TITLE PAGE:

**Title:** Early hint of mNGS and CNVs, an occult case of leptomeningeal metastasis with rapid cognitive decline

**Authors and Affiliation:**

Xueqin Chen, MM1, Haotao Zheng, MD2, Taoli Wang, MD2, Ziyang Feng, MM1, Jia Wang, MM1, Yangsicheng Liu, BM1, Wenxin Qin, BM1, Xiude Qin, MD2, Fanxin Kong, MD2\*.

1. The forth Clinical Medical College of Guangzhou University of Chinese Medicine. No.1, Fuhua Road, Futian District, Shenzhen, Guangdong, China, 518033.

2. Shenzhen Traditional Chinese Medicine Hospital. No.1, Fuhua Road, Futian District, Shenzhen, Guangdong, China, 518033.

**\*Corresponding Author:** Fanxin Kong

**Address**: No.1, Fuhua Road, Futian District, Shenzhen, Guangdong, China, 518033.

**E-mail**: kfx1662@guzcm.edu.cn

# Early hint of mNGS and CNVs, an occult case of leptomeningeal metastasis with rapid cognitive decline

# **Key clinical message**

# We reported a case of lung adenocarcinoma with EGFR L858R mutation complained of rapid cognitive decline, whose imaging showed interstitial brain edema. Under the circumstances of negative cerebrospinal fluid cytology, metagenome next-generation sequencing and copy-number variations analysis were applied, which indicated leptomeningeal metastasis and was confirmed in the subsequent cytology.

# **Key words:** Leptomeningeal metastasis, Copy-number variations, Metagenome next-generation sequencing, Case report.

**Introduction**

# Leptomeningeal metastasis (LM), as a devastating complication of metastatic cancer, was characterized by the dissemination of tumor cells throughout subarachnoid space and leptomeninges[1]. Up to 10% of individuals diagnosed with EGFR-positive non-small cell lung cancer (NSCLC) may experience LM, whose median overall survival is only 4-6 weeks if untreated after diagnosis[2]. Early identification and intervention were critical for improving the prognosis of LM. Cerebrospinal fluid (CSF) cytology of tumor cells is still the golden standard for diagnosing LM with higher specificity compared with the hints of neuroimaging and neurological symptoms. However, early diagnosis of LM remains challenging given the limited sensitivity of CSF cytology that may require multiple samples and repeat lumbar punctures, as well as heterogeneous signs and symptoms at presentation. Therefore, exploring more sensitive diagnostic technologies is a potential research direction for LM.

# Here, we introduced the integration of metagenome next-generation sequencing (mNGS) and copy-number variations (CNVs) analysis as a diagnostic tool for early-stage LM.

# Case presentation

# A 54-year-old male was referred to the encephalopathy department from the oncology department due to his complaint of rapid cognitive decline. The patient was first diagnosed with lung adenocarcinoma with EGFR L858R mutation in September 2020, with metastasis to the pleura, contralateral lung, and bones. He was initially treated with Osimertinib, a third-generation EGFR tyrosine kinase inhibitor (TKI), which produced a positive biochemical response and clinical improvement. Gradually, with resistance to TKIs and disease progress according to the evaluation of positron emission tomography/computed tomography (PET/CT) scan results, several therapies were adjusted.

# Most recently, one week after receiving Bevacizumab and Pemetrexed Disodium, the patient developed asthenia and dizziness and was admitted to the oncology department after the symptoms had persisted for a week. Following admission, contrast-enhanced CT of the chest was assessed as continued stable disease (Fig. 1). Besides, electromyography revealed peripheral nerve injury, especially in his lower limbs, which was considered to be related to the previous chemotherapy. However, abrupt rapid cognitive decline prompted the patient's referral to the encephalopathy unit on 2 August 2023.

