Observational Study

Medicine

OPEN

Diagnostic value of mNGS in patients with suspected tumor

An observational study

Haiyan Zhang, MSc[a,*]

Abstract

Some patients suspected of infection may have potential causes, such as tumors, but conventional examination methods are negative. Copy number variation (CNV) analysis based on metagenomics next generation sequencing (mNGS) can simultaneously detect pathogenic microorganisms and tumors signals. Patients with suspected infection in our department were retrospectively analyzed, and mNGS and chromosomal CNV analysis were performed simultaneously. A total of 9 patients with positive tumor signal were included in the study. This study was divided into 2 parts: in the first part, patients suspected of infection were finally diagnosed with a tumor by CNV assisted analysis; in the second part, the accuracy of this analysis was verified again by patients with a history of cancer. Three of five patients without a history of tumor were diagnosed with hematological malignancy. All patients with active tumor had obvious abnormal CNV signals. The chromosomal abnormalities mainly included multiple chromosome duplication and deletion, arm level duplication and deletion, and chromosome aneuploidy. mNGS-based CNV analysis had clinical value for patients with underlying tumor.

Abbreviations: CNV = copy number variation, CT = computed tomography, EBV = Epstein–Barr virus, FUO = fever of unknown origin, mNGS = metagenomics next generation sequencing, WBC = white blood cell.

Keywords: copy number variation, hematological malignancy, metagenomics Next generation sequencing, suspected infection, tumor signals

1. Introduction

Fever of unknown origin (FUO) is a common puzzle encountered by clinicians. Differential diagnosis of FUO is difficult. One study indicated that infection (16–55%) and neoplasia (7–35.4%) are 2 major causes.[1] Based on the current traditional detection technology, it is difficult to obtain timely and accurate diagnosis. Some tumor patients may have secondary infections, and primary symptoms were concealed by infectious symptoms, especially in patients with hematological malignancies. Compared to conventional methods, the role of metagenomics next generation sequencing (mNGS) is significant for patients with FUO. mNGS not only can discover novel or emerging pathogens,[2] but also can give some clues for neoplasm diagnosis by detecting abnormal DNA clones. DNA copy number variation (CNV) reflects genomic instability and is involved in the development of various types of neoplasm.[3-5] The aim of this study was to assess the clinical significance of mNGS in detecting tumor signals and pathogens in patients with suspected infection.

2. Materials and methods

2.1. Study participants

Patients suspected of infection with positive tumor signals by mNGS-based CNV analysis were included in the study. Patients without a history of tumor were finally diagnosed with tumors by CNV-assisted analysis, which was then validated by patients who have a history of malignancy.

Ultimately, a total of 9 patients were included. Suspected infection is mainly based on a comprehensive assessment of blood routine, inflammatory markers, imaging findings. Clinical data of the patients were collected including age, sex, underlying disease, routine blood examination, clinical microbiological results (cultures for bacteria, virus, acid-fast bacilli, atypical pathogen, and fungi), and pathological reports. The study was approved by the Ethics Committee of the third affiliated Hospital of Sun Yat-sen University. The ethics committee approval number was II2025-280-01. Written informed consent to participate in this study was obtained.

The author has no funding and conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a] Department of General Practice, The Third Affiliated Hospital of Sun Yat-Sen, University Lingnan Hospital, Guangzhou, Guangdong Province, People's Republic of China.

** Correspondence: Haiyan Zhang, Department of General Practice, The Third Affiliated Hospital of Sun Yat-Sen University Lingnan Hospital, 2693 Kaichuang Road, Guangzhou 510000, Guangdong Province, People's Republic of China (e-mail: zhanghy46@mail.sysu.edu.cn).*

How to cite this article: Zhang H. Diagnostic value of mNGS in patients with suspected tumor: An observational study. Medicine 2026;105:7(e47379).

Received: 7 October 2023 / Received in final form: 25 August 2025 / Accepted: 21 October 2025

http://dx.doi.org/10.1097/MD.0000000000047379

Key Points

mNGS can simultaneously identify more possible microorganism and neoplasm DNA in blood or other samples, which is helpful for the diagnosis of malignancy.

