Heliyon 10 (2024) e35681

Contents lists available at ScienceDirect

Heliyon

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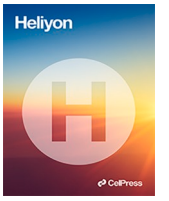

Case report

Multiple lymph nodes enlargement and fever as main manifestations of nocardiosis in immunocompetent individuals: Two case reports

Le Lu [1], Zhiming Zhao [1], Chunli Liu [1], Beibei Zhang, Mengya Fu, Dongyi Wang, Junyi Shen, Hui Cai, Wei Shang [*]

Department of Integrative Medicine, Jinling Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing, 210002, China

ARTICLE INFO

Keywords:
Nocardiosis
FUO
Immunocompetent
Lymph nodes
mNGS
Case report

ABSTRACT

Nocardia farcinica is an aerobic gram-positive bacterium that is pathogenic to humans. It usually causes local and adjacent tissues' diseases at the entry of infection (most commonly occur in the lungs, skin, or central nervous system), which can also spread to other organs through the bloodstream such as joints, kidneys, and liver. However, these infections are often seen as opportunistic that occur in immunocompromised patients. Here, we report for the first time two immunocompetent patients lacking evidence of local infections, with multiple lymph node enlargements and fever as main clinical manifestations, finally diagnosed as nocardiosis by Metagenomic Next-Generation Sequencing testing (mNGS) from formalin-fixed and paraffin-embedded (FFPE) lymph node tissue, after all the other standard tests were negative. Both patients recovered after receiving anti-nocardia therapies. These two cases indicates that in healthy population, there may be more potential nocardia infections than we expected. Multiple lymph node enlargements and fever suggest a possibility of nocardiosis, especially in patients with fever of unknown origin (FUO). mNGS detection from FFPE lymph node tissue is an accurate, reliable and traceable method for diagnosis of nocardiosis.

1. Introduction

Nocardia, a filamentous, gram-positive bacterium, is commonly found in the environment [1]. It causes rare opportunistic infection, usually occur in immunocompromised individuals, such as organ transplant recipients, patients receiving immunosuppressive therapies, patients with human immunodeficiency virus (HIV) infection, and those with hematologic malignancies [2–5]. Much less cases are reported in immunocompetent hosts. Those patients usually suffer from structural lung diseases like bronchiectasis and cystic fibrosis [6–8]. Inhalation of organisms and direct contact through broken skin are most common ways to infect humans, leading to pulmonary, skin and soft tissue infections [9–11]. It also can disseminate to other organs via the bloodstream especially the central nervous system [12]. Poor prognosis is associated with a delay in diagnosis [13].

Due to the subtle and non-specific clinical manifestations, as well as the limitations of the traditional bacterial culture method, early diagnosis of nocardiosis always remains a challenge [12]. Although there are advances in molecular diagnostics, the understanding of

* Corresponding author.
E-mail address: zy_shangwei@163.com (W. Shang).
[1] These authors contribute equally to the manuscript.

https://doi.org/10.1016/j.heliyon.2024.e35681
Received 26 May 2024; Received in revised form 8 July 2024; Accepted 1 August 2024
Available online 6 August 2024

nocardiosis, especially the incidence in general populations is still insufficient. Here we report two previous healthy patients with multiple lymph node enlargement and fever as main manifestations, while lacking local infection manifestations such as pneumonia, skin ulceration and central nervous system infections. Both two patients were initially considered as FUO without an established etiology despite detailed and intensive evaluation, but finally identified as nocardiosis by mNGS from FFPE lymph node tissue. Both patients recovered after receiving anti-nocardia therapies.

2. Case 1

A 49 years old woman was admitted to our hospital on September 2022 for fever and "enlarged lymph nodes" during last 9 days. When admitted, she was suffering from a continues fever at highest 41 °C accompanied with shiver. She was healthy previously and reported no family history. She frequently worked in the fields. On examination, enlarged lymph nodes were touched with tenderness on posterior neck, right armpit, and bilateral inguinal region. Scattered red rash was detected on her skin.

