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Research article

Diagnostic value of tuberculosis-specific antigens ESAT-6 and CFP10 in lymph node tuberculosis

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ABSTRACT

Objective: To assess the diagnostic value of immunohistochemical (IHC) staining for detecting the tuberculosis-secreted antigens ESAT-6 and CFP10 in lymph node tuberculosis.
Methods: Archived, paraffin-embedded lymph node specimens from 72 patients diagnosed with lymph node tuberculosis and 68 patients with lymphoma were retrospectively collected from the Department of Pathology at the Affiliated Hospital of North Sichuan Medical College, Nanchong, Sichuan Province, China between January 2016 and March 2023. These specimens were subjected to acid-fast and immunohistochemical staining to compare the effectiveness of these methods, with their sensitivity and specificity evaluated against a comprehensive reference standard.
Results: Acid-fast staining demonstrated a sensitivity of 12.3% and a specificity of 100%. IHC staining for ESAT-6 showed a sensitivity of 87.5% and a specificity of 85.3%, whereas IHC staining for CFP10 exhibited a sensitivity of 75.0% and a specificity of 89.7%.
Conclusion: The study indicates that IHC detection of ESAT-6 and CFP10 in paraffin-embedded lymph node tuberculosis tissues has a markedly higher sensitivity compared to acid-fast staining. Thus, IHC staining may serve as a supplementary diagnostic tool for the pathological evaluation of lymph node tuberculosis.

Tuberculosis, a chronic infectious disease caused by *Mycobacterium tuberculosis* (*MTB*), can affect various organs, with the exception of teeth, hair, and nails. Extrapulmonary tuberculosis refers to *MTB* infections outside the lungs, with lymph nodes being the most frequently involved organs, accounting for approximately 15.8%–21.8% of cases [1,2]. Tuberculous lymphadenitis (TBL) presents with nonspecific clinical symptoms and a low rate of etiological confirmation, complicating the clinical diagnosis. Pathological examination remains the gold standard for diagnosing lymph node tuberculosis. Techniques such as hematoxylin and eosin (HE) and acid-fast staining are in common use but suffer from limited sensitivity and specificity. Molecular pathology advancements have improved the detection rates, yet issues such as PCR contamination susceptibility and stringent laboratory requirements hinder their widespread adoption in primary care settings [3]. The 2017 Chinese consensus on the pathological diagnosis of tuberculosis highlights the diagnostic significance of detecting *MTB*-specific antigens via immunohistochemistry (IHC) in tissue specimens [4]. Furthermore, the 2023 consensus on diagnosing superficial lymph node tuberculosis suggests that positive *MTB* antigen tests can assist in diagnosis

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[5]. Despite the focus on antigens such as MPT64, 38 kDa, ESAT-6, Ag85, and BCG components [6–9], a search for which yielded a total of 35 articles from specific databases, there is still a notable lack of IHC-specific antibodies and standardized interpretive criteria for clinical use, underscoring the need for further translational research [4].

Early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP10) are key *MTB* secretory proteins, characterized by their specific complex formation and absence in most nontuberculous mycobacteria and BCG strains, granting them high specificity [10,11]. While the Interferon Gamma Release Assay (IGRA) clinically employs this feature, its immunological basis means that results are influenced by the host's immune response [12]. This study aims to assess the expression of ESAT-6 and CFP10 in lymph node tuberculosis tissue using IHC and evaluate their diagnostic utility.

1. Materials and methods

Study Population: Archived paraffin-embedded lymph node tissue samples collected from January 2016 to March 2023 at the hospital's Pathology Department, Nanchong, Sichuan Province, China were retrospectively analyzed. This included 72 tuberculosis cases and 68 control cases (lymphoma). Tuberculosis diagnoses were consistent with the 2017 〝Expert Consensus on Pathological Diagnosis of Tuberculosis in China" [4] Class I or II criteria. Class I criteria encompass a definitive diagnosis with pathological evidence of tuberculosis, while Class II criteria suggest a diagnosis based on tuberculosis-like pathological features without direct etiological proof; however, all patients in this category responded to anti-tuberculosis therapy. Lymphoma diagnoses followed the 2018 〝Chinese Guidelines for the Diagnosis and Treatment of Malignant Lymphoma" [13], with all patients lacking a history of tuberculosis and testing negative for IGRAs.

Procedures: Four consecutive 4-μm sections were prepared from each paraffin block: one for HE staining, one for acid-fast staining, and two for IHC. Pathologists reviewed the HE-stained slides for diagnostic accuracy before performing acid-fast and IHC staining.

Acid-fast Staining: The process followed the manufacturer's protocol (AFB Stain Kit, Baso, Zhuhai, China), starting with deparaffinization and including carbolfuchsin staining, decolorization with acidic alcohol, counterstaining with methylene blue, washing, and mounting.