2.2. NGS sequencing and CNV analysis

mNGS involves several steps. First, sample collection, such as from body fluids or tissues. Then, nucleic acid extraction. Next, library preparation for sequencing. Finally, high-throughput sequencing and bioinformatics analysis to identify microorganisms.

Steps for CNV analysis refer to mNGS analysis by lung biopsy tissue.[6] During mNGS, information on the CNV was obtained from the calling method, which was based on XHMM[7] and Canoes.[8] Then, all the normalized features were fitted to dozens of preprogrammed neuron networks. They were structured using 3 classical neural network and different in hyper-parameters, which is suitable for large-scale-fold changed data of read counts. Twenty models were selected. Each model was used to predict the possibility of cancer in the sample (0 or 1). The predicted score was calculated using the total number of positive results from the 20 models.

2.3. Diagnosis of neoplasm

Hematological tumor was diagnosed by bone marrow smear, molecular biological examination and pathological examination of lymph node biopsy tissue. Previous liver malignant tumor was diagnosed according to imaging results, tumor markers, hepatitis history, etc.

2.4. Statistical methods

Statistical analyses were performed using SPSS 20.0 software (Guangzhou, China). Descriptive statistics were used to describe baseline characteristics. Categorical variables were analyzed using the Fisher's exact test. P value<.05 was considered statistically significant.

3. Results

3.1. Disease diagnosis

Obvious abnormal CNV signals were observed in 4 cases with a history of liver cancer or lung cancer.

Among 5 patients without the history of cancer, 3 cases were diagnosed with hematological malignancy, one with severe pneumonia and one with Epstein-Barr virus associated hemophagocytic syndrome (Table 1).

3.2. CNV analysis for tumor signals

In 3 patients with an initial diagnosis of suspected infection, we detected tumor signals and this was consistent with the histopathological results. Tumor signals were detected in all 4 patients with active tumor. There were 2 cases that were false positive. Abnormal CNV signals were observed in both peripheral blood and bone marrow in 2 patients; 1 case in peripheral blood and tissue; 5 cases in peripheral blood; 1 case in ascites. Abnormal chromosome abnormalities were observed including genome-wide multiple chromosome duplication and deletion (cases 1, 5, 7–9), arm level duplication and deletion (cases 3, 4, 6), and chromosome aneuploidy (case 2). CNV signals of all patients were showed in Figure 1. The CNV position of each case is shown in Table 2.

3.3. Diagnosis of tumor

Three patients without recorded history of malignant tumors were observed to have abnormal CNV signals, and they were diagnosed with chronic mononuclear leukemia acute leukemia, and lymphoma by bone marrow smear, molecular biological examination of bone marrow and pathological result of lymph node biopsy tissue. In this study, 4 patients had a history of malignant tumors, and their blood mNGS were observed to have tumor signals, all of them were assessed as active condition.

Table 1

Course of treatment and final diagnosis of all patients.

No	Sex, age	Past history	Diagnosis of admission	Diagnosis of discharge	Symptom	Antimicrobial drugs use
1	Male /33	No	Maxillofacial infection	Lymphoma	Fever, maxillofacial swelling and pain	Moxifloxacin, vancomycin levofloxacin, linezolid, amikacin isoniazid sulfamethoxazole
2	Male/66	Left clavicle fracture with internal fixation, anemia and duodenal ulcer	Bacteremia	Lymphoma	Fever	Moxifloxacin levofloxacin vancomycin and oral SMZ
3	Male/65	Diabetes, emphysema	Severe pneumonia	Severe bacterial pneumonia	Cough, sputum and fever	Azithromycin, doxycycline, imipenem cilastatin, amikacin
4	Male/50	Primary liver cancer, hepatitis B, pulmonary tuberculosis	Pulmonary tuberculosis	Pulmonary tuberculosis	Cough, sputum and chest pain	Rifampicin, ethambutol, isoniazid, levofloxacin
5	Male/41	Liver cancer, hepatitis B	Abdominal infection	Abdominal infection	Right upper abdominal pain	Levofloxacin and tigecycline
6	Male/24	No	FUO	EB virus associated HPS	Fever	Levofloxacin and amikacin
7	Male/71	Lung cancer emphysema	Pulmonary infection	Pulmonary infection	Cough and short of breath	Levofloxacin and imipenemcenotaxin
8	Male/71	Stable multiple myeloma, hypertension, diabetes	FUO	ALL and pulmonary infection	Fever, cough	Moxifloxacin, meropenem, caspofungin, SMZ
9	Male/59	Hypertension, hepatitis B, liver cancer with lung metastasis, liver transplantation	Pulmonary infection	Pulmonary fungal infection	Cough, fever	Voriconazole

