Original Article
Pathologic characteristics of spinal tuberculosis: analysis of 181 cases

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Received March 1, 2020; Accepted April 14, 2020; Epub May 1, 2020; Published May 15, 2020

Abstract: Objective: This study aimed to provide a basis for the diagnosis of spinal TB by analyzing its pathologic characteristics. Methods: The data of 181 patients with spinal TB who underwent surgery from January 2013 to January 2019 at the General Hospital of Ningxia Medical University were retrospectively analyzed. The participants comprised 80 men and 101 women with an average age of 45.1 ± 16.5 (range: 14-78) years. Based on the assessment of tissue samples, five patients had cervical TB, 49 had thoracic TB, 86 had lumbar TB, 22 had thoracolumbar TB, and 19 had lumbosacral TB. Tuberculous granulation tissue, sclerotic bone, sequestrum, and intervertebral disc tissue were collected for hematoxylin and eosin staining. The proportion of patients with atypical and typical pathologic characteristics was identified and compared for statistical analysis. Results: The typical pathologic characteristics included tubercles, granulomas, caseous necrosis, multinuclear giant cells, infiltration of acute inflammatory cells, sequestration, and fibroblastic proliferation. A total of 119 patients had caseous necrosis, 95 had multinuclear giant cells, 68 had granulomatous inflammation, and 21 had tubercles. Moreover, 46 (25.4%) patients had at least three pathologic characteristics and only 12 (6.6%) exhibited all the pathologic characteristics. Of the 35 (19.3%) patients with atypical pathologic characteristics, 17 had lymphocyte infiltration, 10 had fibroblastic proliferation, 2 had hyaline changes, 1 had local hemorrhage, 1 chronic inflammatory change, 2 had sequestration, 1 had dilated and congested vessels, and 1 had acute suppurative inflammation. Conclusions: The most common pathologic characteristics were caseous necrosis, multinuclear giant cells, granulomatous inflammation, and tubercles. Moreover, multiple pathologic characteristics were observed in patients with spinal TB and one type of these characteristics was dominant. However, atypical pathologic characteristics were also noted. Thus, both pathologic examination and clinical analysis must be performed to improve the diagnostic rate of spinal TB.

Keywords: Spinal tuberculosis, tissue sample, pathology

Introduction

Tuberculosis (TB) is a chronic infectious disease that poses a serious threat to human health [1, 2], and the most common type of bone and joint TB is spinal TB, which accounts for approximately 5% of all TB cases [3, 4]. Moreover, it has a poor prognosis and is associated with high morbidity and mortality [1, 5]. Imaging is considered to be the main diagnostic test for the diagnosis of spinal TB, although it is associated with varying degrees of false negative results. Diagnoses based on clinical symptoms and laboratory tests have low specificity and those based on genetic tests have low sensitivity. Unfortunately, although histopathology is regarded as the gold standard in the diagnosis of pulmonary tuberculosis (TB), it is not the first choice since the pathologic characteristics of spinal TB are atypical. And it has long culture cycle and low positivity rate [6, 7]. In addition, the abuse of anti-TB drugs, pathologic structure of compact focal sclerosis, unreasonable use of antibiotics prior to consultation, and complexity of pathogenesis contribute to the atypical pathologic characteristics of spinal TB. The pathogenesis of spinal TB is closely correlated to type IV hypersensitivity, which is a complex inflammatory reaction involving multiple cytokines and signaling pathways [8, 9]. The basic pathologic characteristics of spinal TB can be divided into three types: exudation, hyperplasia, and necrosis. These characteristics often exist concurrently.
However, we found that one type of characteristic is dominant, and the other two characteristics are mixed to form the polymorphisms of pathologic changes. The three characteristics can transform each other. The presence of these three basic pathologic characteristics is correlated to the quantity, virulence, allergic reaction, and immune response of the organism. Caseous necrosis is also associated with type II cytokines and MMPs [10]. Previously, we found significant differences in tissue distribution of anti-TB drugs in spinal TB vertebrae. The concentrations of isoniazid (INH), rifampicin (RFP), pyrazinamide (PZA), and other first-line anti-tuberculosis drugs were found to be very low or undetectable in the vertebral sclerosis area and the enclosed tuberculosis lesions. Routine doses of anti-TB drugs were unable to remain in the lesional area for a long time, which made it difficult to maintain effective drug concentration [11]. This may be an important trend contributing to the emergence of drug-resistant bacteria and atypical pathology. Furthermore, the number of patients with spinal TB and AIDS increase with the prevalence of HIV, whose CD4+ T cell and IL-4 counts are significantly lower [12]. Interestingly, CD4+ T cells plays a key role factor in cellular immunity against Mycobacterium tuberculosis (M. tuberculosis), and IL-4 can subvert mycobacterial containment in infected macrophages, probably by perturbations in Treg and Th1-linked pathways [13], resulting in atypical pathology of spinal TB. For these reasons, the typical pathologic characteristics of spinal TB, such as presence of tubercles, caseous necrosis, and tuberculous granuloma, are not always observed, and occasionally, only atypical characteristics are found.