Immunohistochemical Staining (Streptavidin peroxidase, SP): Antigen retrieval was conducted using a microwave before applying primary antibodies—ESAT-6 rabbit polyclonal antibody (bs-13107R, Bioss, Beijing, China, 1:300 dilution) and CFP10 rabbit polyclonal antibody (ABIN285580, Antibodies-online, Philadelphia, PA, USA, 1:400 dilution). The UltraSensitive SP IHC Kit (KIT-9710, MXB, Fujian, China) directed the staining process. Next DAB chromogen development, hematoxylin counterstaining, and final slide preparation. Phosphate-buffered saline (PBS) served as the negative control.

Statistical Analysis: The SPSS 27.0 software facilitated the data analysis, with P-values less than 0.05 denoting statistical significance. Qualitative data were presented as frequencies (%), and the chi-square test was used for intergroup comparisons.

Ethical Considerations: Approval was obtained from the Medical Ethics Committee of North Sichuan Medical College's Affiliated Hospital for this study (approval number : 2023ER88-1).

2. Results

Patient Demographics and Clinical Characteristics: Table 1 outlines the demographic and clinical attributes of the participants. The study analyzed 140 lymph node tissues, including 72 from patients with lymph node tuberculosis and 68 from lymphoma cases. In the tuberculosis group, there were 26 males (36%) and 46 females (64%), with ages ranging from 4 to 93 years and an average age of 45.69 ± 17.97. The lymphoma group comprised 38 males (56%) and 30 females (44%), aged between 28 and 86 years, with an average age of

Table 1
Demographic and clinical characteristics of patients.

Characteristics	TB Cases	Non-TB Cases
	N = 72(%)	N = 68(%)
Gender		
Male	26(36)	38(56)
Female	46(64)	30(44)
Age (average)	45.6 ± 17.97	61.91 ± 13.89
Symptoms		
Fever	4 (5)	6 (9)
Night sweat	6 (8)	5 (7)
Weight loss	5 (7)	7 (10)
No symptoms	62 (86)	53 (78)
TB history		
Positive	8 (11)	0
Negative	64 (89)	68 (100)
Site of lymph node		
Cervical	64 (89)	50 (74)
Axillary	8 (11)	6 (9)
Inguinal	0	7 (10)
Celiac	0	5 (7)

61.91 ± 13.89. A majority of the tuberculosis group, 89% (64/72), presented with enlarged cervical lymph nodes. Most (86%, 62/72) were asymptomatic, while 10 patients exhibited symptoms like fever, night sweats, or weight loss.

Hematoxylin and Eosin (H&E) Staining: Of 72 lymph node tuberculosis specimens examined, 62 (86.11%) exhibited granulomatous inflammation with caseous necrosis (Fig. 1A). Nine specimens (12.50%) presented solely with granulomatous inflammation, and one specimen (1.38%) displayed scattered histiocytes with phagocytic nuclear debris. Activated immunoblasts were observed in the subcapsular sinus of this specimen, with an absence of caseous necrosis or granuloma structures.

Acid-Fast Staining: Red, slender, slightly curved rod-shaped acid-fast bacilli in tuberculosis lesions were indicative of a positive stain (Fig. 1B). Of the tuberculosis cohort, 9 of 72 cases (12.3%) tested positive for acid-fast bacilli, while all 68 control specimens were negative. The sensitivity, specificity, positive predictive value, and negative predictive value of acid-fast staining in this study were calculated as 12.3%, 100.0%, 100.0%, and 51.9%, respectively (Table 2).

Immunohistochemical Staining: Positive staining was characterized by dark brown granules, appearing as dots, clusters, or patches,

