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ORIGINAL RESEARCH

Lymphocyte Profile Analysis in Lymph Node Puncture Fluid of Mediastinal Reactive Lymphadenopathy and Its Value in Differential Diagnosis with Tuberculous Lymphadenitis

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Background: Lymphocyte profile (LP) analysis in mediastinal lymph nodes for differentiating reactive lymphadenopathy (RL) from tuberculous lymphadenitis (TBLA) remains understudied.

Methods: Patients with intrathoracic lymphadenopathy undergoing endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) were enrolled. Lymph node puncture fluid (LNPF) was analyzed via flow cytometry to compare LP characteristics between TBLA and RL. Receiver operating characteristic (ROC) analysis identified optimal diagnostic cut-offs, and sensitivity, specificity, and accuracy were calculated.

Results: A total of 41 TBLA cases and 45 RL cases were included. Compared with the TBLA group, the RL group exhibited increased proportions of $CD4^+$ T cells and B cells, and decreased proportions of $CD8^+$ T cells, natural killer (NK) cells, and natural killer T (NKT) cells in LNPF. Among single parameters, the CD4/CD8 ratio demonstrated the highest diagnostic performance for TBLA, with sensitivity of 88.89%, specificity of 70.73%, and accuracy of 80.23%. Among three-parameter combinations, the $CD4^+$ T cell, $CD8^+$ T cell, and NK cell ratio combination achieved optimal diagnostic performance, with sensitivity of 80.5%, specificity of 86.7%, and accuracy of 82.6%.

Keywords: tuberculous lymphadenitis, lymphocyte profile, mediastinal lymph node, reactive lymphadenopathy, lymph node puncture fluid

Introduction

Intrathoracic lymphadenopathy (IL) can arise from malignant or benign etiologies[1]. Benign etiologies encompass granulomatous disorders (eg, tuberculosis and sarcoidosis) and other inflammatory conditions, which require distinct therapeutic strategies, posing significant challenges for both pathologists and clinicians.[1–3]

Among granulomatous diseases, mediastinal tuberculous lymphadenitis (TBLA) presents diagnostic difficulties due to nonspecific clinical manifestations and challenges in obtaining adequate lymph node samples for microbiological and pathological analysis.[4] TBLA is typically paucibacillary, further complicating microbiological diagnosis.[5]

Lymph node enlargement may also occur as a reactive response to underlying comorbidities, designated as reactive lymphadenopathy (RL). RL has been associated with various chronic conditions, including emphysema, chronic bronchitis,[6,7] interstitial lung disease,[8,9] bronchiectasis,[10] pulmonary arterial hypertension,[11] heart failure,[12] and connective tissue diseases.[13]

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Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is currently the preferred minimally invasive technique for evaluating undiagnosed mediastinal adenopathy, diagnosing sarcoidosis and TBLA, and staging lung cancer. Specimens obtained via EBUS-TBNA enable multi-modal analysis, including cytology, flow cytometry, immunohistochemistry, molecular testing, and microbiological assays (eg, GeneXpert MTB/RIF).[14]

To date, the lymphocyte profile (LP) characteristics in LNPF of patients with mediastinal RL remain unreported, and the value of LP analysis in differentiating RL from TBLA has not been comprehensively investigated.

Materials and Methods

Study Patients

This prospective diagnostic accuracy study consecutively enrolled 396 patients with enlarged mediastinal/hilar lymph nodes and pulmonary lesions detected via chest computed tomography (CT) at our hospital between May 2020 and April 2024. Following rigorous selection criteria, 41 patients with tuberculous lymphadenitis (TBLA)[15] and 45 patients with reactive lymphadenopathy (RL) were finally included (Figure 1). All RL patients required a minimum of 6 months of follow-up. A diagnosis of RL was confirmed only if: (1) pathological sampling (including lymph node aspiration, biopsy, or cytology) failed to identify an alternative diagnosis; (2) clinical follow-up for ≥6 months did not reveal any new diagnostic findings; and (3) the treating physician excluded other etiologies based on clinical, radiological, and laboratory evaluations. Among the excluded cases was one patient with a final diagnosis of TBLA who could not be included in the analysis due to loss to follow-up, resulting in incomplete clinical data.

