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Case Report

Metagenomic sequencing expedites diagnosis of disseminated BCG in an infant with BRAFV600E mutation

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Abstract

Introduction: Disseminated bacillus Calmette-Guérin (BCG) disease is a rare but serious BCG complication in children. Early diagnosis and timely interventions are essential to improve prognosis. However, its manifestations can closely mimic those of Langerhans cell histiocytosis (LCH), which usually leads to a high rate of misdiagnoses. Herein we report the first case of successful application of biopsy tissue metagenomic next-generation sequencing (mNGS) in the differential diagnosis of disseminated BCG disease and LCH.

Case study: A 5-month-old female infant was transferred to our center for the treatment of paroxysmal cough, intermittent hematochezia and trunk rash. Examination on admission showed moderate anemia, erythropenia, thrombocytopenia and hepatosplenomegaly. The immunohistochemistry of her intestinal biopsy samples showed CD1a (+) and Langerin (+). Genetic testing of both peripheral blood and bone marrow samples suggested BRAFV600E mutation. Hence, she was initially diagnosed with LCH. However, no improvement was observed after a course of systemic chemotherapy. The left axillary lymph node and colonic mucosal biopsy specimens were sent for mNGS which resulted in sequence reads of *Mycobacterium bovis*-BCG. Triple antimycobacterial therapy was started according to the diagnosis.

Results: The diagnosis of this case was corrected as disseminated BCG disease by mNGS. Currently, she is doing well clinically and continues to follow-up at our outpatient clinic.

Conclusions: This case suggests that mNGS is a valuable tool in the differential diagnosis of disseminated BCG disease and LCH, which can improve the early diagnosis rate of disseminated BCG disease.

Key words: BCG; LCH; mNGS; biopsy.

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Introduction

As the only effective vaccine against tuberculosis (TB), Bacillus Calmette-Guerin (BCG) is generally safe and well tolerated, and the most common response to BCG vaccine is regional or sub-clinical [1]. However, some infants may develop severe disseminated BCG disease presenting as generalized lymphadenopathy, skin rash, peripheral cytopenias and hepatosplenomegaly [2]. Early diagnosis and timely interventions are essential to improve prognosis. Notably, the manifestations of disseminated BCG disease can closely mimic those of Langerhans cell histiocytosis (LCH), which often poses a diagnostic challenge to clinicians [3].

Metagenomic next-generation sequencing (mNGS) is emerging as a promising technique for pathogen detection. In this study, we report the first case of successful application of biopsy tissue mNGS to confirm the diagnosis of disseminated BCG disease in an infant with BRAFV600E mutation that was initially highly suspected as LCH.

