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CASE REPORT

Soft tissue tuberculosis detected by next-generation sequencing: A case report and review of literature

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Abstract

BACKGROUND

Soft tissue tuberculosis is rare and insidious, with most patients presenting with a localized enlarged mass or swelling, which may be factors associated with delayed diagnosis and treatment. In recent years, next-generation sequencing has rapidly evolved and has been successfully applied to numerous areas of basic and clinical research. A literature search revealed that the use of next-generation sequencing in the diagnosis of soft tissue tuberculosis has been rarely reported.

CASE SUMMARY

A 44-year-old man presented with recurrent swelling and ulcers on the left thigh. Magnetic resonance imaging suggested a soft tissue abscess. The lesion was surgically removed and tissue biopsy and culture were performed; however, no organism growth was detected. Finally, Mycobacterium tuberculosis was confirmed as the pathogen responsible for infection through next-generation sequencing analysis of the surgical specimen. The patient received a standardized anti-tuberculosis treatment and showed clinical improvement. We also performed a literature review on soft tissue tuberculosis using studies published in the past 10 years.

CONCLUSION

This case highlights the importance of next-generation sequencing for the early diagnosis of soft tissue tuberculosis, which can provide guidance for clinical treatment and improve prognosis.

Key Words: Mycobacterium tuberculosis; Soft tissue infection; Next-generation sequencing; Extrapulmonary tuberculosis; Diagnosis; Case report

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Core Tip: The diagnosis of extrapulmonary tuberculosis can be challenging, especially for tuberculosis in rare sites such as soft tissues. Soft tissue tuberculosis is rare and easily misdiagnosed. A delay in soft tissue tuberculosis diagnosis may worsen the disease, increase tuberculosis transmission, and accelerate the evolution of drug resistance. This case report emphasizes the importance of next-generation sequencing for early diagnosis of soft tissue tuberculosis, which can provide guidance for clinical treatment and improve prognosis.

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INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by mycobacterium TB (MTB). Extrapulmonary TB (EPTB) in China constitutes 15%-20% of all TB cases, it can involve any organ, with the most usual sites of infection being the pleura (49.8%), bronchi (14.8%), lymph nodes (8.56%), meninges (7.6%), thoracic vertebra (2.55%), and skeletal joints (0.56%)[1,2]. Isolated soft tissue TB is rare and accounts for only 1-2% of all pulmonary TB (PTB) and EPTB cases. Current knowledge of soft tissue TB is largely based on the analysis of a single patient and case series[3,4]. Therefore, the low incidence and lack of typical symptoms of soft tissue TB may lead to difficulties and delays in diagnosis.

The traditional gold standard for TB diagnosis is the MTB culture assay, which has a lower positivity rate of 23.03%. In EPTB, the positivity rate is approximately 18.45%[5]. The MTB culture assay demands laboratory biosafety requirements, and rapid diagnosis is challenging. Molecular biology techniques, such as real-time fluorescent polymerase chain reaction (PCR) and cross-primer amplification, have facilitated the rapid diagnosis. However, the diagnostic sensitivity of these methods for EPTB is limited and varies among specimen types[6]. For example, the sensitivity of Xpert MTB/ rifampin (RIF) assay for PTB and EPTB was found to be 95.5% and 76.5%, respectively[7].

Next-generation sequencing (NGS) is an emerging and promising technology that is used for disease diagnosis, drug resistance determination, and epidemiological investigations. It has the potential to significantly reduce response time for the identification of pathogens, such as bacteria, viruses, tuberculosis, fungi, and parasites[8-12]. For TB, next-generation sequencing can be used for early diagnosis, identification of drug resistance gene mutations associated with conventional anti-TB drugs, and detection of mixed infections[13]. However, there are only a few reports on the rapid diagnosis of MTB infection in soft tissue using NGS. In this report, we present a case of soft tissue TB in an immunocompetent patient with no history of TB. We used NGS technology to detect the surgically resected lesion tissue, and the patient was finally diagnosed with soft tissue TB. In addition, we reviewed the main features of soft tissue TB cases reported in the past decade.