FUO = fever of unknown origin, HPS = hemophagocytic syndrome, SMZ = sulfamethoxazole.

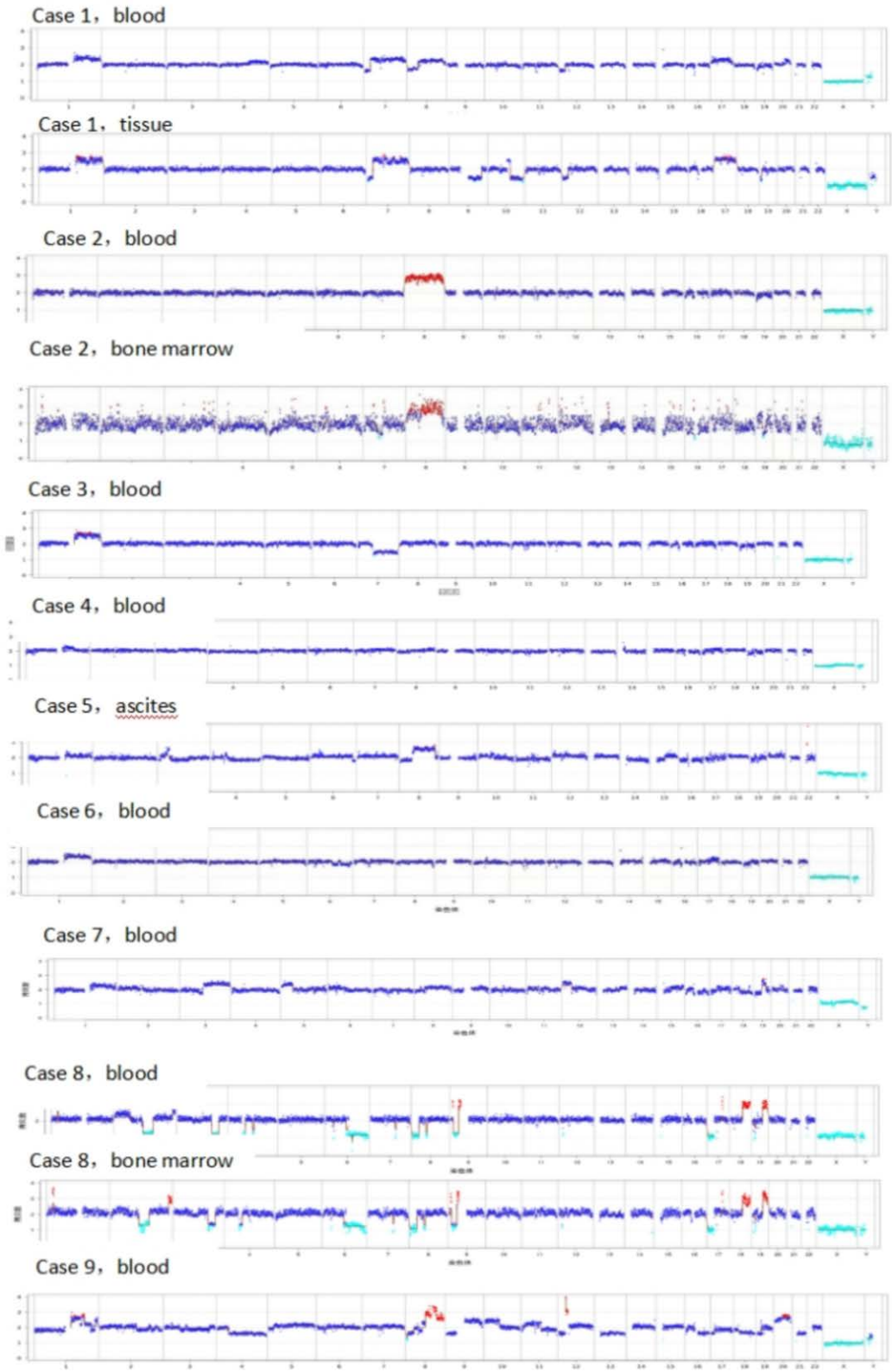

Figure 1. CNV signals of all patients. (1) For each case, the horizontal axis represents the chromosome number, and the vertical axis represents the chromosome copy number; (2) light blue represents a chromosome copy number <1.3, red represents a chromosome copy number >2.7, blue represents a chromosome copy number ranging from 1.3 to 2.7; (3) for each case, the biological specimen harboring copy number variation (CNV) abnormalities are specified. CNV abnormalities include gains/losses of chromosomal segments or whole-chromosome aneuploidies, with genomic coordinates detailed in the main analysis. Specimen types: peripheral blood (blood), solid tissue (tissue), bone marrow aspirate (bone marrow), and abdominal fluid (ascites). Cases 1, 2, and 8 show multi-source abnormalities, while other cases demonstrate abnormalities in a single specimen type. CNV = copy number variation.

Table 2

The CNV location of each case.