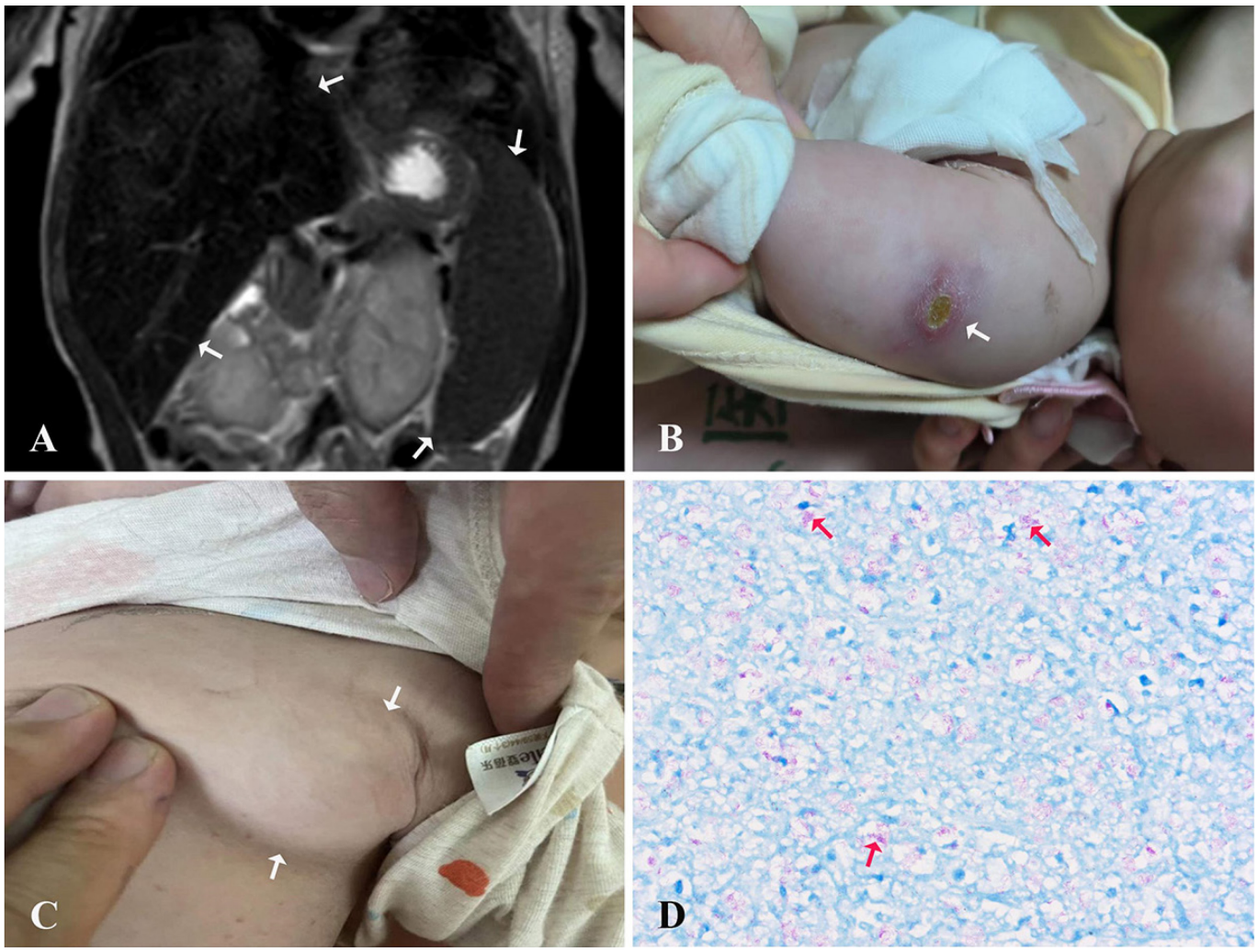
Case report

A 5-month-old female infant was transferred to our children's medical center for the treatment of paroxysmal cough (lasting for one month), intermittent hematochezia (lasting for more than two months) and trunk rash (lasting for more than two months). She was born by normal vaginal delivery without perinatal complications. She received routine bacillus Calmette-Guérin (BCG) vaccination at birth, and there were no reported complications after the vaccination. On physical examination, she was hemodynamically stable, pale, and malnourished. There were scattered pink papules on the neck and trunk. Moderate anemia (Hb = 87 g/L), erythropenia (3.09×10^{12}/L) and thrombocytopenia (126×10^{9}/L) were noted. No abnormalities were found in Coombs' test and the bone marrow smear was unrevealing. Thoracoabdominal

computed tomography (CT) showed slight inflammation in the apical segment of the right upper lobe and in the posterior segment of the right lower lobe with severe hepatosplenomegaly (Figure 1A). Further pathological consultation of her intestinal biopsy samples obtained from colonoscopy examination indicated neutrophil infiltration of colonic mucosa with multiple scattered erosions; the immunohistochemistry showed CD1a (+), CD 68 (+), S-100 (+) and Langerin (+). Genetic testing of both peripheral blood and bone marrow samples suggested BRAFV600E mutation. In view of bicytopenia, skin rashes, hepatosplenomegaly, and immunohistochemical findings of colonoscopy biopsy, as well as BRAFV600E mutation, the infant was diagnosed with LCH. A course of systemic chemotherapy (prednison 40mg/m^2/d for 4 consecutive weeks and vincristine 1.5mg/m^2/w for 6 consecutive weeks) according to CCHG-LCH-2019 [a regimen for childhood LCH in China issued by the Chinese Children's Histiocytic Group (CCHG) in 2019] was decided. Subsequently, she was discharged from hospital and received chemotherapy (oral prednisone and intravenous vincristine) along with other supportive therapies including antibiotic treatment and red blood cell transfusion in a local hospital with plans for close follow-up at our outpatient clinic.

However, no significant improvement in her symptoms including cough, hematochezia and rash along with peripheral cytopenias and hepatosplenomegaly was observed during the first four weeks of chemotherapy, and she developed recurrent fever (maximum body temperature: 38.9 ℃), with a skin ulcer gradually appearing at the vaccination site (0.9 × 0.5 cm; Figure 1B) within the next two weeks.