After admission, laboratory tests demonstrated increased erythrocyte sedimentation rate, 90 mm/h (normal value, 20 mm/h), C-reactive protein 187.2 mg/L (normal value, 0–8 mg/L). The full blood count revealed white blood cells at 24.51 × 10^9/L with neutrophil percentage 85.7 %, and hemoglobin 11.4 g/dL. No bacterial growth was observed in blood and sputum cultures. Blood was then collected for mNGS. Epstein-barr (EB) virus were detected. Computed tomography (CT) scan of the chest showed no signs of infection (Table 1). No response to antiviral therapy. Tuberculosis was also suspected. However, anti-tuberculosis treatment was ineffective. PET-CT showed enlarged lymph nodes with increased FDG metabolism of bilateral neck, submandibular, anterior and posterior ear, left occipital posterior, armpit, posterior edge of pectoral muscle, clavicle area, retroperitoneum, pelvic wall, and groin. Diffuse increased FDG metabolism in the spleen and bone marrow was also observed. A biopsy of the lymph node from the left axillary area was then performed. The pathology showed epithelioid granulomatous inflammation, accompanied by multiple focal small pieces of coagulation necrosis with slight proliferation of surrounding lymphoid tissue. Detection of Mycobacterium tuberculosis by PCR-fluorescent probe was negative. The diagnosis tended towards specific infection. After discussion, mNGS was performed using FFPF lymph node samples and *N. farcinica* was detected (Table 1). Trimethoprim-sulfamethoxazole (TMP-SMX) and intravenous amikacin

Table 1
General information and clinical characteristics of two patients with Nocardia.

	Patient 1	Patient 2
Gender	Female	Male
Age (years)	49	65
Comorbidities	None	Hypertension
Active malignancy	None	None
Immunosuppression	None	None
Duration (months)	1	2
Clinical manifestation	Fever, multiple enlarged lymph nodes	Fever, night sweat, multiple enlarged lymph nodes
CT scan of chest and abdomen	No signs of infection	No signs of infection
MRI scan of brain	/	No signs of infection
PET-CT	multiple enlarged lymph nodes with symmetrical distribution (SUVmax = 10.15), increased diffuse FDG metabolism in spleen and bone marrow	multiple lymph nodes with symmetrical distribution, (SUVmax = 7.41)
Leukocyte (× 10^9/L)	24.51	10.07
CRP (mg/L)	187.2	53.5
ESR (mm/h)	90	75
PCT (μg/L)	0.296	0.214
IL-6 (ng/L)	173.4	36.82
Blood and sputum culture	–	–
EB virus	+	+
Ferritin (ng/ml)	257.2	545.5
Histopathology of lymph node	Epithelioid granulomatous inflammation	Reactive hyperplasia of lymph nodes
mNGS from blood	EB virus	/
mNGS from lymph node, number of sequences (abundance)	*N. farcinica* 12108 (39.97 %)	*N. farcinica* 1042 (34.83 %)
Suspected colonization and/or background microorganisms	*Cutibacterium acnes,* *Stenotrophomonas maltophilia,* *Corynebacterium tuberculostearicum,* *Staphylococcus hominis,* *Streptococcus mitis,* *Streptococcus oralis,* *Moraxella pneumoniae,* *Klebsiella pneumoniae,* *Candidia parapsilosis,* *Human herpes virus-7,* *Human herpes virus-4*	*Corynebacterium striatum,* *Acinetobacter baumannii,* *Pseudomonas aeruginosa,* *Stenotrophomonas maltophilia*
Antibiotics	TMP-SMZ, amikacin	TMP-SMZ, amikacin
Outcome	Recovery	Recovery

PCT: Procalcitonin. IL-6: Interleukin-6.

were used for one week. The patient's temperature returned to normal and her symptoms improved significantly. Her TMP-SMX therapy lasted for six months, and has no recurrence since follow-up. No adverse events were observed.