Case no	Specimen type	The position of CNV
1	Blood	+17(mos),XY,+Y(mos),1q21.1-q44(dup[mos]_105.25Mb),7p22.3-p21.1(del[mos]_20.3Mb),7p21.1-p11.1(dup [mos]_37.8Mb),7q11.21-q36.3(dup[mos]_96.2Mb),8p23.3-p11.22(del[mos]_39.1Mb),8q11.1-q24.3(dup [mos]_99.5Mb),12p13.33-p12.2(del[mos]_21.3Mb),19p13.3-p11(del[mos]_24.5Mb),20q11.1-q13.33(dup[mos]_33.6Mb)
	Tissue	+17(mos),XY,+Y(mos),1q21.2-q44(dup[mos]_99.55Mb),7p22.3-p21.1(del[mos]_20.3Mb),7p21.1-p11.1(dup[mos]_37.7Mb),7q11.21-q36.3(dup[mos]_94.6Mb),9q13-q33.2(del[mos]_56.5Mb),10q11.1-q23.1(dup[mos]_42.1Mb),10q23.1-q26.3(del[mos]_51.1Mb),12p13.33-p12.2(del[mos]_21.3Mb)
2	Blood	+8(mos)
	Bone marrow	+8(mos)
3	Blood	1q21.1-q44(dup[mos]_103.65Mb),7q11.1-q36.3(del[mos]_97.6Mb)
4	Blood	1q, 3q, 7q, 8q
5	Ascites	+12(mos),−14(mos), −16(mos),+20(mos),3p26.3-p22.1(dup[mos]_41.0Mb), 4q11-q35.2(del[mos]_138.4Mb), 8p23.3-p11.1(del[mos]_43.7Mb), 8q11.1-q24.22(dup[mos]_89.5Mb)
6	Blood	1q21.1-q44(dup[mos]_105.25Mb)
7	Blood	XY,−Y(mos),3q11.1-q29(dup[mos]_104.4Mb),5p15.33-p12(dup[mos]_45.1Mb),12p13.33-p11.1(dup[mos]_34.4Mb),19p13.3-p11(del[mos]_24.5Mb), 19q11-q13.2(dup[mos]_14.9Mb)
8	Blood	6q15-q21(del_14.5Mb), 6q21-q27(del_64.3Mb),8p23.3-p21.2(del_26.6Mb),17p13.3-p11.1(del_22.3Mb),18q21.1-q23(dup_30.68Mb),19q13.2-q13.43(dup_18.2Mb),2p25.3-p16.1(dup[mos]_60.6Mb),2q13-q23.3(del[mos]_41.7Mb),2q37.1-q37.3(dup[mos]_11.6Mb),3q22.3-q26.1(del[mos]_29.2Mb),9p24.1-p21.1(del[mos]_21.8Mb),4q11-q13.1(del[mos]_13.0Mb)
	Bone marrow	2q37.1-q37.3(dup_11.6Mb),8p23.3-p21.2(del_26.6Mb),17p13.3-p11.1(del_22.3Mb),18q21.1-q23(dup_30.68Mb),19q13.2-q13.43(dup_18.3Mb),2q13-q23.3(del[mos]_41.6Mb),3q22.3-q26.1(del[mos]_29.3Mb),6q15-q27(del[mos]_79.6Mb),9p24.1-p21.1(del[mos]_21.8Mb),15q11.1-q13.1(del[mos]_10.3Mb),4q11-q13.1(del[mos]_13.0Mb)