Lymph node histopathology in RL cases exhibited features consistent with reactive hyperplasia, including: (1) follicular hyperplasia with increased follicle count, variable size/shape, and distinct boundaries; (2) prominent germinal centers with abundant transformed lymphocytes (large nuclei, frequent mitoses, and phagocytic cells containing cellular debris); (3) a surrounding mantle zone of small lymphocytes; (4) interfollicular infiltration of plasma cells, histiocytes,

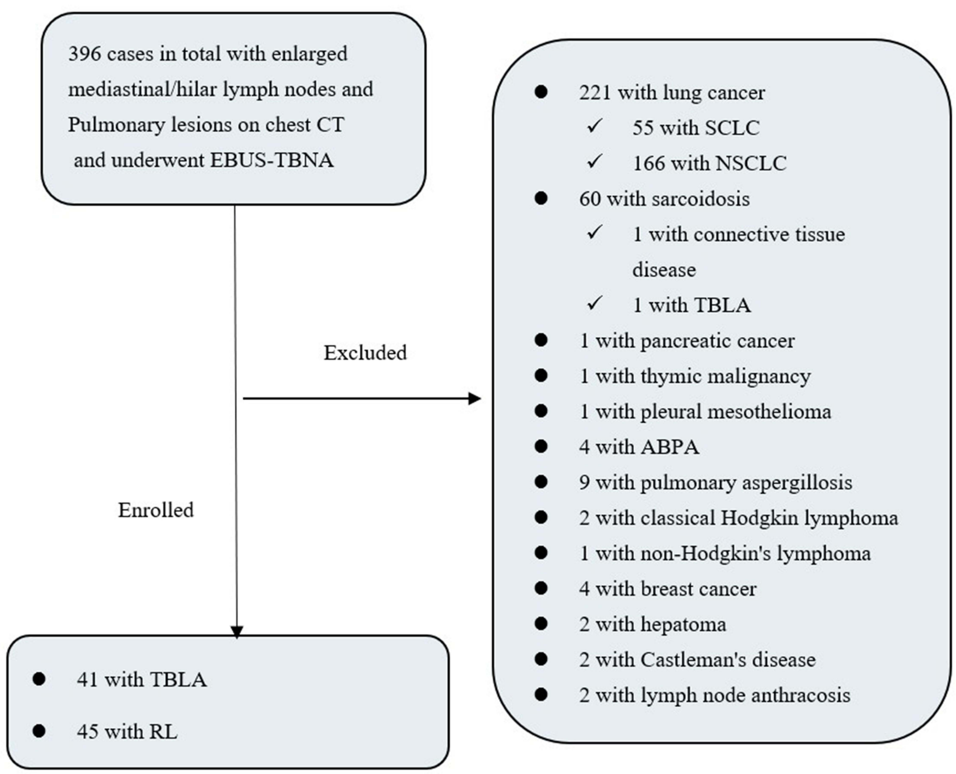

Figure 1 Study Flow Chart for Patient Inclusion.

Abbreviations: ABPA, allergic bronchopulmonary aspergillosis; CT, computed tomography; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; NSCLC, non-small cell lung cancer; RL, reactive lymphadenopathy; SCLC, small cell lung cancer; TBLA, tuberculous lymphadenitis.

and occasional neutrophils/eosinophils; and (5) proliferation of reticular cells and endothelial cells within lymphatic sinuses.[16]

Exclusion criteria were: (1) pregnancy; (2) acute infectious diseases or use of immunosuppressants/immunostimulants within 2 weeks prior to enrollment; (3) severe comorbidities or organ system dysfunction; (4) incomplete clinical data; (5) loss to follow-up; (6) confirmed AIDS diagnosis.

Demographic and clinical data were collected and analyzed. This study was conducted in accordance with the ethical principles outlined in the 1964 Declaration of Helsinki and its subsequent amendments and was approved by the Ethics Committee of our Hospital (Approval No. ke-616). Written informed consent was obtained from all patients or their legal guardians.

EBUS-TBNA Procedure

EBUS-TBNA was performed by operators with proven proficiency using dedicated EBUS equipment (Olympus Medical Systems, Japan) and 21G fine needles. The procedure was conducted in a bronchoscopy suite under intravenous anesthesia with propofol and fentanyl.