3. Case 2

A 65-year-old previously healthy man was referred to our hospital in March 2024, for a two weeks history of fever and night sweat. He complained of moderate fever (38–39 °C) every night following with night sweats. During the day, his temperature was within normal and had no discomfort. He lost 10 kg of weight before admission. A review of systems was negative for upper respiratory symptoms, cutaneous lesions and urinary tract irritation symptoms. He reported no family history. And he hasn't been injured or traveling recently. On examination, vital signs such as blood pressure, pulse and respiration were normal. Enlarged lymph nodes were reached on both sides of the neck, chin, right armpit, and bilateral inguinal region.

After admission, laboratory tests showed increased erythrocyte sedimentation rate, 75 mm/h (normal value, 20 mm/h), C-reactive protein 53.5 mg/L (normal value, 0–8 mg/L) and ferritin, 545.5 (normal value, 23.9–336.2 ng/ml). Multiple blood and sputum cultures were negative. The full blood count revealed white blood cells at 10.07 × 10^9/L, hemoglobin 12.5 g/dL. An antibody screening was negative. Thyroid function was normal. A tuberculin skin test (Mantoux) was used and the induration was 5 mm (Table 1). He had no response to moxifloxacin.

CT scan of the chest revealed no pneumonia. Brain magnetic resonance imaging showed no infection or abscess. To rule out lymphoma, PET-CT was performed. Increased FDG metabolism in bilateral neck, submandibular, right armpit, right clavicle area, and bilateral inguinal lymph nodes. A biopsy of the lymph node from the right axillary area was then performed. The pathology showed reactive hyperplasia of lymph nodes. Detection of Mycobacterium tuberculosis by PCR-fluorescent probe was negative. mNGS test using lymph node tissue was then considered. *N. farcinica* was detected on the second day (Table 1). Considering the patient's clinical manifestations and the mNGS test results, he was diagnosed with Nocardia infection. Systemic infection was considered as generalized lymph nodes were involved. TMP-SMX tablets and intravenous amikacin were used from March 21st to 30th. Four days after the usage of TMP-SMX, the patient's temperature returned to normal and his symptoms improved significantly. He continued with TMP-SMX therapy and has no recurrence since follow-up. ESR, CRP value and leukocyte counts in blood returned to normal and ferritin decreased significantly after two months (Fig. 1A, B, 1C, 1D). Dizziness after taking TMP-SMX were observed but tolerable.

4. Discussion

As many as 54 species of Nocardia bacteria cause disease in humans [14]. However, the prevalence of different strains varies in different regions. *N. farcinica* is the most common strain in China [15]. Both two cases we reported were infected by *N. farcinica*.

The development of nocardiosis depends on the interplay between host defenses and nocardial infections. Both two patients suffered from chronic fever and no clear infection sites were detected. We speculate that after the initial acute infection at infectious sites, the interplay between the host and bacteria concentrates in the lymph nodes, leading to a chronic state. Therefore, for chronic nocardia patients, bacteriological testing using lymph node tissue becomes a reliable detection method.

Nocardiosis is generally considered to be an opportunistic infection that is common in immunocompromised patients [16]. The onset of Nocardia is insidious, and the systemic symptoms may include fever, night sweats and leukocytosis [5]. The symptoms are