CASE PRESENTATION

Chief complaints

A 44-year-old man admitted to Department of Orthopaedics of the First Affiliated Hospital of Nanjing Medical University presented with a history of left thigh ulceration and swelling for 10 d.

History of present illness

The patient showed similar symptoms in 2018, some tests including bacterial and tuberculosis culture, acid-fast staining, and tuberculosis infection T-lymphocyte spot test (T-SPOT). TB tests, were negative at that time. And the patient recovered gradually after debridement. Through careful history investigation, we found that the patient had an open wound on the left thigh caused by trauma 18 years ago, which improved after debridement and suturing.

History of past illness

There is no relevant history of past illness.

Personal and family history

The patient's personal and family histories were unremarkable.

Physical examination

Two old scars were observed on the patient's left thigh, and there was a red and swollen ruptured wound above them. The skin temperature of the limb was normal, and no movement limitation was observed. A physical examination revealed no other positive signs. Breath sounds of both lungs were clear, no obvious dry or wet rales were heard, and there was no pleural friction rub.

Laboratory examinations

On day 2 after admission, laboratory tests, such as routine blood examination, coagulation function tests, erythrocyte sedimentation rate, and serum biochemical indicators, did not show any significant abnormalities.

Imaging examinations

A radiograph of the left femur indicated no bone erosion (Figure 1A). Bone single-photon emission computed tomography did not reveal any abnormalities in bone metabolism (Figure 1B). On day five after admission, magnetic resonance imaging (MRI) of the left hip was performed, which revealed abnormal signals in the soft tissue of the left upper femur and oedema of the subcutaneous soft tissue. In addition, MRI revealed a chronic abscess with sinus tract formation (Figure 1C-E). Computed tomography of the chest showed scattered nodules in both lungs (Figure 1F). But the symptoms of tuberculosis are not obvious.

Further diagnostic work-up

Considering that the abscess was large, we performed abscess resection of the left thigh and vacuum sealing drainage therapy. We observed inflammation and degeneration in subcutaneous tissue and cystic infected tissue wrapped in the deep layer, which had tough capsule walls and a size of approximately 8 cm × 6 cm (Figure 2A and B). After incision of the purulent cyst, gelatinous necrotic tissue which was grey and white, was observed. Furthermore, we performed histological examination, bacterial culture, and NGS testing of the specimen.

Histological examination revealed fibrous connective tissue hyperplasia with necrosis, acute and chronic inflammatory cell infiltration, and focal granulomatous inflammation with multinucleated giant cell formation (Figure 2C). Bacterial culture of the surgically excised specimen was negative on day seven after admission. However, MTB was detected by NGS simultaneously. The distribution of bacteria and fungi identified by NGS revealed that MTB was the main pathogen (Figure 3A), and 24 sequence reads were identified (Figure 3B).

The pathology department was then contacted for an additional acid-fast bacillus staining test of the intraoperative specimen, and scattered suspicious antacid staining positive rods were observed microscopically (Figure 2D). Further, the T-SPOT. TB test results were positive.

FINAL DIAGNOSIS

Combined with the patient's medical history and outcome of NGS, the patient was finally diagnosed with soft tissue tuberculosis.

TREATMENT

We planned a standardized anti-tuberculosis treatment with four drugs, isoniazid, rifampicin, ethambutol, and pyrazinamide, for 2 mo. Further isoniazid and rifampicin were given for four months. But the patient stopped the drug because of gastrointestinal discomfort during the 3rd month of treatment and suspended for 2 wk, then he continued to take the medicine again for 3 mo.

OUTCOME AND FOLLOW-UP

Currently, the wound has healed adequately and the patient is undergoing follow-up. A summary of the timeline is shown in Figure 4.