9	Blood	−13(mos),−17(mos),−18(mos),+20(mos),−21(mos),XY,+Y(mos),8q13.3-q24.3(dup_73.0Mb),1p36.33-p11.2(del [mos]_120.8Mb),1q21.1-q25.3(dup[mos]_35.9Mb),1q31.1-q31.3(dup[mos]_10.6Mb),1q31.3-q41(dup[mos]_24.1Mb),1q43-q44(dup [mos]_10.25Mb),4q11-q35.2(del[mos]_138.2Mb),5q11.1-q35.3(dup[mos]_131.4Mb),8p23.2-p21.1(del[mos]_ 24.4Mb),9p24.3-p13.1(del[mos]_38.7Mb),9q13-q34.3(dup[mos]_74.6Mb),10p15.3-p11.1(dup[mos]_38.5Mb),10q11.1-q21.1(dup [mos]_14.4Mb),11p15.5-p11.12(dup[mos]_51.5Mb),11q11-q13.5(dup[mos]_20.7Mb),11q13.5-q25(del[mos]_ 59.2Mb),12p13.33-p12.1(del[mos]_24.6Mb),16q11.2-q24.3(del[mos]_43.9Mb),19p13.3-p13.12(del[mos]_14.0Mb)

CNV = copy number variation.

3.4. Comparison of the positive rate of CNV-based mNGS in patients with or without tumor disease

For patients with and without tumor, the positive rate of CNV-based mNGS was respectively 100% and 60%, and the difference was not statistically significant ($P = .444$).

3.5. Cases in which CNV-mNGS yielded a malignant diagnosis

3.5.1. Case 1. A 35-year-old man was admitted to hospital for fever with swelling and pain of left neck and maxillofacial region for 2 months. At first, the patient had nasal obstruction, runny nose, pharyngeal discomfort, and mild cough. The surface of the maxillofacial region was red, swollen and had tenderness. Moreover, swelling ranges tended to expand. During the first hospitalization, methylprednisolone (40 mg/d) was rendered, the facial swelling and pain basically disappeared, but 3 days after discharge, the patient had fever, maxillofacial swelling and pain again, so he was admitted to another hospital. The interferon release test of mycobacterium tuberculosis was positive, so he was given regular antituberculosis treatment for 1 month, however, the treatment response was not significant. He was admitted to our hospital on July 24.