At least six needle passes were obtained: four from one lymph node, two from another, or six from a single accessible node. Aspirated samples were expelled into a sterile collection bottle containing 20 mL normal saline, with the needle rinsed into the bottle to recover residual cells. Solid tissue fragments were retrieved using a sterile needle, fixed in 10% buffered formalin, and used for routine histopathology and acid-fast staining. The remaining 20 mL fluid (lymph node puncture fluid, LNPF) was aliquoted: 5 mL for flow cytometry, 5 mL for cytology, and 5 mL for *Mycobacterium tuberculosis* (Mtb) DNA and rifampicin resistance gene detection via the GeneXpert MTB/RIF platform (GeneXpert® DX System R2, GX-XVI, Cepheid, CA, USA).

TBLB and EBB Procedure

Endobronchial biopsy (EBB) and transbronchial lung biopsy (TBLB) were performed at the bronchoscopist's discretion. EBB was indicated for endobronchial abnormalities or clinical suspicion of sarcoidosis; TBLB was performed for parenchymal lesions on CT. Specimens underwent histopathological examination and acid-fast staining.

Flow Cytometry of LNPF

LNPF was filtered through a metal mesh and centrifuged (300g, 1296 rpm) to pellet cells. The supernatant was discarded, and the cell pellet was resuspended in phosphate-buffered saline (PBS), centrifuged again, and resuspended for flow cytometry. Analysis was performed using a Beckman Coulter DXFlex flow cytometer (Beckman Coulter Commercial Enterprise Co, CA, USA) with monoclonal antibodies against CD3, CD4, CD8, CD16, CD19, and CD56 (same manufacturer). To minimize the impact of variable LNPF dilution, lymphocyte subsets (eg, $CD4^+$ T cells) are reported as percentages of total lymphocytes.

Tuberculin Skin Test (TST)

TST was performed with 5 tuberculin units of purified protein derivative (PPD). Induration diameters were measured at 48 and 72 hours post-administration, with positivity defined as induration ≥10 mm.

GeneXpert MTB/RIF Assay

Detection of Mtb DNA and rifampicin resistance genes in LNPF was performed using a standardized protocol on the GeneXpert MTB/RIF platform, primarily for patients suspected of non-neoplastic diseases (eg, sarcoidosis, tuberculosis, RL).

Histological Examination

Routine histopathological analysis was performed on specimens from TBNA, TBLB, EBB, and superficial lymph nodes. Hematoxylin and eosin (H&E) staining was performed first, followed by immunohistochemistry as needed. Acid-fast staining was applied to TBNA, TBLB, and EBB specimens. In cases where granulomatous inflammation was observed, Periodic acid-Schiff (PAS) and Grocott's methenamine silver (GMS) staining were also performed to rule out fungal infections.

Statistical Analysis

Categorical variables are presented as counts and percentages (%). Continuous variables are reported as mean ± standard deviation (SD) or median with interquartile range (IQR). Normality was assessed using the Shapiro–Wilk test. Group differences (TBLA vs RL) were evaluated using the two-sample *t*-test for normally distributed variables and the Mann–Whitney *U*-test for non-normally distributed variables.

Receiver operating characteristic (ROC) curve analysis was conducted to determine optimal cut-offs for TBLA screening using statistically significant parameters. Sensitivity, specificity, accuracy (π), and area under the ROC curve (AUC) were calculated. The sample size was prospectively determined based on an a priori power analysis. To detect a difference in the CD4/CD8 ratio between the TBLA and RL groups with a statistical power of 80% and a two-sided significance level of 0.05, a minimum of 40 patients per group was required. All statistical analyses were performed using SPSS software (version 22.0; IBM Corp., Armonk, NY, USA). A two-tailed p-value <0.05 was considered statistically significant.

Results

Demographics and Clinical Information

A total of 396 consecutive patients with enlarged mediastinal/hilar lymph nodes and pulmonary lesions (detected via chest computed tomography, CT) underwent endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA). After exclusion of malignancies and other non-reactive/non-tuberculous etiologies, 41 patients with tuberculous lymphadenitis (TBLA) and 45 patients with reactive lymphadenopathy (RL) were enrolled (Figure 1).