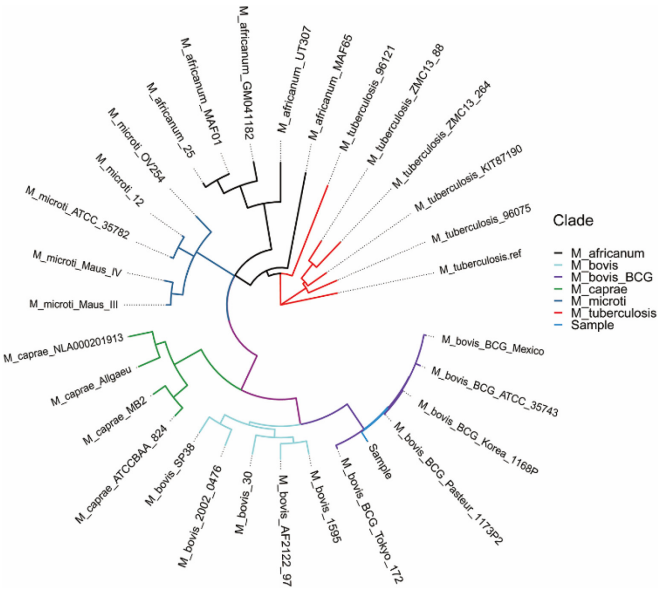
Figure 1. Physical examination and laboratory findings during hospitalization.



A: CT abdomen revealed severe hepatosplenomegaly; **B:** Skin ulcer (0.9×0.5 cm) appeared at the vaccination site during her second hospitalization; **C:** Soft, well-defined, immovable, tender mass (4×2.5 cm) noted in the left axilla during her second hospitalization; **D:** Histopathologic examination of her left axillary lymph node biopsy sample showed acid-fast bacilli (AFB).

Meanwhile, an approximately 4 × 2.5 cm, soft, well-defined, immovable, tender mass in the left axilla, subsequently confirmed as lymph node enlargement by ultrasonography, had developed over the last week of chemotherapy (Figure 1C). As a result, she was hospitalized again. On examination, the infant had tachycardia, tachypnea and pallor. A tissue biopsy was obtained of her left axillary lymph node. Histopathologic examination of her biopsy revealed granulomatous inflammation, and the biopsy was negative for CD1a, CD68, S-100 and Langerin immunohistochemistry, but showed acid-fast bacilli (AFB) positivity (Figure 1D). The presence of BCG scar ulceration and BCG lymphadenitis as well as persistent systemic symptoms raised the suspicion of disseminated *Mycobacterium bovis*-BCG infection. The formalin-fixed paraffin-embedding (FFPE) samples of lymph node and colonic mucosa (obtained from colonoscopy examination during her first hospitalization) were sent to the Cellular and Molecular Diagnostics Center for mNGS, which were both positive in 48 hours for *Mycobacterium tuberculosis* (MTB) complex. The sequencing strategy was SE75 (NextSeq 550, Illumina, San Diego, USA), sequencing of the total reads was 20 million; there were 19795936 clean reads; and 21605 reads were mapped to the MTB complex. To further clarify whether they were *Mycobacterium bovis*-BCG infections, we amplified the genomic DNA of the FFPE samples with the following primers: F-primer: GCGGATGTGGTGCTGGAT; R-primer: GACGGAAACCTTTGCGAAGTC. The amplified products were sequenced by Sanger, and the Sanger sequences were analyzed with Chromas software (Technelysium, South Brisbane, Australia) (Supplementary Figure 1). The read of Sanger sequence was mapped by Basic Local Alignment Search Tool (BLAST) of National Center for Biotechnology Information (NCBI), which strongly suggested *Mycobacterium bovis*-BCG infection. Meanwhile, the library was re-sequenced, and the results were as follows: the sequencing strategy was PE150 (NovaSeq 6000Dx, Illumina, San Diego, USA) a total of 356214504 reads were sequenced; there were 325216492 clean reads; and 476880 reads mapped to the MTB complex. The 476880 reads were then spliced by SPAdes [4]. FASTA files for six mycobacteria were downloaded from the NCBI database. The phylogenetic tree (Figure 2) constructed using IQtree (version 2) software indicated that the infant was infected with *Mycobacterium bovis*-BCG.

Figure 2. Phylogenetic tree constructed using IQtree software indicated that the infant was infected with Mycobacterium bovis-BCG.