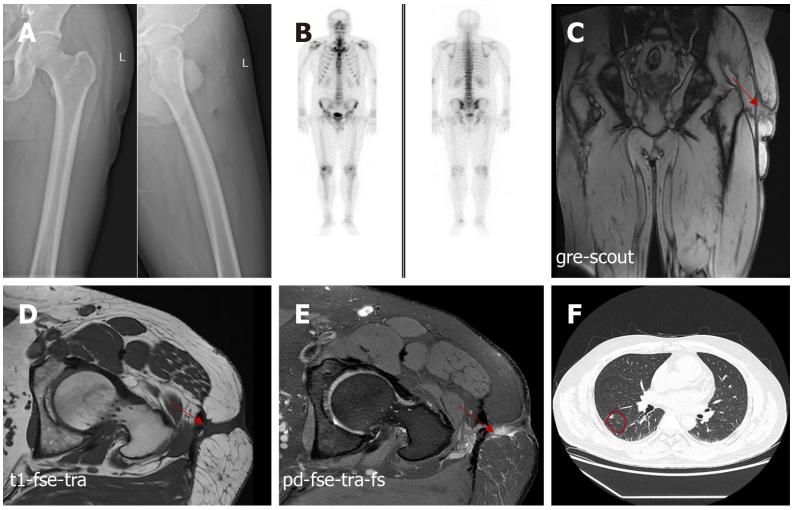

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Figure 1 Imaging pictures of the patient. A: Anteroposterior and lateral radiographs of the left femur are normal; B: Bone single-photon emission computed tomography did not reveal any abnormalities in bone metabolism; C-E: Magnetic resonance imaging of the left hip showing abnormal signals in the soft tissue of the left upper femur and suggesting a chronic abscess with sinus tract formation; F: Computed tomography of the chest showing scattered nodules in both lungs, the largest nodule in the right lung with a length of 8 mm.