The 45 RL cases were associated with diverse chronic conditions: interstitial lung disease (ILD; 33 cases, including 11 with connective tissue disease-related ILD, 9 with cryptogenic organizing pneumonia, 4 with antineutrophil cytoplasmic antibody-associated vasculitis, 4 with idiopathic interstitial pneumonia, 2 with allergic pneumonia, 2 with eosinophilic granulomatosis with polyangiitis, and 1 with IgG4-related disease), chronic bronchitis (5 cases), bronchiectasis (4 cases), and chronic obstructive pulmonary disease (3 cases).

The demographic and clinical characteristics of the TBLA and RL groups are summarized in Table 1. The two groups were comparable in terms of age, sex, smoker status, and BMI. However, the TBLA group showed significantly higher

Table 1 Demographics and Clinical Characteristics of Study Participants

Variable	TBLA (n=41)	Reactive Lymphadenopathy (n=45)	P value
Age (years, mean [range])	60.9 (23–84)	60.6 (37–78)	0.867
Sex (M/F)	18/23	29/16	0.129
Smoker status (current/former/never)	9/6/26	13/6/26	0.640
BMI (mean ± SD)	24.11 ± 3.61	25.22 ± 3.61	0.833
ESR (mm/h, mean ± SD)	42.5 ± 18.2	21.3 ± 11.5	<0.001
CRP (mg/L, median [IQR])	28.5 [15.0, 45.0]	9.8 [5.2, 16.5]	<0.001
Positive TST (≥++) [n]	30/41	3/45	–
GeneXpert MTB/RIF positive [n]	32/41	0/45	–
Acid-fast staining positive [n]	12/41	0/45	–
Non-necrotizing granuloma [n]	8/41	2/45*	–
Necrotizing granuloma [n]	14/41	0/45	–
Granuloma detected [n]	22/41	2/45*	–
TBNA granuloma [n]	14/41	2/45*	–
TBLB granuloma [n]	8/12	0/45	–
EBB granuloma [n]	8/13	0/45	–

Note: *Atypical non-necrotizing granuloma.

Abbreviations: BMI, body mass index; CRP, C-reactive protein; EBB, endobronchial biopsy; ESR, erythrocyte sedimentation rate; TBLA, tuberculous lymphadenitis; TBNA, transbronchial needle aspiration; TBLB, transbronchial lung biopsy; TST, tuberculin skin test.

Table 2 Diagnostic Profile of Tuberculous Lymphadenitis Patients (n=41)

Diagnostic Test	N (%) of Positive cases (n=41)
Pathology	
Granuloma Detected	22 (53.7)
- Necrotizing Granuloma	14 (34.1)
- Non-necrotizing Granuloma	8 (19.5)
Microbiology & Molecular	
Acid-Fast Staining Positive	12 (29.3)
GeneXpert MTB/RIF Positive	32 (78.0)
- Rifampicin resistance detected	0 (0)
- DNA Load: Very Low (of 32 positives)	15 (46.9)
- DNA Load: Low (of 32 positives)	11 (34.4)
- DNA Load: Medium (of 32 positives)	6 (18.8)
Immunology	
Tuberculin Skin Test (TST) Positive (≥++)	30 (73.2%)
Test Combination Diagnostic Yield	
At least one positive (GeneXpert, AFB, or Granuloma)	37 (90.2)
GeneXpert-positive AND AFB-negative	23 (56.1)
Granuloma-positive AND GeneXpert-negative	3 (7.3)

Note: Percentages for DNA load are calculated out of the 32 GeneXpert-positive cases. All other percentages are out of the total 41 TBLA cases. Combinations were selected to highlight the complementary value of molecular tests, microbiology, and histopathology in diagnosing TBLA.

Abbreviations: AFB, acid-fast bacilli; TBLA, tuberculous lymphadenitis.

levels of inflammatory markers, including Erythrocyte Sedimentation Rate (ESR) and C-reactive protein (CRP), compared to the RL group ($P < 0.001$ for both).