Materials and methods

Case selection

A total of 181 specimens were collected from patients with spinal TB who were diagnosed by the assessment of clinical symptoms, laboratory examination, imaging, pathologic examination, and bacterial culture from January 2013 to January 2019 at General Hospital of Ningxia Medical University. The participants comprised 80 men and 101 women with an average age of 45.1 ± 16.5 (range: 14-78) years. Five patients presented with cervical TB, 49 with thoracic TB, 86 with lumbar TB, 22 with thoracolumbar TB, and 19 with lumbosacral TB.

The inclusion criteria were patients with spinal TB who underwent surgery in our department. However, those with TB in other parts of the body, other infectious diseases, tumors, trauma, immunodeficiency diseases, and metabolic diseases were excluded. All patients had varying degrees of the clinical manifestation, poor nutritional status, local pain, kyphosis, local tenderness, and neurological symptoms. Laboratory tests showed elevated erythrocyte sedimentation rate and C-reactive protein level. The TSPOT assay was positive, whereas the Brucella agglutination test was negative. The typical characteristics of spinal TB, such as stenosis of intervertebral space, bone destruction, paravertebral abscess, and kyphosis, were observed on radiography, computed tomography (CT) scan, and magnetic resonance imaging (MRI). In addition, the TB culture of each patient was positive.

Preoperative anti-TB treatment

The anti-TB protocol comprises 300 mg INH, 450 mg RFP, 750 mg PZA, and 750 mg ethambutol. The drugs were taken every day before breakfast. The liver and kidney functions of the patients were assessed after 2-4 weeks of taking the anti-TB medications.

Treatment approaches and sample collections

The treatment approaches used in this study were debridement, bone graft, and instrumentation using the anterior, posterior, and combined anterior and posterior approaches. Pathologic specimens were collected when debridement was completed, during which the specimens of typical TB foci were selected by experienced physicians. The samples of tuberculous granulation tissue, sclerotic bone, sequestrum, and diseased disc were sent to the pathology department by postgraduates who specialize in the immediate collection of specimens.

Pathologic examination

The pathological tissue specimens, including tuberculous granulation tissue, sclerotic bone, sequestrum, and intervertebral disc tissue were separated and fixed in 4% formalin solution for 30 minutes after surgery and were sent to the pathology department of our hospital for examination. The pathologists selected tissues that were 0.5-1 cm in size in the central area
Pathologic characteristics of spinal tuberculosis

The main pathologic characteristics were caseous necrosis, multinuclear giant cells, granulomatous inflammation, tuberculosis, acute inflammatory response, inflammatory cell exudate, osteocyte degeneration and necrosis, and fibrous tissue proliferation. In terms of the typical pathologic characteristics of spinal TB, 68 patients presented with granulomatous inflammation, 21 with tubercle, 95 with multinuclear giant cells, and 119 with caseous necrosis. Meanwhile, 35 had atypical characteristics (See Table 1). Granulomatous inflammation, caseous necrosis, tubercles, multinuclear giant cells, and atypical characteristics were found in one, two, one, three, and one of five patients with cervical TB; 20, 39, 7, 29, and 4 of 49 patients with thoracic TB; 36, 51, 11, 39, and 21 of 86 patients with lumbar TB; 6, 12, 2, 13, and 6 of 22 patients with thoracolumbar TB; and 5, 15, 0, 11, and 3 of 19 patients with lumbosacral TB, respectively. A total of 89 (49.2%) patients presented with caseous necrosis concurrent with granulomatous inflammation, multinucleated giant cells, or tubercles.