On initial exam, the patient couldn't recognize his family. The mini-mental state examination (MMSE) was 13. Unsteady gait and dysmetria on the finger-to-nose test were observed. Except for positive Romberg tests, his cranial nerve examination exhibited intact, as were his strength, sensation, meningeal irritation signs, and reflexes throughout his extremities. Furthermore, enhanced cranial magnetic resonance imaging (MRI) revealed interstitial brain edema (Fig. 2). CSF was also examined and a pressure of 220 mm H2O was recorded. CSF analysis showed normal protein (196.0 mg/dL), decreased glucose (2.70 mmol/L), and lactate dehydrogenase(4U/L). Additionally, CSF culture, smears of Cryptococcus neoformans and acid-fast bacilli, immunoglobulin M for Toxoplasma, Rubella, Cytomegalovirus, Herpes Simplex Virus, and syphilis were all negative. CSF cytology discovered only a few lymphocytes.

Mannitol and hypertonic haline therapies were applied to release brain edema and reduce intracranial pressure, but little efficacy was achieved. More examinations were conducted to figure out the etiology. On 8 August, CNVs of CSF discovered 9 chromosomal abnormalities (Fig. 3), which indicated central nervous system (CNS) tumors. Besides, one sequence of Cytomegalovirus and Epstein-Barr virus were found by mNGS of CSF. Based on the indication of mNGS and CNVs analysis, a repeat cytological examination of CSF was conducted, where tumor cells were discovered (Fig. 4). The patient was eventually diagnosed with LM and referred to the oncology department for intravenous Bevacizumab (15 mg/kg, q21d) and Osimertinib (160 mg, qd), since he couldn't withstand the potential side effects of intrathecal therapy. When being discharged from the hospital, the patient's cognitive function improved significantly, but other neurological examinations did not reveal obvious improvement, probably due to the blood-brain barrier reducing Bevacizumab and Osimertinib's concentration.

# Discussion

Parenchymal brain metastases and leptomeningeal metastases are the most prevalent adult intracranial malignancies, with increasing incidence on account of innovation and advancement in early identification. LM may occur in various advanced cancer patients, most of which arise from breast carcinoma, lung cancer, and lymphoma[3]. Due to the restrictions created by the blood-brain barrier, both cancerous cells and most therapeutic agents are prevented from accessing the CNS. Besides, significantly hypoxic CSF with low concentrations of micronutrients obstructs tumor cells from traveling through the leptomeningeal[4]. Despite the harsh microenvironments in CSF, aggressive tumor cells survive by competing for iron with macrophages[5], and spread to leptomeningeal via direct extension from brain metastases, hematogenous or lymphatic dispersion, as well as endoneural and perineural diffusion[6].

The diagnosis of LM is based on comprehensive assessments of clinical manifestations, imaging, and CSF cytology. Neurological symptoms at the time of brain metastasis diagnosis are a strong, independent predictor of survival time[7]. However, patients with LM have diverse symptoms that reflect different sites invaded by the tumor cells involving neuroaxial involvement, cerebral, cranial nerve, and spinal complaints. Heterogeneous clinical manifestations of LM pose challenges to clinicians, such as headache, ensuing nausea and vomiting, weakness, rapid weight loss, mental obtundation, excessive sleepiness, and speech disorder[8]. Notably, the early signs of LM can be subtle, and difficult to distinguish from the toxic effects of radiotherapy or chemotherapy used to treat the primary tumor. Therefore, clinicians should be vigilant when receiving patients with suspected LM and conduct careful neurological assessments.

Gadolinium-enhanced MRI, sensitive to minor lesions, edema, and meningeal enhancement, is the best imaging technique to evaluate LM. Characteristic MRI findings that suggest LM are the enhancement of leptomeningeal nodules or cranial nerve root, sulcal and folial obliteration or enhancement, and linear enhancement of ependyma [9]. Nonetheless, a normal or false-negative MRI is present in 20-30% of patients diagnosed with LM [10]. Besides, an MRI should be performed before any irritation to leptomeninges such as lumbar punctures or neurosurgery, for which could induce false-positive enhancement.