A detailed diagnostic profile of the 41 TBLA patients is presented in Table 2. Granulomas were detected in 22 patients (53.7%), of which 14 (34.1%) were necrotizing. Acid-fast staining was positive in 12 patients (29.3%), and GeneXpert MTB/RIF assay was positive in 32 patients (78.0%). Among the 32 TBLA patients who were positive by GeneXpert MTB/RIF, no cases of rifampicin resistance were detected. The semi-quantitative results indicated a paucibacillary state in most cases, with DNA loads categorized as very low in 15 cases (46.9%), low in 11 cases (34.4%), and medium in 6 cases (18.8%). TST was positive in 30 patients (73.2%).

Lymphocyte Profile (LP) in Lymph Node Puncture Fluid (LNPF)

LP parameters in LNPF were compared between TBLA and reactive lymphadenopathy (RL) patients (Table 3). Compared with TBLA patients, RL patients exhibited increased proportions of B cells (BC%), $CD4^+$ T cells (CD4%), and CD4/CD8 ratio, decreased proportions of $CD8^+$ T cells (CD8%), natural killer (NK) cells (NK%), and natural killer T (NKT) cells (NKT%), and no significant difference in total T cell proportion (TC%). Notably, the CD4/CD8 ratio was significantly higher in RL patients than in TBLA patients.

ROC Analysis for Single-Parameter Diagnosis of TBLA

Diagnostic performance of statistically significant LP parameters (sensitivity, specificity, accuracy, and area under the ROC curve [AUC]) is presented in Table 4. Among single parameters, the CD4/CD8 ratio demonstrated the highest diagnostic utility for TBLA, with sensitivity of 88.89%, specificity of 70.73%, accuracy of 80.23%, and AUC of 0.811 ($P<0.001$). This was followed by CD4% (AUC=0.796) and NK% (AUC=0.781). ROC curves for CD4%, CD8%, BC%, NK%, NKT%, and CD4/CD8 ratio are shown in Figure 2.

Diagnostic Performance of Three-Parameter Combinations

To enhance diagnostic precision, we analyzed combinations of three LP parameters. A patient was classified as TBLA if ≥2 parameters met their respective TBLA cut-offs. Among tested combinations, the CD4% + CD8% + NK%

Table 3 Lymphocyte Profile (LP) in Lymph Node Puncture Fluid (LNPF)

LP Parameter	TBLA (n=41)	Reactive Lymphadenopathy (n=45)	P value
LC (%)	32.55 (26.73, 40.95)	46.37 ± 15.59	0.064
TC (%)	64.28 ± 14.33	67.36 ± 14.80	0.331
CD4 (%)	36.34 ± 15.13	51.52 ± 12.40	<0.001
CD8 (%)	16.60 (13.85, 24.55)	14.43 ± 5.83	0.009
CD4/CD8 ratio	1.63 (1.13, 3.19)	3.53 (2.89, 5.76)	<0.001
BC (%)	14.30 (6.35, 30.45)	23.52 ± 13.15	0.042
NK (%)	5.40 (2.20, 8.00)	1.70 (1.06, 3.20)	<0.001
NKT (%)	3.50 (0.90, 6.65)	1.10 (0.60, 2.35)	0.015
NKT/NK ratio	0.57 (0.24, 0.88)	0.63 (0.22, 1.75)	0.557

Abbreviations: BC%, B cell proportion; CD4%, $CD4^+$ T cell proportion; CD8%, $CD8^+$ T cell proportion; LC%, lymphocyte/total cell ratio; NKT%, natural killer T cell proportion; NK%, natural killer cell proportion; TC%, T cell ($CD3^+CD19^-$)/lymphocyte ratio.

Table 4 Diagnostic Accuracy of Single LP Parameters for TBLA

LP Parameter	Sensitivity (%)	Specificity (%)	Accuracy (π, %)	AUC (P value)	Cut-off
CD4%	95.56	60.98	77.38	0.796 (<0.001)	37.90
CD8%	88.89	41.46	65.48	0.664 (0.009)	19.70
BC%	73.33	63.41	68.60	0.627 (0.042)	17.30
NK%	91.11	58.54	77.91	0.781 (<0.001)	4.50
NKT%	88.89	39.02	65.12	0.652 (0.015)	3.85
CD4/CD8 ratio	88.89	70.73	80.23	0.811 (<0.001)	2.33

Abbreviations: AUC, area under the ROC curve; BC%, B cell proportion; CD4%, $CD4^+$ T cell proportion; CD8%, $CD8^+$ T cell proportion; LP, lymphocyte profile; NKT%, natural killer T cell proportion; NK%, natural killer cell proportion; TBLA, tuberculous lymphadenitis.

combination achieved the highest accuracy (82.56%), with sensitivity of 80.5%, specificity of 86.7%, and AUC of 0.843 (95% CI: 0.755–0.932; Table 5).