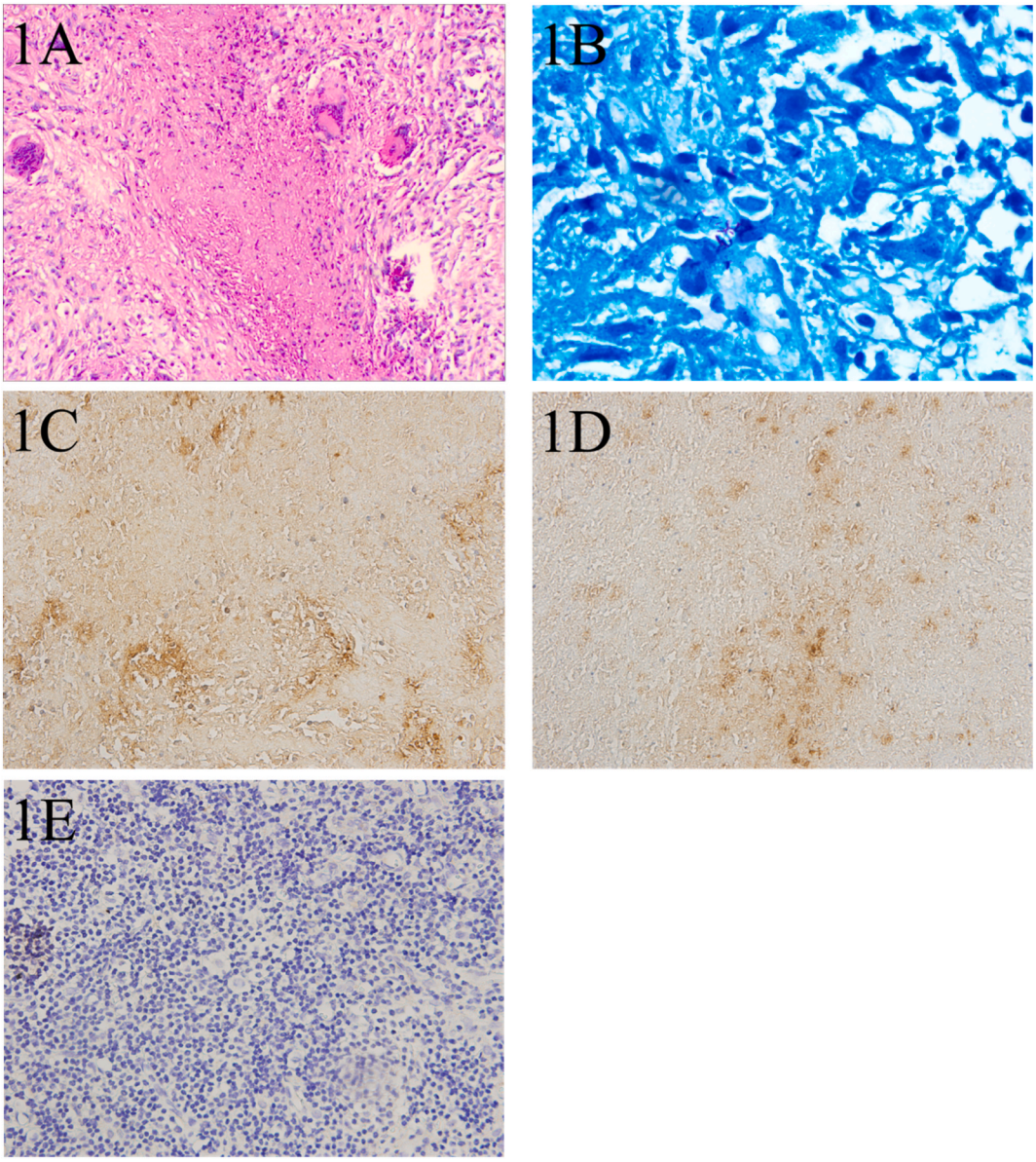

Fig. 1. Histological and Immunohistochemical Staining of Lymph Node Tissue Samples
Note: Fig. 1A–D represent serial sections from the same paraffin block. Fig. 1A illustrates H&E staining of lymph node tuberculosis tissue; Fig. 1B depicts acid-fast bacilli staining (oil immersion microscopy); Fig. 1C and D exhibit immunohistochemical staining for ESAT-6 and CFP10, respectively, with typical dark brown precipitates visible in necrotic lesions of tuberculosis(SP × 400); Fig. 1E serves as the negative control. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Comparative analysis of detection Efficiency and diagnostic value among three methods.

Diagnostics		TB N = 72	Non-TB N = 68	Sensitivity%	Specificity%	PPV%	NPV%	P-value
ZN stain	Positive	9	0	12.3	100	100	51.9	0.003
	Negative	63	68					
ESAT-6	Positive	63	10	87.5[a]	85.3	86.3	86.6	< 0.001
	Negative	9	58					
CFP10	Positive	54	7	75.0[b]	89.7	88.5	77.2	< 0.001
	Negative	18	61					

Notes: The superscript letters a and b denote $P < 0.05$ for sensitivity comparisons among the Ziehl-Neelsen stain, ESAT-6, and CFP10. Abbreviations: TB, tuberculosis; Non-TB, non-tuberculous conditions; PPV, positive predictive value; NPV, negative predictive value; ESAT-6, early secretory antigenic target-6; CFP-10, culture filtrate protein-10; AFB, acid-fast bacilli.

predominantly within the necrotic zones of the tuberculosis lesions and adjacent to macrophages and multinucleated giant cells (Fig. 1C and D). The negative control immunohistochemical staining exhibited no specific pattern of distribution and presented minimal light brown signals (Fig. 1E). Within the tuberculosis group, ESAT-6 staining was positive in 63 out of 72 cases and CFP10 staining in 54. Conversely, among the 68 control cases, ESAT-6 and CFP10 staining were positive in 10 and 7 cases, respectively. ESAT-6 displayed a sensitivity of 87.5%, specificity of 85.3%, positive predictive value of 86.3%, and negative predictive value of 86.6%. CFP10 staining demonstrated a sensitivity of 75.0%, specificity of 89.7%, positive predictive value of 88.5%, and a negative predictive value of 77.2% (Table 2).

