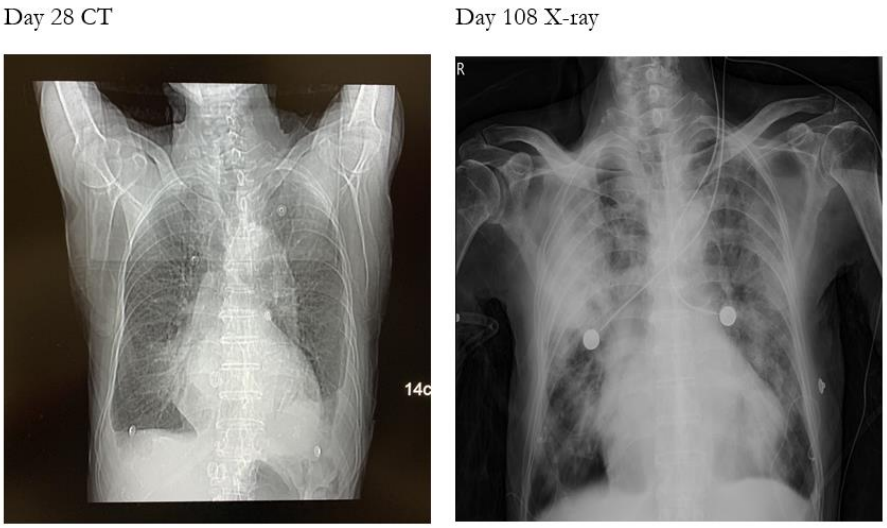

Fig. 1: Comparison of frontal chest images

Table 1: Changes in blood tests

Variable	*Day1*	*Day34*	*Day69*	*Day86*	*Day 108*
Blood routine (normal range)					
WBC# (4.0-10.0×10^9/L)	6.4	5.9	4.5	4.1	12.5
Neutrophils (50.0-70.0%)	69.1	67.5	65.5	63.1	96.0
Eosinophils (0.5-5.0%)	1.5	0.13	1.1	2.4	0.1
Basophils (< 1.0%)	0.5	0.0	0.1	0.1	0.0
Monocytes (3.0-12.0%)	8.8	7.1	9.0	7.3	2.5
Lymphocytes (20.0-40.0%)	20.1	22.8	24.3	27.1	1.4
RBC# (4.50-5.50×10^{12}/L)	4.51	4.39	4.24	4.26	4.61
Hemoglobin (120-160g/L)	146	144	139	138	148
PTL# (100-300×10^9/L)	137	125	96	120	124
Blood biochemistry and myocardial enzyme spectrum (normal range)					
TP# (60.0-80.0g/L)	71.4	71.8	65.1	58.8	55.7
Albumin (35.0-55.0g/L)	44.7	36.5	34.4	34.2	27.8
TBIL# (2.0-18.0μmol/L)	36.2	18.3	23.1	14.3	30.2
ALT# (< 42.0U/L)	30.4	20.9	18.0	30.4	57.4
AST# (< 40.0U/L)	46.0	35.8	40.8	38.0	53.2
LDH# (109.0-245.0U/L)	247.9	248.7	223.7	212.2	-
CK# (24.0-195.0U/L)	169.1	137.2	130.7	72.1	-
CKMB# (< 24.00U/L)	27.56	-	-	9.88	-
Troponin-T# (0-0.3ng/mL)	-	<0.1	-	<0.1	-
UREA# (1.7-8.3mmol/L)	11.9	9.2	13.8	9.8	29.1
Creatinine (35.0-105.0μmol/L)	104.8	116.2	142.3	96.8	109.7
Sodium (136.0-145.0mmol/L)	139.1	144.0	144.4	140.6	140.4
Potassium (3.50-5.20mmol/L)	4.03	3.62	4.14	3.94	4.94
Potassium (96.0-108.0mmol/L)	104.5	107.2	107.1	103.4	104.3
Glucose (3.89-6.11mmol/L)	5.37	5.3	5.7	4.4	-
Coagulation spectrum (normal range)					
PT# (10.0-14.0s)	13.0	13.6	13.6	12.8	16.1
INR# (0.80-1.50)	1.00	1.04	1.04	0.98	1.24
APTT# (25.0-38.0s)	28.6	26.3	25.4	26.5	27.0
TT# (14.0-21.0s)	19.1	20.1	22.7	19.0	18.1
FIB# (2.00-4.00g/L)	2.19	2.32	1.75	2.45	5.09
D-Dimer (< 0.50ug/mL)	2.27	4.12	4.23	3.55	4.77
CRP# (normal range <10.0 mg/L)	-	7.5	0.8	7.5	134.5

#WBC: White blood cell count. RBC: Red blood cell count. PLT: Platelet count. TP: Total protein. TBIL: Total bilirubin. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. LDH: Lactate dehydrogenase. CK: Creatine Kinase. CKMB: Creatine Kinase-MB. Troponin-T: Due to different testing methods, the same index has different reference value ranges. UREA: Urea nitrogen. PT: Prothrombin time. INR: International standard ratio. APTT: Partial thromboplastin time. TT: Thrombin time. FIB: Plasma fibrinogen. CRP: C reactive protein.