Discussion

Our results demonstrated that compared with tuberculous lymphadenitis (TBLA) patients, reactive lymphadenopathy (RL) patients exhibited increased proportions of B cells (BC%), $CD4^+$ T cells (CD4%), and CD4/CD8 ratio, along with decreased proportions of $CD8^+$ T cells (CD8%), natural killer (NK) cells (NK%), and natural killer T (NKT) cells (NKT %) in lymph node puncture fluid (LNPF). No significant difference was observed in total T cell proportion (TC%). Notably, the CD4/CD8 ratio was significantly higher in RL patients than in TBLA patients. These findings highlight the potential of lymphocyte profile (LP) analysis in LNPF as a complementary tool for differentiating RL from TBLA, with single parameters such as the CD4/CD8 ratio (accuracy: 80.23%) and NK% (accuracy: 77.91%) showing robust diagnostic performance. Furthermore, combining CD4%, CD8%, and NK% enhanced diagnostic precision (accuracy: 82.56%), underscoring the value of multi-parameter analysis.

Global Tuberculosis Burden and Diagnostic Challenges

Since 2020, global tuberculosis (TB) incidence has rebounded, with 10.8 million cases (95% uncertainty interval [UI]: 10.1–11.7 million) reported in 2023, re-establishing TB as the leading infectious cause of death worldwide after a 3-year hiatus due to COVID-19.[17] Among extrapulmonary TB, TBLA remains diagnostically challenging due to nonspecific clinical features and limitations of conventional methods. Current diagnostic gold standards rely on detecting *Mycobacterium tuberculosis* (Mtb) in sputum, bronchoalveolar lavage fluid (BALF), or tissue via acid-fast staining, molecular assays, or culture. However, these methods are constrained by low sensitivity (eg, sputum smear microscopy: 32–94% pooled sensitivity;[18] Mtb culture in TBLA: ~50% sensitivity[19]) and prolonged turnaround times. The Xpert