Based on moderately elevated white blood cells (WBCs), inflammation biomarkers and maxillofacial infection indicated by MRI and multiple pulmonary infection indicated by chest computed tomography (CT), the patient was rendered anti-infective therapy. The specific anti-infective program was moxifloxacin (0.4/day) for 5 days, and vancomycin (2.0 g/d) for 4 days. However, the patient's symptoms and signs did not improve. The anti-infection regimen was adjusted to venous levofloxacin (0.5/day, 8 days), linezolid (1.2/day, 8 days), amikacin (0.6/day, 4 days), and isoniazid (0.6/day, 8 days) without obvious therapeutic effect. Maxillofacial swelling may be caused by Nocardia infection, so sulfamethoxazole (6 tables/day) was added. Unfortunately, all anti-infective treatments were ineffective. After multidisciplinary conference, rare and special pathogen infection was still considered, but the patient's blood mNGS suggested tumor clues, so reactive enlarged cervical lymph nodes was biopsied. Pathological result of lymph nodes was suggestive of natural killer T cell lymphoma. After a course of chemotherapy, the patient's body temperature returned to normal, the maxillofacial swelling subsided, and the lung lesions were basically absorbed.

3.5.2. Case 2. A 66-year-old patient presented with recurrent fever over the past 6 months. His past history included left clavicle fracture with internal fixation, anemia and duodenal ulcer. His temperature ranged from 37℃ to 38.5℃, with normal ranges of pulse rate and blood pressure. Physical examination showed conjunctiva pale. Cardiopulmonary and abdominal examination showed no abnormalities. On admission blood routine test showed a WBC count of 12.75×10^9/L, absolute value of lymphocyte of 1.06×10^9/L, absolute value of neutrophil of 8.47×10^9/L, the percent of neutrophile granulocyte of 66.5%, monocyte percentage 24.4%, monocyte absolute value 3.11×10^9/L, hemoglobin level of 70 g/L, platelet count of 61×10^9/L. Liver and kidney function are normal. Inflammatory indicators showed C-reactive protein of 113 mg/L, and procalcitonin of 13.04 ng/mL. Serum 1,3-β-D-glucan and galactomannan level was negative. DNA quantification of Epstein–Barr virus (EBV) was normal. The multiple blood cultures for both fungus and bacteria were negative. Abdominal ultrasound showed enlargement of the liver and spleen. Chest CT revealed slight chronic inflammation and mild bronchiectasis in both lungs. Positron emission tomography-CT scan showed the bone marrow metabolism

Table 3

Inflammatory markers and microbiological results of 3 patients with suspected infection.

No	WBC (×10⁹/L)	PCT (ng/mL)	CRP (mg/L)	Microbiology	mNGS result
1	2.93	0.06	44.5	N	EBV
2	12.75	13.04	113	N	Stenostomonas maltophilia and EBV
8	9.69	3.18	164.7	N	Negative

CRP = C-reactive protein, EBV = Epstein–Barr virus, mNGS = metagenomics Next Generation Sequencing, PCT = procalcitonin, WBC = white blood cell.

was diffusely active in the axial bones and the 4 limbs, and the metabolism of multiple small lymph nodes in the right lower abdominal mesentery, bilateral neck and mediastinum were mildly active. Bone marrow mNGS result was negative, but indicated presence of neoplasm. A bone marrow smear revealed secondary anemia with thrombocytopenia. Bone marrow pathology revealed hyperactive hyperplasia and did not indicate tumor. Blood mNGS identified 6 sequencing reads of stenostomonas maltophilia and 8 sequencing reads of EBV. After 7 days of moxifloxacin treatment, the patient's symptoms did not improve. The patient still had high fever (39.8℃) with chills. Reexamined blood mNGS did not detect stenostomonas maltophilia, but 7 sequencing reads of EBV still existed. According to the mNGS result, the treatment regimen was adjusted to intravenous levofloxacin for 7 days, vancomycin for 5 days and oral sulfamethoxazole trimethoprim for 7 days and venous immunoglobulin for 3 days. One day later, the patient's temperature returned to normal. However, after discharge, the patient suffered from recurrent fever and then was admitted to the department of hematology. Blood routine test showed a WBC count of 13.31×10^9/L, absolute value of lymphocyte of 1.25×10^9/L, absolute value of neutrophil of 8.78×10^9/L, the percent of neutrophile granulocyte of 65.9%, monocyte percentage 23.6%, monocyte absolute value 3.14×10^9/L, hemoglobin level of 72 g/L, platelet count of 63×10^9/L. procalcitonin was 1.07 ng/mL. After admission, cefoperazone and sulbactam were given for anti-infection treatment. However, the patient had recurrent fever during hospitalization, the anti-infection regimen was changed to linezolid and imipenem cilastatin sodium. The morphological, pathological and molecular findings of bone marrow were suggestive of chronic mononuclear leukemia. Hence, chemotherapy with cytarabine was started. Since then, the patient had no fever.