The infant was managed with broad-spectrum antibiotics, antituberculosis therapy, and supportive treatment. Her initial antimycobacterial treatment regimen included rifampicin, isoniazid and linezolid. After one week of treatment, her fever, cough and hematochezia were relieved and the surface of the skin ulcer gradually healed, but without significant improvement in other symptoms. Two weeks into treatment, the infant developed severe myelosuppression, prompting discontinuation of antituberculosis therapy, during which time she suffered from severe pulmonary infection with intermittent fever (maximum body temperature: 40.4 °C). Then she was started on intravenous imipenem, amikacin, and teicoplanin combined with blood transfusions and recombinant human granulocyte colony-stimulating factor (rhG-CSF) administration. Two weeks later, her condition improved, and the antituberculosis therapy was restarted: isoniazid was continued, while rifampicin and linezolid were substituted with ethambutol and rifapentine. Meanwhile, given the potential possibilities for malignancies associated with BRAFV600E mutation, she was treated with oral vemurafenib (10 mg/kg twice daily) simultaneously. Moreover, to further investigate the status of infant immunodeficiency, we considered a trio of whole-exome sequencing analyses (including mother, father, and infant) and found some heterozygous genetic variants of unknown clinical significance, including TNFRSF13B (p.Ser144Leu), NOTCH1 (p.Gly1473Ser), MMAA (p.Ile335Val), and

NBEAL2 (c.1113+1G>A). However, no pathogenic genes related to immune deficiency were found.

Results

The diagnosis of this case was ultimately determined as disseminated BCG disease by mNGS. Triple antimycobacterial therapy was started according to the diagnosis. Her condition improved remarkably (pneumonia was under control; rash, axillary lymph node swelling and hepatosplenomegaly subsided gradually; laboratory parameters returned to normal gradually) after four months of antimycobacterial therapy along with oral vemurafenib. Currently, she is doing well clinically and continues to follow-up at our outpatient clinic. The dynamic courses of her laboratory parameters are presented in Table 1.

Discussion

This case report is the first to describe the application of biopsy tissue mNGS in the differential diagnosis of disseminated BCG disease and LCH in children. It highlights the value of mNGS in rapid and accurate diagnosis of disseminated BCG disease and serves as a reference for further utilization of mNGS in the diagnosis of pediatric infectious diseases.

In countries where BCG is routinely administered in infancy, disseminated *Mycobacterium bovis*-BCG infection is commonly seen in children with underlying inborn error of immunity (IEI) like severe combined immunodeficiency (SCID) and chronic granulomatous disease [5]. Wang and colleagues evaluated 22 cases of disseminated BCG disease in China for immunodeficiency, and found that at least 16 of them (72.7%) suffered from definite immunodeficiency simultaneously [6]. Moreover, the disease may occur occasionally in children without significant immunodeficiency, as in our case. Disseminated BCG disease is generally severe, with a reported mortality rate of 70-80% [6]. Most of the cases become symptomatic within 12 months of vaccination, and the mortality rate among children who received the BCG vaccine at < 1-month age ranked the highest [7]. The infant in our study received BCG vaccination at birth and developed progressively aggravating systemic symptoms at approximately 5 months of age. Fortunately, timely correction to the diagnosis and early intervention, with close monitoring for adverse effects, improved her clinical outcome.

The manifestations of disseminated BCG infection depend on the sites of infection and the immunocompetence of the host [8]. Infected children usually fail to gain weight, develop recurrent pneumonias, BCG scar ulceration and bone pain. Peripheral cytopenia, hepatosplenomegaly and skin rash also occur, which can closely mimic the manifestations of LCH. In this case, the infant presented with almost all the above symptoms and immunohistochemistry findings closely related to LCH were also noted in the colonoscopy biopsy samples during her first hospitalization. Hence, she was highly suspected as a case of LCH. LCH is an inflammatory myeloid malignancy with unknown etiology characterized by abnormal proliferation of histiocytic cells [9]. The granulomatous lesions of LCH comprise CD1a (+) and Langerin (+) histiocytes and abundant inflammatory cells, indicating that CD1a and Langerin are potential specific diagnostic markers for LCH [10]. Actually, none of these markers, including CD1a, CD68, S-100 and Langerin, are exclusively specific to LCH, because they are expressed by mononuclear precursors and other derivatives. The diagnosis of LCH requires a combination of clinical presentation, histology, and immunohistochemistry after exclusion of other causes [11]. Besides, simultaneous genetic

Table 1. The dynamic courses of laboratory parameters.

Time points	CRP (mg/L)	PCT (ng/mL)	WBC (10^9/L)	N (%)	RBC (10^{12}/L)	Hb (g/L)	PLT (10^9/L)	RET (%)	Chest CT	Abdominal CT	
										Maximum oblique diameter of the right liver lobe (mm)	Length of the spleen (mm)
First admission	18.2	-	9.36	37.7	3.09	87	126	13.56	Slight inflammation in the apical segment of the right upper lobe and in the posterior segment of the right lower lobe	93	98
First diagnosis	16	0.11	8.13	54.3	3.85	111	376	2.63	The inflammatory lesions were further absorbed than before	97	102
Development of side effects	42.7	1.43	2.47	74.6	3.05	87	62	1.35	Multiple inflammatory lesions in the right lung and the left lower lobe；small amounts of pleural effusion	115	102
Initiation of treatment for the second time	32.2	0.88	4.86	47.5	2.44	70	31	3.18	The inflammatory lesions and pleural effusion were further absorbed than before	113	103
Recovery	< 5	< 0.05	6.28	20.1	4.46	132	257	2.38	No obvious inflammatory lesions were observed	77	94