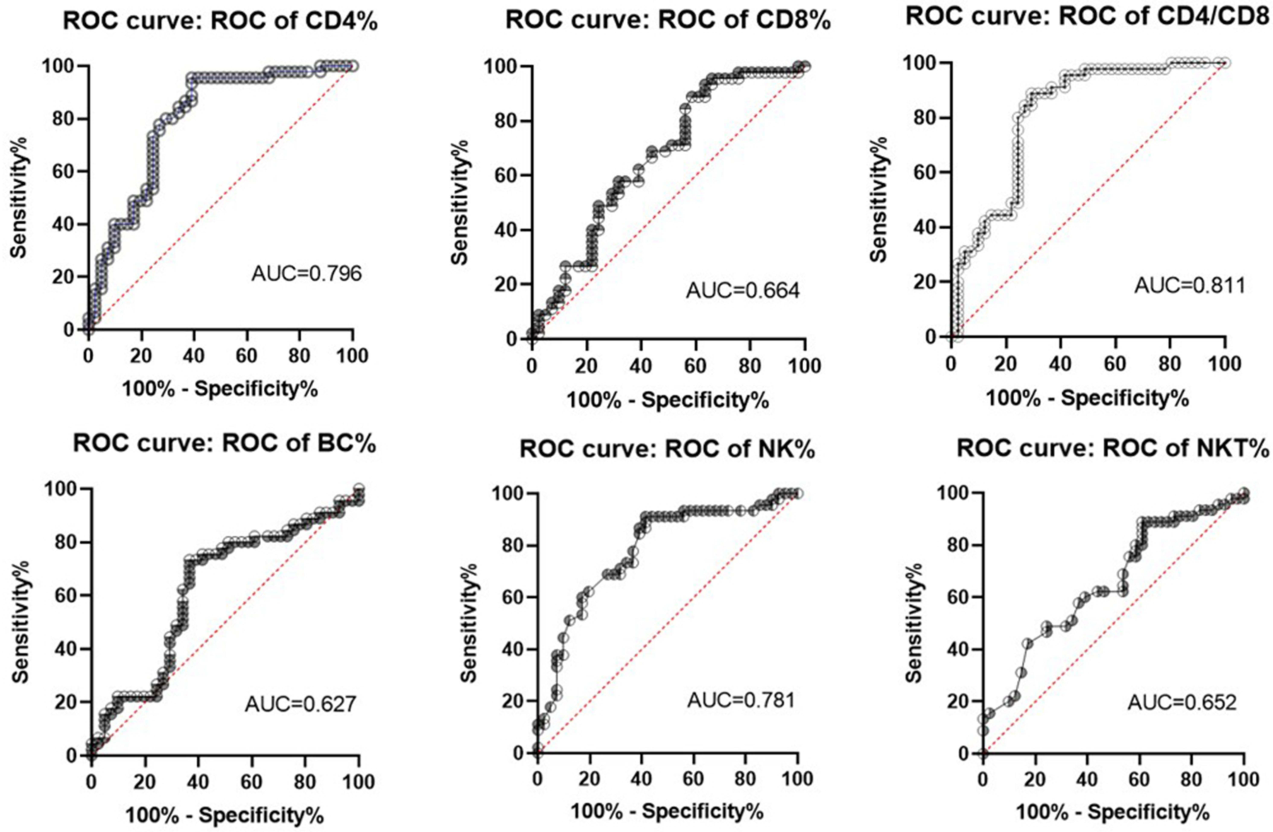

Figure 2 ROC Curves of LP Parameters for TBLA Diagnosis.
Abbreviations: LP, lymphocyte profile; ROC, receiver operating characteristic; TBLA, tuberculous lymphadenitis.

MTB/RIF assay, a WHO-recommended rapid PCR-based test targeting the *rpoB* gene, enables concurrent detection of Mtb and rifampicin resistance but has variable performance in extrapulmonary TB.[20] For instance, in mediastinal lymphadenopathy, Xpert MTB/RIF in LNPF showed 49.1% sensitivity and 97.9% specificity for TB, with occasional false positives in sarcoidosis.[21] These limitations emphasize the need for adjunctive diagnostic tools, particularly when histopathology lacks granulomatous features.

LP Analysis in LNPF: A Novel Diagnostic Avenue

EBUS-TBNA has emerged as a key modality for sampling mediastinal lymph nodes, yielding both tissue and LNPF for multi-modal analysis. While prior studies focused on LNPF for bacteriological testing,[22–24] our study expands its utility to LP analysis via flow cytometry. This approach addresses a critical gap: RL diagnosis traditionally relies on histopathology and exclusion of malignancies or TB, with no prior reports on LP in mediastinal RL.

Notably, our findings align with and extend prior work on CD4/CD8 ratios in mediastinal lymph nodes. For example, Peng et al reported higher CD4/CD8 ratios in sarcoidosis than TB (7.3±1.8 vs 3.6±1.1, $P<0.001$) using

Table 5 Diagnostic Accuracy of Three-Parameter Combinations for TBLA

Parameter Combination	AUC	95% CI	Sensitivity (%)	Specificity (%)	Accuracy (π, %)
CD4% + CD8% + NK%	0.843	(0.755–0.932)	80.50	86.70	82.56
CD4% + CD8% + BC%	0.820	(0.725–0.914)	75.60	88.90	82.55
CD4% + CD8% + NKT/NK ratio	0.829	(0.737–0.922)	78.00	86.70	82.35

Abbreviations: AUC, area under the ROC curve; BC%, B cell proportion; CD4%, $CD4^+$ T cell proportion; CD8%, $CD8^+$ T cell proportion; NKT%, natural killer T cell proportion; NK%, natural killer cell proportion; TBLA, tuberculous lymphadenitis.

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immunohistochemistry,[25] consistent with our prior observations.[26] By using flow cytometry on LNPF, we provide a more objective, quantitative assessment of lymphocyte subsets, avoiding potential biases from tissue section sampling variability.