3.5.3. Case 8. A 71-year-old man was admitted to hospital due to cough for 1 week and fever for 4 days. Other accompanying symptoms included coughing yellow sticky sputum, chills and fatigue. Maximum body temperature was 40℃, with nausea and vomiting. Previous medical history included 10 years of hypertension, 7 years of diabetes, and 7 years of multiple myeloma. The patient had recurrent fever, hemoptysis, and chest CT suggested that the pulmonary infection was aggravated. Successive anti-infective treatment with moxifloxacin, meropenem, caspofungin, sulfamethoxazole were rendered. However, the reexamination of blood routine suggested that WBCs, hemoglobin and platelets had a downward trend, and blood CNV analysis suggested tumor signals, so bone marrow puncture was performed to exclude hematological diseases. Bone marrow smear and molecular biological examination of bone marrow indicated acute lymphoblastic leukemia. After infection was controlled well, unfortunately, the patient suddenly presented with shortness of breath, and his heart rate and blood pressure decreased progressively on the second day of chemotherapy, and finally died. Inflammatory markers and microbial outcomes of the 3 patients were showed (Table 3).

4. Discussion

FUO was ascribed to many factors, such as infection, neoplastic disease, and so on.[9] Patients suspected of infection may also have potential unknown causes, for instance, malignant tumors. Sometimes traditional serological detection methods and imaging techniques are difficult to make timely diagnosis for patients with chronic fever.[10] Conventional microbial tests such as microscopy, culture, and serologic assays, had low sensitivity,[11] narrow detectable bacterial spectrum and time-consuming.[12] Delayed identification of pathogens may lead to treatment failure and further increase drug resistance and medical costs.[13] Delayed diagnosis of tumors can lead to diseaseprogression and affect the prognosis of patients.

To date, many mNGS-related molecular screening methods have been developed.[14] Chromosomal instability can be used to auxiliarily diagnose tumor.[15-17] Genome instability can be as an important tumor marker.[18] mNGS-based CNV analysis can rapidly identify the suspected pathogens and abnormal DNA clones without prior suspicious.[17,19] Moreover, patients do not require additional blood tests and can simultaneously detect tumors and pathogens at acceptable prices. In this study, 3 patients without a history of malignancy were observed with abnormal CNV signals, and ultimately was diagnosed with hematological tumor. Therefore, this technology can provide reliable clues for the diagnosis of patients with unexplained fever, and provide a clear direction for further examination. Some studies found the detection of chromosomal abnormalities usually suggested an active or recurrent state of tumor.[20] In this study, 4 patients with a history of malignancy were in an active state, and their tumor signals were positive by CNV analysis.

For patients with suspected infection whose tumors cannot be completely ruled out, CNV analysis can be a significant auxiliary diagnostic tool for clinicians, and missed diagnosis of underlying neoplastic disease can be avoided. A clinical challenge of mNGS or mNGS-based CNV analysis is whether the detected microorganism sequences or tumor signals is of clinical significance. The results need to be reevaluated basing on the clinical manifestations, routine examination results and follow-up results of the patients.

This study has several limits: first, due to the small sample size, the predictive value of CNV analysis for tumor was difficult to determine; second, positive tumor signal can not identify the primary elocation of the tumor.

In summary, this study suggests the novel value of mNGS in the diagnosis of malignancy. In clinical cases with suspected infection, the improving availability of mNGS can simultaneously identify more possible microorganism and neoplasm DNA in blood or other samples.

Author contributions

Conceptualization: Haiyan Zhang.
Data curation: Haiyan Zhang.
Formal analysis: Haiyan Zhang.
Methodology: Haiyan Zhang.