CRP: C-reactive protein; PCT: procalcitonin; WBC: white blood cell; N: neutrophil; RBC: red blood cell; Hb: hemoglobin; PLT: platelet; RET: reticulocyte; CT: computed tomography.

testing of the infant revealed BRAFV600E mutation, which have further led to her misdiagnosis. BRAF is an important kinase molecule in the RAS-RAF-MAPK signaling pathway, and BRAF mutations have been implicated in the formation of many types of cancer such as thyroid cancer, non-small cell lung cancer, LCH, etc. It has been reported that nearly 50% of LCH patients carry BRAFV600E mutation [12]. Therefore, the infant was initially misdiagnosed as LCH. However, treatment with initial chemotherapy of the CCHG-LCH-2019 regimen was not effective and new symptoms gradually appeared, which prompted further evaluation. Eventually, mNGS on biopsy tissues of left axillary lymph node and colonic mucosa provided the correct diagnosis.

To date, mycobacterial cultures remain the gold standard for tuberculosis diagnosis, however, it is limited by the long duration to a positive result and drug susceptibility testing [13]. MTB complex-specific nucleic acid amplification is also a rapid detection technique in diagnosing various infectious diseases, which can be positive for any species in the MTB complex, thus expediting evaluation and treatment. However, this technique cannot differentiate between MTB, *Mycobacterium bovis* and BCG [14]. In recent years, mNGS has emerged as a novel technique in pathogen detection [15]. The strengths of mNGS include avoidance of invasive diagnostic procedures, simplification of various infectious diseases evaluations, identification of pathogens otherwise undetected by conventional techniques [16], and contribution to the identification of MTB complex strains as in our study. Recently, some case reports have described the application of plasma mNGS in the early diagnosis of BCG-associated infection [17,18]. Given the long-term history of exposure to broad-spectrum antibiotics in our case, we did not perform plasma mNGS due to a high risk of false-negative results, which was the main limitation of our study. In addition to plasma sample, biopsy tissues are also considered as ideal samples for mNGS to detect pathogens [19,20]. However, a positive mNGS result on axillary lymph node alone is not enough to diagnose disseminated BCG disease. In our study, mNGS on biopsy samples of left axillary lymph node and colonic mucosa (evidence of distant site infection) eventually established the correct diagnosis of disseminated *Mycobacterium bovis*-BCG infection. Notably, despite the tremendous potential of mNGS in detecting pathogens, many limitations persist: (1) This expensive technology is not easily accessible in most hospitals; (2) The sensitivity of mNGS needs to be further improved; (3) The result interpretation of mNGS remains a complicated issue due to the double-edged sword of unbiased testing [16]. Currently, most of these limitations mentioned above are continuously improving, which will improve its feasibility in clinical practice. In order to optimize its use and implementation, further studies are still needed, and the collaboration between molecular diagnostic laboratory, bioinformatics, and clinicians should also be strengthened.

The medical management of disseminated BCG disease can be challenging for potential adverse drug events [18]. In our study, the infant developed severe myelosuppression, consistent with toxicity from antimycobacterial agents including rifampicin, isoniazid and linezolid, which prompted further adjustments to the antituberculosis regimens. Thus, both selection of antimycobacterial agents based on susceptibility testing and close monitoring for adverse effects were necessary for successful treatment of disseminated BCG disease.

Conclusions

The clinical manifestations of disseminated BCG disease can closely mimic those of LCH. Clinicians should be alert for the possibility of disseminated BCG disease in infants and children with unexplained infectious symptoms. This case suggests that mNGS is a valuable tool in the differential diagnosis of disseminated BCG disease and LCH, which can improve the early diagnosis rate of disseminated BCG disease. In addition, much research is needed to further identify specific genetic markers of disseminated BCG disease and LCH by analyzing and comparing the genomic profiles of plasma or biopsy tissue samples from these patients.

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Authors' contributions

Que Yang drafted the manuscript. Baojing Wu and Wenxia Wang reviewed and revised the manuscript. Ni Tan and Huarong Huang conceptualized the study and reviewed and revised the manuscript.

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Conflict of interests: No conflict of interests is declared.

Annex – Supplementary Items

Supplementary Figure 1. Result of Sanger sequencing.