Comparison between Immunohistochemical (IHC) and Acid-Fast Staining: IHC staining for ESAT-6 and CFP10 was positive in all 9 cases with positive acid-fast staining and additionally positive in 62 and 54 cases, respectively, that were acid-fast-negative. The sensitivity of IHC surpassed that of acid-fast staining (Table 2).

3. Discussion

The definitive diagnosis of lymph node tuberculosis relies principally on pathological examination. Characteristically, granulomatous inflammation, with or without caseous necrosis, is identified, yet such patterns are not exclusive to tuberculosis and may also manifest in conditions such as non-tuberculous mycobacterial disease [14], fungal infections [15], leprosy [16], sarcoidosis [17], and foreign body granuloma [18], among others. Thus, integrating pathological findings with mycobacterial etiology is pivotal for enhanced diagnostic precision.Acid-fast staining is a conventional method for pathogen detection in laboratory settings, albeit with a variable positive rate of 0%–34.4% as documented in literature [7,9,19]. This study corroborates these findings, demonstrating a 12.3% positive rate (9/72) for acid-fast staining, aligning with previous research.

Immunohistochemical (IHC) analysis in this study revealed that ESAT-6 and CFP10 antigens predominated in the necrotic regions of tuberculous lesions and were associated with macrophages and multinucleated giant cells. In contrast, the control group (comprising lymphoma cases) exhibited no or minimal light brown staining, without a specific pattern. The sensitivity and specificity of IHC for ESAT-6 were 87.5% and 85.3%, respectively, which is generally consistent with prior studies: S. Sumi et al. [20] reported a sensitivity of 88.6% and specificity of 100% using IHC for ESAT-6 in lymph node tuberculosis; whereas studies by Sun Li [21], Nan Zhao [22], and Yong Fang [23] on renal tuberculosis tissue yielded a sensitivity range of 75%–100% and a specificity of 91.1%–94.2%. The examination of CFP10 via IHC is less documented; only Yong Fang [23] reported a sensitivity of 63.9% and specificity of 84.6% in renal tuberculosis tissue. This study is novel in its detection of CFP-10 in lymph node tuberculosis tissue, achieving 75.0% sensitivity and 89.7% specificity. Discrepancies in these results may stem from variations in tissue origin or the types of antibodies utilized (monoclonal, polyclonal).

In the non-tuberculosis group, there were positive results for ESAT-6 (10 cases) and CFP10 (7 cases), with the presence of light brown particles in the lesions lacking a specific distribution pattern. This outcome was considered positive, although it was uncertain whether the staining was due to the target antigen or nonspecific. The possibility of non-specific staining or antibody cross-reactivity exists, but latent tuberculosis infection cannot be excluded. Enhancing the quality of IHC antibodies could further improve the sensitivity and specificity of immunohistochemistry in tuberculosis diagnosis.

IHC detection of both antigens significantly exceeded the sensitivity of acid-fast staining. The methodology is straightforward, feasible in standard laboratories, and does not necessitate oil immersion techniques, facilitating the identification of positive signals at high magnification, which can support the pathological diagnosis of tuberculosis, especially in culture-negative pulmonary or extrapulmonary cases.

Nonetheless, this study is not without limitations. Being retrospective, it relied on paraffin-embedded tissues, precluding bacterial culture, and lacked a definitive diagnostic gold standard for tuberculosis. Future research should entail a prospective design utilizing fresh tissue samples for comparative analyses with tuberculosis culture and molecular diagnostics to validate the antigen detection performance. Moreover, this study's use of lymphoma cases as the sole negative control and its limited sample size warrant an expansion of the comparative spectrum to include other pathologies and a larger cohort for comprehensive evaluation.

In conclusion, IHC detection of ESAT-6 and CFP-10 in paraffin-embedded lymph node tuberculosis tissue demonstrated a markedly higher sensitivity compared to acid-fast staining and could serve as an ancillary diagnostic tool.

Date availability statement

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

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CRediT authorship contribution statement

Xueqian Chen: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Shaoqi Duan:** Writing – original draft, Data curation, Conceptualization. **Xinchun Zhou:** Writing – original draft. **Shiyu Fang:** Writing – original draft. **Guihua Gu:** Writing – original draft. **Jie Sun:** Writing – original draft. **Fengjun Liu:** Writing – review & editing, Conceptualization.

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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