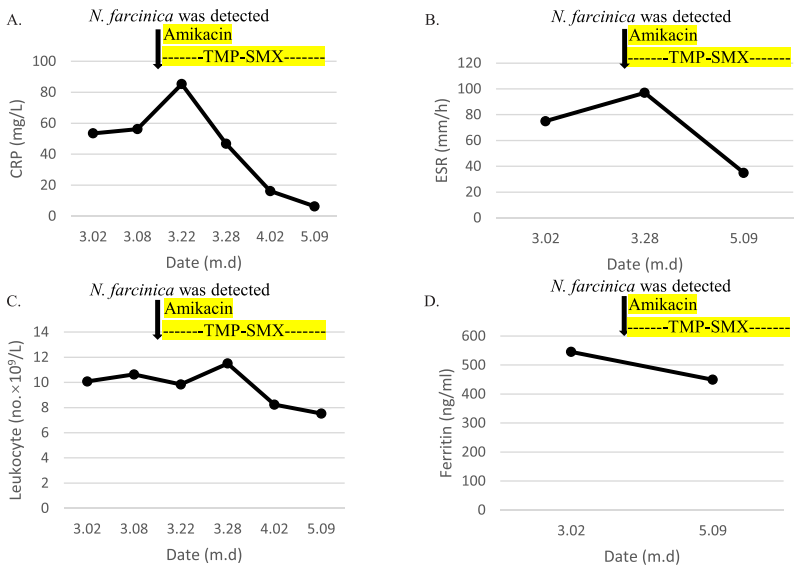

Fig. 1. The values of CRP (A), ESR (B), leukocyte (C) and ferritin (D) before and after the treatment on patient 2 with nocardiosis.

often related to the site of infection [17]. These two patients we reported were immunocompetent. Except for fever and lymph node enlargement, there were no specific clinical manifestations of local infection. This makes our diagnosis extremely difficult to be distinguished from malignant tumors and autoimmune diseases. We initially focused on the possibility of tuberculosis and lymphoma, but neither etiological nor histological examination support those diagnosis. We were also not able to culture the appropriate bacteria with traditional blood or sputum cultures. In one case, mNGS test from blood samples were used, but it also failed to identify the pathogen. As a new method with high sensitivity and specificity, mNGS finally helped us in distinguish this atypical infectious disease. Since we thought about using lymph node tissue for mNGS testing, we've diagnosed two cases of nocardia infection within a short period. This indicates that, insidious infection of Nocardia in immunocompetent individuals may be more than we expected. For immunocompetent patients with fever and multiple lymph nodes enlargement, especially the patients with FUO, norcardiosis needs to be considered and excluded.

Finally, FFPE lymph node samples were used for mNGS screening, and the diagnosis was confirmed in just 1 day. In view of the difficulty of diagnosis and specificity of treatment of nocardiosis, traditional diagnostic methods have limitations. Blood mNGS has the advantage of convenience, speed and accuracy, but in our case, it is still not sensitive enough. Thus, histological mNGS is particularly necessary and important. The reliability of this method was demonstrated by our two consecutive cases of accurate and rapid diagnosis. In addition, a great advantage of histological mNGS is easy to preserve and can be traced at any time. In cases that were not considered of at the time of diagnosis, when the disease is highly suspected, we can still screen the samples at any time.

There are also several limitations in our study. First, the invasiveness of lymph node biopsy limits its use as a first-line diagnostic tool, particularly in patients who are weak or have comorbidities. Second, mNGS also has its limitation. It is costly and will be a burden to some patients. FFPE processing leads to DNA fragmentation and degradation. Thus, the sensitivity and specificity of mNGS on FFPE samples are generally lower compared to fresh or frozen tissue.

5. Conclusion

These two cases indicates that in healthy population, there may be more potential nocardia infections than we expected. For immunocompetent patients with FUO, nocardiosis should also be considered. Multiple lymph nodes enlargement and fever could be a hint for nocardiosis. mNGS detection using FFPE lymph node samples is an accurate, reliable and traceable method for diagnosis of nocardiosis.

Both patients were very satisfied and grateful with the successful diagnosis and treatment of nocardiosis, after suffering from a period of fever and anxiety about failed initial treatment and lack of a clear diagnosis and outcome. Their compliance was quite well that we have maintained follow-up till date.

Disclosure statement

Written informed consent was obtained for anonymized patient information to be published in this article.
Our institution does not require ethics approval for reporting individual cases or case series.
No potential conflict of interest was reported by the authors.

Funding source

This report did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

The data are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Le Lu: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Zhiming Zhao:** Visualization, Validation, Resources, Methodology. **Chunli Liu:** Validation, Resources, Investigation, Formal analysis. **Beibei Zhang:** Methodology, Investigation, Data curation. **Mengya Fu:** Methodology, Investigation, Data curation. **Dongyi Wang:** Methodology, Formal analysis. **Junyi Shen:** Investigation, Data curation. **Hui Cai:** Supervision, Resources, Project administration. **Wei Shang:** Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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