A total of 46 (25.4%) patients presented with at least three pathologic characteristics and all four typical pathologic characteristics were observed in 12 (6.6%) patients. Typical characteristics of spinal TB were as follows: fibrocartilage tissue and spongy bone tissue; patchy caseous necrosis of varying degrees observed between the bony trabeculae in some regions. In addition, not only lymphocytes, epithelioid cells, and multinuclear giant cells but also granulomatous nodules were found around the necrotic foci (see Figure 1). The atypical pathologic characteristics were as follows: spongy or loose bone tissue, proliferation of fibrovascular tissues between the trabeculae, dilatation of blood vessels and hyperemia, and numerous lympho-

Table 1. Pathologic characteristics of different types of spinal tuberculosis

<table>
<thead>
<tr>
<th>Types of spinal tuberculosis</th>
<th>Number of patients</th>
<th>Granulomatous inflammation</th>
<th>Caseous necrosis</th>
<th>Tubercles</th>
<th>Multinuclear giant cells</th>
<th>Atypical pathologic changes</th>
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<tr>
<td>Cervical tuberculosis</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Thoracic tuberculosis</td>
<td>49</td>
<td>20</td>
<td>39</td>
<td>7</td>
<td>29</td>
<td>4</td>
</tr>
<tr>
<td>Lumbar tuberculosis</td>
<td>86</td>
<td>36</td>
<td>51</td>
<td>11</td>
<td>39</td>
<td>21</td>
</tr>
<tr>
<td>Thoracolumbar tuberculosis</td>
<td>22</td>
<td>6</td>
<td>12</td>
<td>2</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Lumbosacral tuberculosis</td>
<td>19</td>
<td>5</td>
<td>15</td>
<td>0</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>181</td>
<td>68</td>
<td>119</td>
<td>21</td>
<td>95</td>
<td>35</td>
</tr>
</tbody>
</table>

Figure 1. Typical pathologic characteristics of spinal tuberculosis. A: The blue arrow points to a large area of caseous necrosis and the black arrow indicates quantities of lymphocyte infiltration [hematoxylin and eosin (H&E) staining, x100]. B: The arrow shows the formation of tubercles with multinuclear giant cells at the center and surrounding fibrous tissues (H&E staining, x100). C: The black arrow shows dense epithelioid cells, and the blue arrow indicates multinuclear giant cells between the epithelioid cells (H&E staining, x100). D: The overall picture shows granulomatous inflammatory changes, and the arrow indicates inflammatory cell infiltration (H&E staining, x100).

of the lesion for routine decalcification, sectioning, and hematoxylin and eosin staining, and the samples were assessed under a microscope. Immunohistochemical examination was performed when necessary.

Results

The main pathologic characteristics were caseous necrosis, multinuclear giant cells, granulomatous inflammation, tuberculosis, acute inflammatory response, inflammatory cell exudate, osteocyte degeneration and necrosis, and fibrous tissue proliferation. In terms of the typical pathologic characteristics of spinal TB, 68 patients presented with granulomatous inflammation, 21 with tubercle, 95 with multinuclear giant cells, and 119 with caseous necrosis. Meanwhile, 35 had atypical characteristics (See Table 1). Granulomatous inflammation, caseous necrosis, tubercles, multinuclear giant cells, and atypical characteristics were found in one, two, one, three, and one of five patients with cervical TB; 20, 39, 7, 29, and 4 of 49 patients with thoracic TB; 36, 51, 11, 39, and 21 of 86 patients with lumbar TB; 6, 12, 2, 13, and 6 of 22 patients with thoracolumbar TB; and 5, 15, 0, 11, and 3 of 19 patients with lumbosacral TB, respectively. A total of 89 (49.2%) patients presented with caseous necrosis concurrent with granulomatous inflammation, multinucleated giant cells, or tubercles.

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cytes, monocytes, and neutrophil infiltrates, along with acute suppurative reaction (see Figure 2). The atypical pathologic characteristics of TB were observed in 35 (19.3%) patients, including 17 with lymphocyte infiltration, 10 with fibrous hyperplasia, two with hyaline degeneration, one with local hemorrhage, one with chronic inflammation, two with dead bone, one with vascular dilatation and congestion, and one with acute suppurative inflammation. Furthermore, there existed other pathologic changes (see Figure 3).