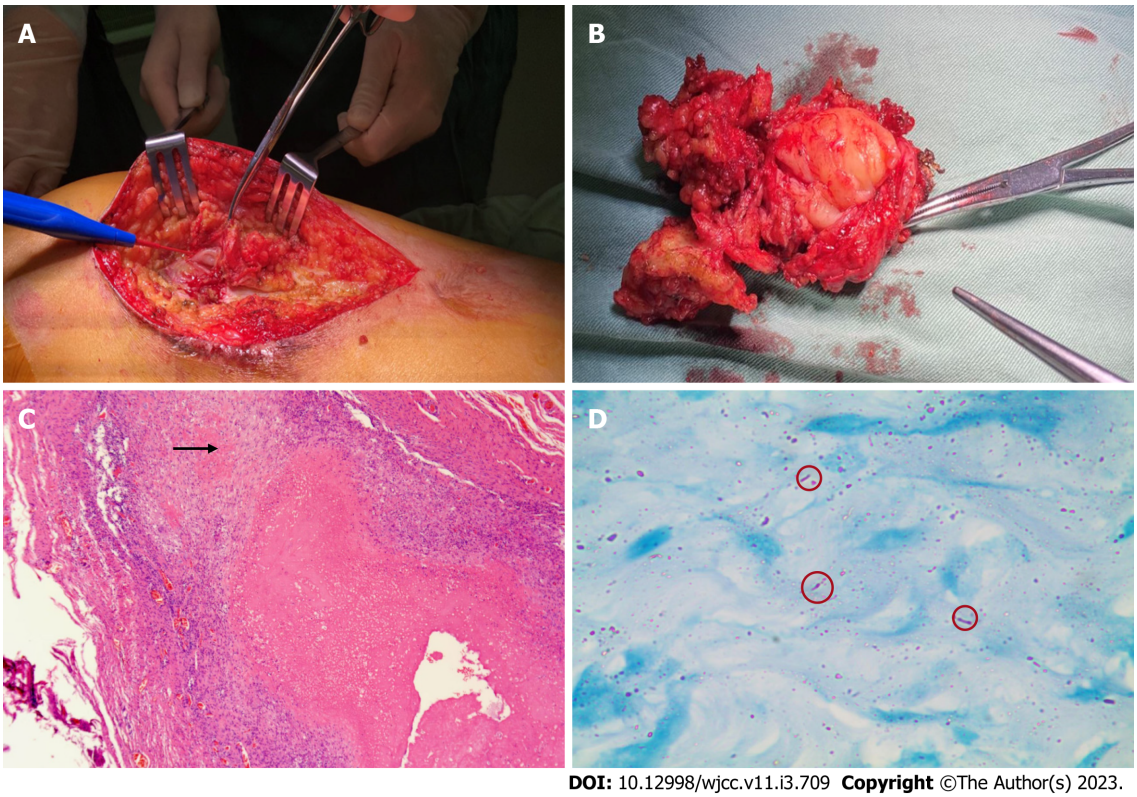

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Figure 2 Intraoperative photographs of the left thigh and histopathological examination of resected specimens. A: Intraoperative photograph; B: Excised specimen (8 cm × 6 cm); C: Granulomas are embedded among the muscle fibers with lymphocyte infiltration and multinucleated giant cell aggregation (× 40); D: The acid-fast bacilli staining test showed scattered, suspicious antacid staining-positive rods (× 1000).

DISCUSSION

According to a global TB report published by WHO, TB deaths have increased because of reduced access to TB diagnosis and treatment in the face of the COVID-19 pandemic for the first time in over a decade[14]. China has the second-highest number of TB cases worldwide, accounting for approximately 9% of the global TB incidence. One study indicated that the prevalence of smear-positive TB in China decreased from 170/100 000 population in 1990 to 59 /100 000 population in 2010, a reduction of more than 50 percent[15]. Despite past success in controlling TB, the limited epidemiologic information

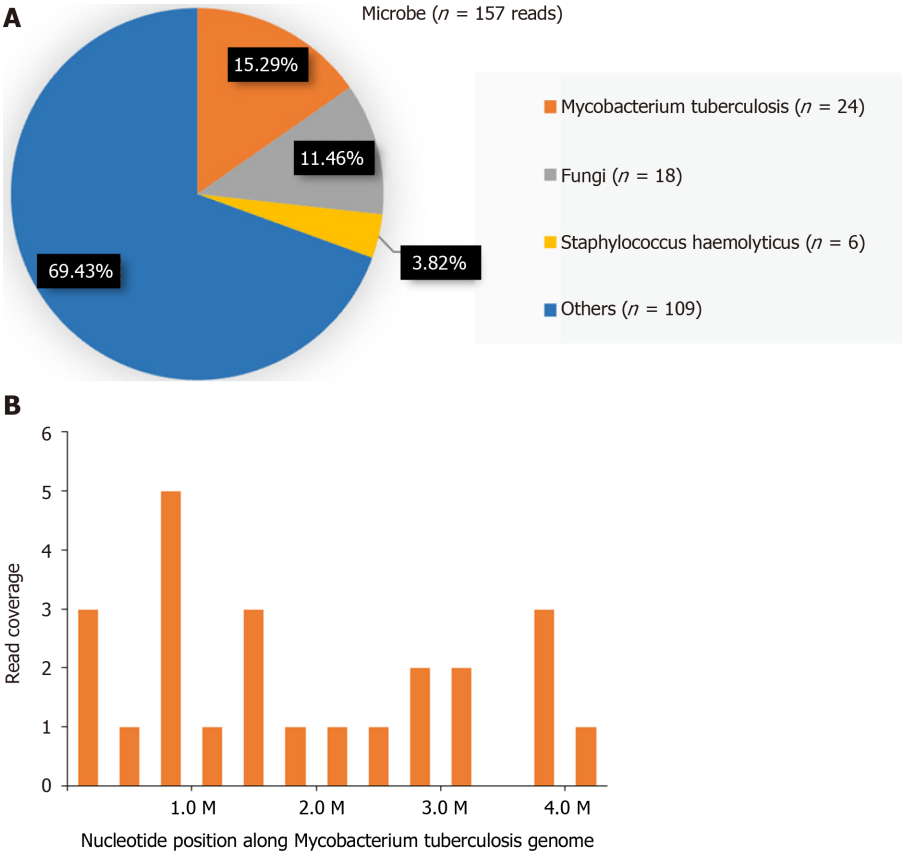

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Figure 3 Next-generation sequencing results of surgically resected specimen. A: Distribution of the sequences detected by next-generation sequencing of surgically excised specimens; B: Twenty-four sequence reads of mycobacterium tuberculosis are observed, with a coverage rate of 0.005%.

available suggests that the incidence of EPTB may be increasing steadily worldwide, including in China [16,17].