Visualization of malignant cells, decreased glucose concentration, increased protein, and lymphocytes are typical CSF findings for LM. However, up to 45% of LM patients tested negative in CSF cytology on the first examination[11]. The sensitivity of CSF cytology results has been demonstrated to increase by withdrawing more than 10.5 mL CSF, processing specimens immediately, obtaining CSF from the most optimal location, and repeating lumbar punctures[12]. Nevertheless, repeated punctures were usually required, which might result in local bleeding or infection, CSF leakage, and hypotensive cranial pressure headache. Our patient's clinical presentations were unique since neither rapid cognitive decline nor interstitial brain edema on imaging are typical of LM. Therefore, LM was not considered at first, especially when the previous CSF cytology was negative. Mannitol and hypertonic haline therapies were applied, aiming to improve the patient’s symptoms, but with little efficacy. MNGS-CNVs brought a turn to our treatment dilemma.

CNVs, as important contributors to genomic alterations, are structurally variant regions characterized by differences in copy numbers across two or more genomes, which confers substantial susceptibility to numerous forms of cancer[13, 14]. Seminal papers have demonstrated that CNVs can be used to predict the presence of tumor cells, or tumor progression, as well as treatment response[15-17]. Peripheral blood is a common detection source for CNVs, but LM lacks detectable CNVs in plasma samples owing to the blood-brain barrier. Instead, CNVs detection of CSF is more sensitive and reliable to LM than plasma[18]. MNGS detects potential pathogens by obtaining genomic information of the sample species through nucleic acid fragments. Currently, appreciation of the role of mNGS in CSF in diagnosing neurological infections has burgeoned[19-21]. The combination of mNGS and CNVs suggested CNS malignancy in the presence of negative CSF flow cytometry or cytology with a sensitivity of 75% (95% CI, 63%-85%) and a specificity of 100% (95% CI, 96%-100%)[22].

In our case, given the patient’s unexplained cognitive impairment and the possibility of false-negative CSF cytology, mNGS-CNVs were applied to analyze CSF for information on pathogenic microorganisms and tumor cell chromosomes ’s CNVs. As a result, 9 variations above 5 Mb were detected in copy number, which indicated chromosome instability, in accordance with the chromosomal features of malignancy. Besides, mNGS detected no high-sequence micro-organisms, providing evidence to rule out infectious meningitis. Furthermore, considering the patient's history of lung cancer, LM was highly suspected, and repeat CSF cytology was performed. Eventually, tumor cells were discovered in the later cytology, confirming the diagnosis of LM and facilitating subsequent treatment. In addition, mNGS-CNVs eliminate the need for preserving cell integrity with lower specimen volume requirements, simultaneously collecting more biological information and providing more diagnostic references for patients to distinguish neuroinfectious diseases and neurotumours with one lumbar puncture. Nonetheless, large clinical trials on the diagnosis of LM using mNGS-CNVs are still lacking. More relative research will be encouraged.

Early screening, identification, and diagnosis were crucial to initiate antitumor therapy and improve the survival rate of LM, especially for cancer patients with sudden onset of unexplained neurological symptoms. As a newly developed technology, mNGS-CNVs may play a significant role in distinguishing LM and intracranial infection. Future studies will be required to determine the efficacy and accuracy of mNGS-CNVs diagnosis of LM.

**Declarations**

**Consent for publication**

The patient provided written informed permission for the publication of this case report and the use of related pictures.

**Availability of data and materials**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Competing interests**

The authors declared no competing interests.

**Funding**

This work was supported by the Traditional Chinese Medicine Bureau of Guangdong Province [grant numbers 20221357]; Sanming Project of Medicine in Shenzhen [grant numbers SZZYSM202111011]; “3030 project” of Clinical Research Program in Shenzhen Traditional Chinese Medicine Hospital in 2021 [grant numbers G3030202132].

**Authors' contributions**

XC and FK conceived and designed the study. HZ and TW treated the patient in the department of encephalopathy and oncology respectively, and collected medical history data.YL, WQ, XQ: analyze the original materials and data. The first draft of the manuscript was written by XC, then ZF and JW revised the manuscript for significant intellectual content. Every author commented on previous versions of the manuscript and devoted to the final draft. All authors read and approved the final manuscript.