Discussion

In the study of He [14] et al., granulomas were found in all patients (n = 126) with pulmonary TB. Thus, the proportion of patients with typical pathologic characteristics was 100%. Meanwhile, in the current study, only 80.7% of the patients presented with typical pathological characteristics. In addition, most patients only presented with a single characteristic. This result indicated a significant difference between patients with pulmonary and spinal TB in terms of pathology, which may be attributed to the differences in the histomorphology of the lungs and spine as well as the emergence of drug-resistant strains. A5 protein secreted by M. tuberculosis can enhance the pathogenicity of the bacteria by identifying and acting with type II alveolar epithelial cells and can significantly increase the production of tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and interleukin 6 (IL-6) [15], and all these cytokines play an important role in the pathogenesis of TB (Figure 4). Macrophages are activated and transformed into epithelioid cells by IFN-γ and TNF-α after reaching the vertebral body or intervertebral disc with the action of chemokines. Then, multiple epithelioid cells fuse to form multinuclear giant cells surrounding the central TB bacillus and caseous necrosis, thereby leading to the formation of a typical tubercle in the lesion.
Moreover, although both pulmonary macrophages and osteoclasts evolve from monocytes in the blood, unlike osteoclasts, the function of pulmonary macrophages is finely regulated by the pulmonary environment [16]. That is, when the lung is infected with TB bacteria, the pulmonary environment may be destroyed, thereby losing its ability to regulate. Pulmonary macrophages can aggregate with the action of chemokines by amoeba-like movement and the systolic and diastolic movements of the alveoli owing to loose pulmonary tissue structure and alveolar pores. This phenomenon makes it easier to form granulomas comprising macrophages and macrophage-derived cells in pulmonary TB. M. tuberculosis is recognized and engulfed by pulmonary macrophages by TLR [17], which can also be confirmed from Figure 4. Besides macrophages, type II alveolar epithelial cells are also the host cells of M. tuberculosis, which may be closely correlated to the presence of TLR2 on the cell membranes of alveolar epithelial cells [18, 19].

The experiment of Shahemabadi [20] has shown that the response of peripheral blood mononuclear cells and CD4+ T cells to M. tuberculosis total lipid antigens significantly decreased in a patient with multidrug-resistant TB (MDR-TB) due to the lack of lipid antigen in drug-resistant M. tuberculosis, which leads to a decrease in the secretion of IFN-γ. Waxy D and 6,6-bifidobacteric acid trehalose are the important lipid components of M. tuberculosis, and they are phagocytized and processed by macrophages or other antigen-presenting cells, thereby forming pMHCII or pMHCII and activating Th1 cells and CD8+ cytotoxic T lymphocyte (CTL) following recognition and binding with TCR. By contrast, waxy D-activated Th1 cells can enhance the function of T lymphocytes and release IL-2, IL-3, and other cytokines. IL-2 activates CD8+ CTL to kill macrophages that engulf M. tuberculosis in the vertebrae and intervertebral discs by the perforin, granulase, or Fas pathway, leading to cell lysis and antigen exposure. This method is important for the removal of M. tuberculosis from the vertebral body and intervertebral disc through cellular immunity. However, no typical tubercle can be formed with the single action of waxy D or tuberculin, and tuberculous granuloma can only be formed through the synergistic action of 6,6-bifidobacteric acid trehalose. Therefore, the lack of lipid antigens in drug-resistant bacteria limits the formation of granulomas.

Likewise, the TNF-α levels of a group of patients with drug-resistant and non-drug-resistant TB were significantly different. The production of IL-12 and STAT from peripheral blood mononuclear cells were decreased in patients with MDR-TB, and the expression of TLR2-dependent TGF-β were increased, thereby enhancing the function of IL-17 and IFN-γ T cells [21, 22]. Some studies have shown that the decrease in granulomas in the vertebral bone tissue is closely correlated to the blockage of the NF-κB pathway. Combined with the pathway map (Figure 4), the blockage of the NF-κB

\*Figure 3. Other pathologic characteristics of spinal tuberculosis. A: Large areas of intertrabecular fibrous tissues and hemorrhage accompanied by massive inflammatory cell infiltration [hematoxylin and eosin (H&E staining), ×100]. B: The arrow pointing to hyaline degeneration of fibrous tissues. Simultaneously, foci of cartilage and bone, necrosis of non-structural tissue (pink stain), and lymphocyte and plasma cell infiltration are observed (H&E staining, ×100). C: Abundant bone marrow hematopoietic tissues were observed between the trabeculae. However, no typical inflammatory reaction was observed (H&E staining, ×100). D: The arrow shows a localized calcification, with numerous lymphocytic and neutrophil infiltrates in some areas (H&E staining, ×100).\*
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Figure 4. Signaling pathways associated with tuberculosis (KEGG Database).
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Malika [23] et al. have found a significant increase in CD4+CD25+FoxP3+ regulated T cells in patients with XDR-TB whose treatment failed, indicating that this may be an important cause of immune dysfunction. Furthermore, as an anti-inflammatory cytokine, the serum IL-10 level of patients with drug-resistant TB increased significantly [24]. The combination of IL-10 and IL-10R on the macrophage membrane inhibits the antigen-presenting function of macrophages, which may eventually lead to an atypical pathology.