Biological Rationale for LP Differences

The observed LP differences likely reflect distinct immune responses in TBLA and RL. In TBLA, NK cells—key effectors of innate immunity—play a critical role in controlling Mtb infection by directly killing Mtb and Mtb-infected cells via surface receptors (eg, NKG2D, DNAM-1).[27] Elevated NK% in TBLA may thus reflect heightened anti-mycobacterial immune activation. For instance, previous studies have shown that NK cell activating receptors are downregulated in active TB, potentially as a result of chronic engagement with Mtb-infected cells, yet their overall proportion may increase in the localized lymph node environment.[28,29] Conversely, RL, driven by reactive lymphocyte proliferation to chronic stimuli (eg, ILD, COPD), is characterized by Th2-polarized or regulatory immune responses, which may suppress NK and $CD8^+$ T cell activation.[30,31] The higher CD4/CD8 ratio in RL aligns with this Th2 bias, as $CD4^+$ T cells dominate in non-infectious, reactive lymphoid hyperplasia.[32]

Clinical Utility and Practical Implications

LP analysis in LNPF offers several advantages: (1) It can be integrated into routine EBUS-TBNA workflows without additional needle passes, as LNPF is already collected for cytology and microbiology; (2) Flow cytometry is widely available in tertiary centers, making this method scalable; (3) Quantitative LP parameters (eg, CD4/CD8 ratio) provide objective metrics to complement subjective histopathological assessment. It is important to position LP analysis as an adjunctive diagnostic tool rather than a replacement for conventional methods. Its greatest utility may lie in diagnostically challenging situations, such as in patients with non-necrotizing granulomas, paucibacillary disease where microbiological tests are negative, or in immunosuppressed individuals where immune responses are atypical. It complements, but does not replace, bacteriological confirmation and drug susceptibility testing. Challenges remain in resource-limited settings, where EBUS-TBNA and flow cytometry may be less accessible. Future studies should explore simplified LP panels or point-of-care assays to enhance applicability.

Limitations and Future Directions

This study has several limitations. First, the sample size (41 TBLA vs 45 RL) was relatively small, potentially limiting statistical power and generalizability, although it met the requirements of our a priori power calculation. While we included RL cases from diverse chronic conditions (eg, ILD, COPD), viral/bacterial infectious etiologies were under-represented, which may affect the generalizability of LP patterns. Second, the relatively small sample size precluded multivariate analysis, limiting our ability to identify optimal parameter combinations. Third, single-center enrollment may introduce institutional bias, despite using standardized EBUS-TBNA and flow cytometry protocols. Fourth, we did not specifically analyze subgroups where this test might be most useful, such as in immunocompromised patients or in cases with indeterminate histopathology, which represents an important area for future investigation.

To address these, future studies should: (1) Enroll larger, multi-center cohorts with RL cases from broader etiologies (eg, viral infections, bacterial lymphadenitis); (2) Perform formal power calculations to justify sample size; (3) Validate LP findings using multivariate models to identify the most predictive parameters; (4) Explore the utility of LP in dynamic monitoring (eg, response to anti-TB therapy).

Conclusion

EBUS-TBNA enables concurrent collection of tissue and LNPF, supporting multi-modal analysis including LP via flow cytometry. Significant differences in LP parameters (eg, CD4/CD8 ratio, NK%) between TBLA and RL enhance differential diagnostic accuracy, particularly when combined. LP analysis in LNPF represents a novel, practical tool to complement histopathology and microbiology in the diagnostic workup of TBLA, with potential to reduce misdiagnosis and guide timely treatment.

 https://doi.org/10.2147/IDR.S542415

Abbreviations

BALF, bronchoalveolar lavage fluid; CT, computed tomography; EBB, endobronchial biopsy; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; LNPF, lymph node puncture fluid; LP, lymphocyte profile; Mtb, mycobacterium tuberculosis; RL, reactive lymphadenopathy; ROC, receiver operating characteristic; TB, tuberculosis; TBLA, tuberculous lymphadenitis; TBLB, transbronchial lung biopsy; TST, tuberculin skin test.

Data Sharing Statement

Data is provided within the manuscript files; further enquiries can be directed to the lead corresponding author, Kewu Huang.

Ethics Approval and Consent to Participate

This prospective study involving human participants was approved by the ethics committees of Beijing Chao-Yang Hospital (NO. KE - 616), Capital Medical University and was in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from patients.

Consent for Publication

Written informed consent was obtained from patients.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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