**Acknowledgments**

We thank all the participants and researchers who devoted to this study. We thank Dr. Huacheng Wang (Clinic neuroscience center, The Seventh Affiliated Hospital, Sun Yat-Sen University) for helping with CSF cytology.

**Abbreviations:**

Leptomeningeal metastasis: LM; non-small cell lung cancer: NSCLC; Cerebrospinal fluid: CSF; metagenome next-generation sequencing: mNGS; copy-number variations: CNVs; tyrosine kinase inhibitors: TKIs; positron emission tomography/computed tomography: PET/CT; mini-mental state examination: MMSE; magnetic resonance imaging: MRI; central nervous system: CNS.

**References:**

1. Le Rhun E, Weller M, Brandsma D, Van den Bent M, de Azambuja E, Henriksson R, Boulanger T, Peters S, Watts C, Wick W et al: EANO-ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up of patients with leptomeningeal metastasis from solid tumours. ANN ONCOL 2017, 28(suppl\_4):iv84-iv99.

2. Grossman SA, Krabak MJ: Leptomeningeal carcinomatosis. CANCER TREAT REV 1999, 25(2):103-119.

3. Clarke JL, Perez HR, Jacks LM, Panageas KS, Deangelis LM: Leptomeningeal metastases in the MRI era. NEUROLOGY 2010, 74(18):1449-1454.

4. Li Q, Lin Z, Hong Y, Fu Y, Chen Y, Liu T, Zheng Y, Tian J, Liu C, Pu W et al: Brain parenchymal and leptomeningeal metastasis in non-small cell lung cancer. SCI REP-UK 2022, 12(1):22372.

5. Chi Y, Remsik J, Kiseliovas V, Derderian C, Sener U, Alghader M, Saadeh F, Nikishina K, Bale T, Iacobuzio-Donahue C et al: Cancer cells deploy lipocalin-2 to collect limiting iron in leptomeningeal metastasis. SCIENCE 2020, 369(6501):276-282.

6. Cheng H, Perez-Soler R: Leptomeningeal metastases in non-small-cell lung cancer. LANCET ONCOL 2018, 19(1):e43-e55.

7. Steindl A, Yadavalli S, Gruber KA, Seiwald M, Gatterbauer B, Dieckmann K, Frischer JM, Klikovits T, Zochbauer-Muller S, Grisold A et al: Neurological symptom burden impacts survival prognosis in patients with newly diagnosed non-small cell lung cancer brain metastases. CANCER-AM CANCER SOC 2020, 126(19):4341-4352.

8. Pan Z, Yang G, He H, Yuan T, Wang Y, Li Y, Shi W, Gao P, Dong L, Zhao G: Leptomeningeal metastasis from solid tumors: clinical features and its diagnostic implication. SCI REP-UK 2018, 8(1):10445.

9. Le Rhun E, Devos P, Weller J, Seystahl K, Mo F, Compter A, Berghoff AS, Jongen J, Wolpert F, Ruda R et al: Prognostic validation and clinical implications of the EANO ESMO classification of leptomeningeal metastasis from solid tumors. NEURO-ONCOLOGY 2021, 23(7):1100-1112.

10. Hyun JW, Jeong IH, Joung A, Cho HJ, Kim SH, Kim HJ: Leptomeningeal metastasis: Clinical experience of 519 cases. EUR J CANCER 2016, 56:107-114.

11. Le Rhun E, Massin F, Tu Q, Bonneterre J, Bittencourt MC, Faure GC: Development of a new method for identification and quantification in cerebrospinal fluid of malignant cells from breast carcinoma leptomeningeal metastasis. BMC Clin Pathol 2012, 12:21.

12. Glantz MJ, Cole BF, Glantz LK, Cobb J, Mills P, Lekos A, Walters BC, Recht LD: Cerebrospinal fluid cytology in patients with cancer: minimizing false-negative results. CANCER-AM CANCER SOC 1998, 82(4):733-739.