The diagnosis of EPTB can be challenging, especially for TB in rare sites such as soft tissues. A delay in soft tissue TB diagnosis may worsen the disease, increase TB transmission, and accelerate the evolution of drug resistance. The conventional gold standards for TB diagnosis are MTB culture and drug sensitivity tests. However, it is tedious and can take 6-8 wk because of slow growth of MTB[18]. Molecular diagnostic techniques, such as Xpert MTB/RIF, loop-mediated isothermal amplification (LAMP), and line probe assay (LPA), can effectively reduce turnaround time and improve diagnostic performance. However, these techniques have limited diagnostic sensitivity for specimens with low bacterial content, such as EPTB[19,20]. For example, a meta-analysis found that the pooled sensitivity of Xpert MTB/RIF for diagnosing abdominal TB was only 23%[21]. Another meta-analysis reported that the pooled sensitivity of LAMP for detecting EPTB was 77%[22]. Only a few studies have evaluated the role of LPA in EPTB specimens. A study from India reported a sensitivity of 46.1% with a specificity of 91% in EPTB specimens, with liquid culture as the reference standard[23].

Soft tissue TB is rare and easily misdiagnosed. Therefore, rapid, efficient, and accurate diagnosis of soft tissue TB is an urgent clinical problem. NGS is a revolutionary development of first-generation sequencing methods that can sequence millions of DNA fragments simultaneously with high throughput and short detection cycles. A study found that the positivity rate of NGS is approximately 15% higher than that of traditional pathogen culture in a pairwise manner for infectious diseases[24]. Xpert MTB/RIF and NGS tests were performed on various samples of sputum, cerebrospinal fluid, and pus from patients with suspected active TB infection. Compared with Xpert MTB/RIF, NGS showed better sensitivity in all clinical (76.9% *vs* 61.5%), pulmonary (87.5% *vs* 75.0%), and extrapulmonary samples (60.0% *vs* 40.0%)[13]. In this case, NGS rapidly detected the sequence of MTB in the sample, which was important for us to confirm the diagnosis of soft tissue TB early and to treat it with anti-tuberculosis drugs in time. Below, we review the relevant literature on soft tissue TB.

Recent case reports on soft tissue TB published in the past 10 years were identified by searching PubMed (Table 1). We found 17 case reports including 10 males and 7 females. The ages of the patients ranged from 7 to 79 years. There were 12 cases distributed in Asian countries, four cases in African countries and one case in America. No underlying diseases were reported in 10 patients. In 13 patients, the lesions were located on the extremities, including the thigh, calf, forearm, and wrist. The other four patients had lesions in the gluteus, back, thorax, and iliopsoas.