References

[1] Wright WF, Auwaerter PG. Fever and fever of unknown origin: review, recent advances, and lingering dogma. Open Forum Infect. Dis. 2020;7:ofaa132.

[2] Zheng Y, Qiu X, Wang T, Zhang J. The diagnostic value of metagenomic next-generation sequencing in lower respiratory tract infection. Front Cell Infect Microbiol. 2021;11:694756.

[3] Xu JF, Kang Q, Ma XY, et al. A novel method to detect early colorectal cancer based o n chromosome copy number variation in plasma. Cell Physiol Biochem. 2018;45:1444–54.

[4] Kumaran M, Cass CE, Graham K, et al. Germline copy number variations are associated with breast cancer risk and prognosis. Sci Rep. 2017;7:14621.

[5] Lenaerts L, Vandenberghe P, Brison N, et al. Genomewide copy number alteration screening of circulating plasma DNA: potential for the detection of incipient tumors. Ann Oncol. 2019;30:85–95.

[6] Guo Y, Li H, Chen H, et al. Metagenomic next-generation sequencing to identify pathogens and cancer in lung biopsy tissue. EBioMedicine. 2021;73:103639.

[7] Fromer M, Moran JL, Chambert K, et al. Discovery and statistical genotyping of copy-number variation from whole-exome sequencing depth. Am J Hum Genet. 2012;91:597–607.

[8] Backenroth D, Homsy J, Murillo LR, et al. CANOES: detecting rare copy nu mber variants from whole exome sequencing data. Nucleic Acids Res. 2014;42:e97.

[9] Fusco FM, Pisapia R, Nardiello S, Cicala SD, Gaeta GB, Brancaccio G. Fever of unknown origin (FUO): which are the factors inflfluencing the final diagnosis? A 2005-2015 systematic review. BMC Infect Dis. 2019;19:653.

[10] Wright WF, Mulders-Manders CM, Auwaerter PG, Bleeker-Rovers CP. Fever of unknown origin (FUO) - a call for new research standards and updated clinical management. Am J Med. 2022;135:173–8.

[11] Gonzalez MD, Chao T, Pettengill MA. Modern blood culture: management decisions and method options. Clin Lab Med. 2020;40:379–92.

[12] Álvarez Artero E, Campo Núñez A, Garcia Bravo M, García García I, Belhassen Garcia M, Pardo Lledías J. Utility of the blood culture in infection of the urinary tract with fever in the elderly. Rev Esp Quimioter. 2021;34:249–53.

[13] Miao Q, Ma Y, Wang Q, et al. Microbiological diagnostic performance of metagenomic next-generation sequencing when applied to clinical Practice. Clin Infect Dis 2018;67:S231–40.

[14] Kim ST, Lee J, Hong M, et al. The NEXT-1 (Next generation pErsonalized tX with mulTi-omics and preclinical model) trial: prospective molecular screening trial of metastatic solid cancer patients, a feasibility analysis. Oncotarget. 2015;6:33358–68.

[15] Watkins TBK, Lim EL, Petkovic M, et al. Pervasive chromosomal instability and karyotype order in tumour evolution. Nature. 2020;587:126–32.

[16] Bolhaqueiro ACF, Ponsioen B, Bakker B, et al. Ongoing chromosomal instability and karyotype evolution in human colorectal cancer organoids. Nat Genet. 2019;51:824–34.

[17] Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. Proc Natl Acad Sci U S A. 2008;105:16266–71.

[18] Lau TY, Poon Randy YC. Whole-genome duplication and genome instability in cancer cells: double the trouble. Int J Mol Sci . 2023;24:3733.

[19] Gu W, Talevich E, Hsu E, et al. Detection of cryptogenic malignancies from metagenomic whole genome sequencing of body fluids. Genome Med. 2021;13:98.

[20] Su J, Han X, Xu X, et al. Simultaneous detection of pathogens and tumors in patients with suspected infections by next generation sequencing. Front Cell Infect Microbiol. 2022;12:892087.