The differences in the proportion of epithelioid cells, multinuclear giant cells, inflammation, caseous necrosis, tubercles, and even atypical changes contribute to the polymorphisms of the pathologic characteristics of spinal TB. Canthus, one may find typical characteristics, such as epithelioid cells, multinuclear giant cells, and tubercles under a microscope; whereas, there may be only lymphocyte and neutrophil infiltration without any typical pathologic characteristics.

Tubercles are mainly proliferative pathologic changes that occur with low bacterial count or a strong immune response. The main cellular components of tubercles are epithelioid cells, multinuclear giant cells, and lymphocytes [25]. Multinuclear giant cells with abundant cytoplasm form by the fusion of epithelioid cells; thus, they often appear between epithelioid cells. In addition, another characteristic is the formation of horseshoe- or flower-shaped nuclei by dozens of epithelioid nuclei, and numerous lymphocyte infiltrates surround the epithelioid cells, and fibroblasts reactively proliferate at the outermost surface of the tubercle, making the tubercle boundary clear. However, in this study, the incidence rate of tubercles was only 11.6%, and such a value was lower than that of granulomatous inflammation, caseous necrosis, and multinuclear giant cells. Typical tubercles were not observed in all 19 patients with lumbosacral TB. In contrast, in this study, the occurrence of caseous necrosis was more common than other characteristics, with the highest positivity rate at 65.7%, followed by polykaryocytes, and this result was consistent with that of Tang [26] et al. Microscopic examination showed granular necrosis of non-structural red dye without any outline of bone tissues. However, multinuclear giant cells were observed at the margin, which may be correlated to the high fat content in bone marrow hematopoietic tissues. Moreover, caseous necrosis has a vital role in the pathologic diagnosis of spinal TB [27].

Notably, the positivity rate of atypical characteristics (19.3%), including certain soft and proliferative TB, was higher than that of tubercles [28]. Soft TB rich in fibrin may develop with the presence of numerous TB bacteria in the lesion, but without the characteristics of typical TB, such as caseous necrosis.

In this study, one pathologic characteristic of the patients (n = 35) with atypical TB was nonspecific inflammation under a microscope. The other characteristics included not only acute exudative inflammation changes, such as the presence of cellulose, neutrophils, and lymphocytes, but also chronic proliferative inflammation changes, such as fibrous tissue proliferation, osteogenesis, calcification, and ossification. No tubercles, caseous necrosis, and other diagnostic characteristics were found. In addition, caseous necrosis was not observed in patients with non-marginal sclerosis, and only inflammatory cells appeared at the center. Such atypical pathologic changes caused certain difficulties in the diagnosis of spinal TB. However, similar conditions could be observed in patients with proliferative TB.

At present, spinal TB is diagnosed by comprehensive analysis of clinical characteristics, imaging examination, laboratory examination, bacterial culture, and pathologic examination, of which pathologic examination is the main diagnostic method [29, 30]. Spinal TB without typical pathologic characteristics should be distinguished from conditions such as pyogenic osteomyelitis of the spine, ankylosing spondylitis, spinal tumor, osteoporosis, and compression fracture. Thus, the analysis of clinical characteristics, laboratory examination, and particularly, imaging examination are very important [31]. Some studies have shown that imaging examinations, such as CT and MRI, are effective for the early diagnosis of spinal TB and positron emission tomography/CT can be performed when necessary [32, 33].
In summary, the most common characteristic of pathology in spinal TB is caseous necrosis, which can occur along with various other characteristics such as multinuclear giant cells, granulomatous inflammation, and tubercles, and having one of these typical pathologic characteristics is considered important. However, the pathologic characteristics can be completely atypical. With comprehensive analysis of clinical characteristics, imaging examination, laboratory examination, bacterial culture, and pathologic examination, the diagnostic rate of spinal TB can be improved.

Acknowledgements
This project is financially supported by the National Natural Science Foundation of China (NSFC 81501903, 81860395), Ningxia Medical University Youth Key Talents Cultivation Program (30230103), the First-Class Discipline Construction Funded Project of NingXia Medical University and the School of Clinical Medicine (NXYLXK2017A05), Autonomous Region Health and Health System Research Project (2019-NW-011).

Disclosure of conflict of interest
None.

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References


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Gopalakrishnan A and Salgame P. Toll-like receptor 2 in host defense against Mycobacterium tuberculosis: to be or not to be—that is the question. Curr Opin Immunol 2006; 42: 76-82.


Qualls JE and Murray PJ. Immunometabolism within the tuberculosis granuloma: amino acids, hypoxia, and cellular respiration. Semin Immunopathol 2016; 38: 139-152.