Table 1 Main features of reported cases of soft tissue tuberculosis

Ref.	Gender/age (yr)	Country	Underlying disease	Main clinical manifestations	Involved sites	Diagnostic methods
Arora *et al*[38], 2012	Male/15	India	None	Swelling, anorexia, weight loss	Left thigh	Mycobacterium tuberculosis culture
Lee *et al*[39], 2013	Male/62	Korea	Right total hip arthroplasty	Mass	Right thigh	Histological examination, and culture
Elshafie *et al*[40], 2013	Male/25	Oman	Exposed to suspected tuberculosis, diarrhea	Enlarging swelling	Right gluteus	Histological examination, and culture
Neogi *et al*[41], 2013	Female/11	India	None	Swelling	Right thigh, right calf, and left arm	Histological examination, and culture
Meena *et al*[42], 2015	Male/25	India	None	Swelling, fatigue, weight loss	Right triceps	Mycobacterium tuberculosis PCR
Dhakal *et al*[43], 2015	Female/9	Nepal	None	Swelling	Forearm, right calf	Histological examination
Sbai *et al*[44], 2016	Male/45	Tunisia	None	Pain, swelling	Right wrist	Tissue biopsy and culture
Al-khazraji *et al*[45], 2017	Female/33	America	Lupus nephritis, hormonal therapy	Pain, weakness, swelling, redness	Right calf	Fluid culture
Alaya *et al*[46], 2017	Female/23	Tunisia	None	Swelling, pain	Left thigh	Mycobacterium tuberculosis PCR
Manicketh *et al*[25], 2018	Female/55	India	Pulmonary tuberculosis	Swelling, fever	Left wrist and right calf	A Ziehl-Nielsen stain
Hashimoto *et al*[27], 2018	Male/79	Japan	None	Swelling, erythema	Left wrist	Histological examination
Zeng *et al*[4], 2019	Male/49	China	Pulmonary tuberculosis; steroid treatment	Pain, mass, swelling	Both thighs and calves	Mycobacterium tuberculosis PCR, Tissue biopsy and culture
Zitouna *et al*[47], 2019	Female/42	Tunisia	None	Mass, swelling	Right mid-back	Tissue biopsy and culture
Moyano *et al*[48], 2019	Male/29	Senegal	None	Pain, increase in size of hemithorax	Right hemithorax	Mycobacterium tuberculosis PCR and culture
Fahad *et al*[28], 2020	Female/45	Pakistan	None	Swelling, pain	Right forearm	Histological examination
Murugesh *et al*[49], 2020	Male/31	India	Renal transplant with immuno-suppressants	Fever, pain, swollen erythematous	Right foot and calf	Nucleic acid amplification test
Tone *et al*[26], 2021	Male/29	Japan	Right tuberculous pleurisy	Fever, pain	Right iliopsoas	Mycobacterium tuberculosis PCR

PCR: Polymerase chain reaction.

Soft tissue TB occurs mostly in the extremities, and patients present with local masses, swelling, and weakness. Only a small percentage of patients present with constitutional symptoms such as fever and weight loss[25,26]. Most cases of soft tissue TB reported in the literature were diagnosed by histological examination and MTB culture, whereas some were confirmed by rapid PCR. Although the culture and gene Xpert were negative, two patients recovered after empirical anti-TB therapy[27,28]. Empirical treatment for TB was initiated without a confirmed bacterial diagnosis. Moreover, factors contributing to the probability of a patient developing TB and experiencing adverse outcomes were weighed against the threshold for initiating anti-TB therapy. This threshold is subjective and may vary among the clinicians. Factors considered in empirical anti-TB treatment include the background TB epidemiology in the geographic area, exposure to TB patients, clinical manifestations suggestive of TB disease, comorbidities such as HIV co-infection, and the results of other diagnostic methods such as imaging outcomes if available[29].

There are many traditional tests for TB, including smear, culture, pathological biopsy, imaging, purified protein derivative testing, interferon-gamma release assay, and TB antibody testing[30,31]. Confirmation of TB over the past few decades often requires a combination of these tests. Over the last decade, advances have been made in the field of TB diagnostics in the form of new molecular tests. Often referred to as nucleic acid amplification tests, these assays rely on amplification of a targeted genetic region of the MTB complex, typically by PCR[32]. GeneXpert MTB/RIF is a fully automated