Table 2: Tests of pleural effusion

Variable	*Day6*	*Day10*	*Day18*	*Day29*
Pleural fluid biochemistry				
Pleural fluid TP	17.1 g/L	20.9 g/L	16.2 g/L	15.3 g/L
Pleural fluid LDH	72U/L	127 U/L	98 U/L	69 U/L
ADA##	5U/L	4 U/L	4 U/L	3 U/L
Pleural fluid glucose	7.39 mmol/L	7.68 mmol/L	8.03 mmol/L	6.31 mmol/L
Pleural fluid routine				
Appearance and transparency	-	Dark yellow Transparent	Yellow Slightly muddy	Dark yellow Muddy
Specific gravity	-	1.010	1.010	1.010
Coagulation	-	with clot	with clot	with clot
Li Fanta Test	-	Positive (+)	Positive (+)	Positive (+)
RBC#	-	0.01×10^{12}/L	0.00×10^{12}/L	0.02×10^{12}/L
WBC#	-	0.49×10^{9}/L	0.61×10^{9}/L	0.48×10^{9}/L
Monocytes	-	1.94%	1.97%	1.97%
Multinucleated cells	-	1.6%	1.3%	1.3%
Bacterial culture and identification	No bacterial growth after 3 d of culture	-	-	-
Fungus culture	-	-	No fungus growth after 5 d of cultiva-tion	-
Pleural fluid smear	-	-	No tumor cells found	No tumor cells found

##ADA：Thymus adenosine dehydrogenase

#WBC: White blood cell count. RBC: Red blood cell count

Replace the untested items with "-" in the test

Discussion

The main clinical manifestations of this case were recurrent chest pain and pleural effusion. No obvious abnormality had shown in the early inflammation test indices, the CT image, and tests of pleural effusion. Through mNGS we found EBV in the pleural effusion. No evidence of lung infection and no basis of acute infection existed in the early stage. This patient had many susceptible causes to EBV, such as diseases of heart, brain and lung, and advanced age. Combined with the situation, we diagnosed as a case of viral pleurisy caused by CAEBV. In the later stage, it progressed rapidly. The effect of antiviral treatment was not well. Even we was transferred to a higher-level hospital, it did not reverse the poor prognosis. Some examinations had not completed, such as gastroenteroscopy and rechecked CT. There may be underlying malignant tumors and other unknown diseases. Without unified imaging examinations, only the frontal image of CT and X-ray could to compare roughly (Fig. 1). In the later stage, inflammation had extended from pleura to both lungs and the right upper lung had become consolidation from the comparison.

The treatments of CAEBV have no unified standard and a bad effect, including traditional

antiviral, anti-tumor chemotherapy and immunotherapy. In the later stage, most patients just have a temporary remission, and only few person could cure.

Compared with conventional culture, mNGS has three advantages in etiological diagnosis: no bias, wide coverage and high efficiency. mNGS can identify known and unexpected pathogens, and even discover new organisms (19). mNGS can detect multiple pathogens at the same time. mNGS can use on targeted approaches, such as using of primers from 16S ribosomal RNA and internal transcribed spacer sequences for bacterial and fungal detection. Besides, mNGS can provide auxiliary genomic information necessary for evolutionary tracking, strain identification and drug resistance prediction (20). However, mNGS also has disadvantages. It takes more time than molecular diagnostic tests. DNA or RNA sequencing on standard Illumina instruments alone takes about 9 times the time spent on molecular diagnostic tests (21).

The complexity of mNGS analysis needs trained personnel and extreme care in sample handling to avoid errors and cross-contamination. It needs to divide the test workflow into discrete steps by rotating shifts to avoid laboratory errors. Even a small deviation in the treatment of the sample can lead to a significant change in the results (22). No user-friendly bioinformatics software and specific reference database exist to analyze mNGS data (23, 24). Specific bioinformatics pipelines often need trained programmers to develop, validate, and uphold for clinical use. Common large reference databases include National Center for Biotechnology Information, GenBank database, genome collection, and microbial reference sequence.

Conclusion

CAEBV is easy to be ignored in the early stage, and progresses rapidly in the later stage, and the prognosis is poor. mNGS covers a wider range of microorganisms, is more efficient, and has no deviation, providing a new generation of detection technology for the early diagnosis of CAEBV.

Ethical considerations

The authors have observed ethical issues, including plagiarism, informed consent, misconduct, data untruth and falsification, double publication and submission, redundancy, etc.

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Conflict of interest

The authors declare that no conflict of interests exists.

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