13. Shlien A, Malkin D: Copy number variations and cancer. GENOME MED 2009, 1(6):62.

14. Dear PH: Copy-number variation: the end of the human genome? TRENDS BIOTECHNOL 2009, 27(8):448-454.

15. Bowcock AM: Invited review DNA copy number changes as diagnostic tools for lung cancer. THORAX 2014, 69(5):495-496.

16. van Boerdonk RA, Daniels JM, Snijders PJ, Grunberg K, Thunnissen E, van de Wiel MA, Ylstra B, Postmus PE, Meijer CJ, Meijer GA et al: DNA copy number aberrations in endobronchial lesions: a validated predictor for cancer. THORAX 2014, 69(5):451-457.

17. van Boerdonk RA, Sutedja TG, Snijders PJ, Reinen E, Wilting SM, van de Wiel MA, Thunnissen FE, Duin S, Kooi C, Ylstra B et al: DNA copy number alterations in endobronchial squamous metaplastic lesions predict lung cancer. AM J RESP CRIT CARE 2011, 184(8):948-956.

18. Pan C, Diplas BH, Chen X, Wu Y, Xiao X, Jiang L, Geng Y, Xu C, Sun Y, Zhang P et al: Molecular profiling of tumors of the brainstem by sequencing of CSF-derived circulating tumor DNA. ACTA NEUROPATHOL 2019, 137(2):297-306.

19. Piantadosi A, Mukerji SS, Ye S, Leone MJ, Freimark LM, Park D, Adams G, Lemieux J, Kanjilal S, Solomon IH et al: Enhanced Virus Detection and Metagenomic Sequencing in Patients with Meningitis and Encephalitis. MBIO 2021, 12(4):e114321.

20. Ramachandran PS, Wilson MR: Metagenomics for neurological infections - expanding our imagination. NAT REV NEUROL 2020, 16(10):547-556.

21. Xing XW, Zhang JT, Ma YB, He MW, Yao GE, Wang W, Qi XK, Chen XY, Wu L, Wang XL et al: Metagenomic Next-Generation Sequencing for Diagnosis of Infectious Encephalitis and Meningitis: A Large, Prospective Case Series of 213 Patients. FRONT CELL INFECT MI 2020, 10:88.

22. Gu W, Rauschecker AM, Hsu E, Zorn KC, Sucu Y, Federman S, Gopez A, Arevalo S, Sample HA, Talevich E et al: Detection of Neoplasms by Metagenomic Next-Generation Sequencing of Cerebrospinal Fluid. JAMA NEUROL 2021, 78(11):1355-1366.

**Figure legends**

**Fig. 1.** Chest scans of mediastinal window (A) and lung window(B). A lobulated massy shadow measuring 51mm×43mm×48mm of uneven density with patchy calcification and burr edges was observed in the upper lobe of the left lung. The left pleura is thickened and stretched, along with the presence of a pleural effusion on the same side.

**Fig. 2.** A. T1-weighted images (T1WI) showed mild dilatation of the ventricular system of the brain and decreased signal changes in the surrounding cerebral white matter. B. T2-weighted imaging (T2WI) represented increased signal. Fluid attenuation inversion recovery (FLAIR) manifested patchy enhancement near the bilateral Lateral ventricle (C), while no relative enhancement was found (D).

**Fig. 3.** Image of copy number variation of CSF from Agene (Fuzhou) Genetic Medical Testing Laboratory). The numbers on the horizontal axis of the table represent the chromosome sequence and the vertical axis represents relative copy numbers. Chr1, Chr5, Chr9, Chr12, Chr14, Chr16, Chr18 and Chr20 were observed chromosomal abnormality.

**Fig. 4.** Tumor cells of CSF were irregular, with abundant cytoplasm and uneven staining. Besides, the nuclei are large, deviated, and deeply stained, and the nucleoli and nuclear membranes are relatively clear.