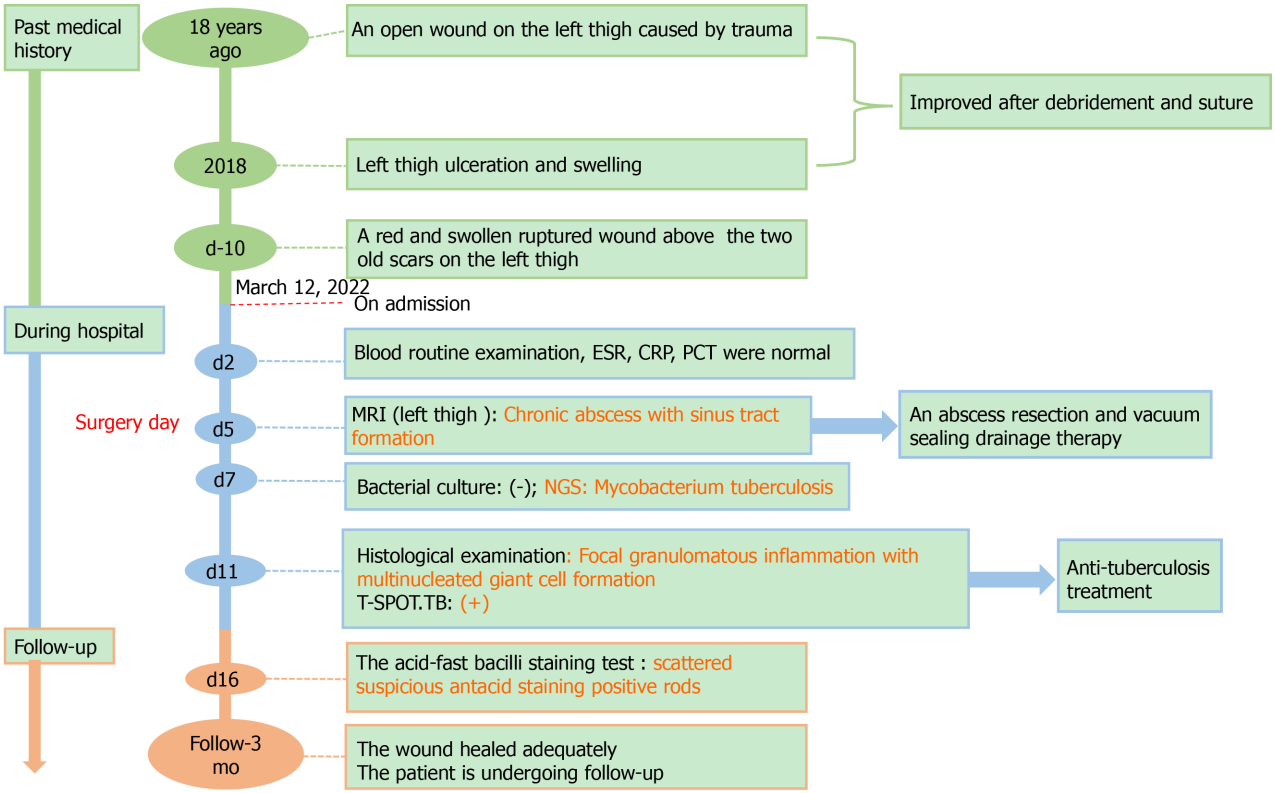

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Figure 4 A timeline showing the progress of the disease and the patient's treatment and follow-up. ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; PCT: Procalcitonin; MRI: Magnetic resonance imaging; NGS: Next-generation sequencing.

closed system that performs sample preparation and real-time PCR and produces results within 2 h. This system can detect RIF resistance (targeting the rpoB gene). In 2011, the WHO recommended GeneXpert MTB/RIF for the early diagnosis of drug-resistant TB, which was further expanded in 2013 to replace smear and culture for the rapid diagnosis of EPTB[33]. The new version of Xpert MTB Ultra improved overall sensitivity and was endorsed by the WHO in 2017[34]. In 2017, the UK used NGS for TB diagnosis, drug resistance detection, and MTB typing for the first time[35]. Several studies have shown its advantages in the diagnosis and treatment of EPTB[36,37]. However, the value of NGS in the rapid diagnosis of TB has not been verified in large samples, and there is a lack of unified standards and procedures. Guidelines for the clinical interpretation of NGS reports need to be improved.

CONCLUSION

We reported the case of a patient who was immunocompetent and had no history of TB and was diagnosed with soft tissue TB by NGS. The patient received timely anti-TB treatment, which improved. Clinicians should consider atypical pathogens such as MTB in the diagnosis of patients with local masses or swelling. NGS may be a useful method for identifying the pathogens responsible for soft tissue infections without typical clinical manifestations.

FOOTNOTES

Author contributions: He YG and Huang YH drafted the manuscript, interpreted the patient data. Xi XL, Qian KL, Wang Y, and Cheng H collected information from the patients and searched the related literature. Liu Y and Hu J contributed to supervision and final approval. All authors have read and approved the final manuscript.

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