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Significance of non-granulomatous cytomorphology on fine needle aspirate in lymphadenitis cases classified as tuberculous by using a composite reference standard

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Abstract

Background: Fine needle aspiration cytology (FNAC) is established as a first line investigation for tuberculous lymphadenitis (TBLA). We aimed to describe the various cytomorphologic features of tuberculosis (TB) on FNAC and their contribution in the diagnostic decision-making in suspected TBLA cases.

Methods: Patients with presumptive TBLA were prospectively enrolled (n = 266) and subjected to routine diagnostic work-up for TB, including FNAC samples, and followed until the end of treatment. Patients were categorized as TB or non-TB cases based on a composite reference standard of which the various cytomorphologic patterns were compared. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy was calculated using cross-tabulation.

Results: Fifty-six patients were categorized as bacteriologically confirmed TB, 102 as clinically confirmed TB and 108 as non-TB. The most common cytomorphologic pattern among TB cases (59%) was granulomatous inflammation with necrosis, however, about one-third of tuberculous lymphadenitis patients presented with non-granulomatous inflammation, with 21% showing only necrosis and 13% presenting with a reactive pattern. The overall sensitivity and specificity of FNAC was 85% and 66%, respectively.

Conclusions: We found that about one-third of TBLA patients presented without granulomas on FNA, highlighting the importance of considering TB in a wide spectrum of cytomorphology in a high TB burden setting. Our study supports the use of FNAC as a first-line investigation tool for diagnosing TBLA in a low-resource setting due to its relative simplicity and good sensitivity. However, the low specificity of FNAC, emphasizes the need for a second-tier confirmatory test with improved specificity.

KEYWORDS
composite reference standard, extrapulmonary tuberculosis, fine needle aspiration cytology, granulomas, tuberculous lymphadenitis

1 | INTRODUCTION

Tuberculosis (TB) remains a major cause of mortality and morbidity worldwide, with extrapulmonary TB (EPTB) accounting for approximately 16% of all TB cases globally.[1] Tuberculous lymphadenitis (TBLA) is the most common presentation of EPTB, accounting for approximately 35%, and is one of the most common causes of lymphadenopathy in a high TB endemic setting.[2]

In determining whether a lymph node swelling is due to TB infection or other etiologies, e.g. malignancy, histopathology has been central, with an emphasis on the presence of granulomas. In recent decades, however, fine needle aspiration cytology (FNAC) has emerged as a first line of investigation for routine practice[3] and is recommended by the TB control programme in India for the diagnostic work up in suspected TBLA.[4] The test has clear advantages in being relatively cheap, without the need for advanced laboratory facilities, and could provide an alternative diagnosis within hours. Furthermore, it is minimal invasive and with reduced risk of serious complications compared to excision biopsies.[5] Albeit not as accurate as a biopsy to differentiate between *m. tuberculosis* and other granulomatous conditions, the high cost of molecular based tests, and poor sensitivity of routine TB diagnostic tools to analyze paucibacillary lesions supports FNAC as a first choice of investigations for clinicians in routine practice in low-resource settings.[6–8]

Similarly to histopathology, the typical cytomorphology of TB on FNAC have been centered around the findings of granulomas or epithelioid cells with or without necrosis. This reliance on the presence of epithelioid cells as a surrogate of granulomatous patterns, however, might be a simplification, as several studies have found that presence of some atypical features on FNAC can be the only findings suggestive of TB.[2,9–11] In a TB endemic setting, features resembling suppurative lymphadenitis are known to be a challenge for pathologists to rule out TB, especially in immunocompromised patients.[9] Furthermore, reactive patterns may represent TB due to the relative small amount of tissue samples on FNA.[12]

In this study, from the high TB burden setting of India, the aim was to describe the various cytomorphologic features of TBLA on FNAC and the contribution of non-granulomatous patterns in the diagnostic decision-making in suspected TBLA cases.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

This cross-sectional study was nested in a prospective cohort study conducted at Chandrikaben Rashimikant Gardi Hospital (CRGH), associated with Ruxmaniben Deepchand Gardi Medical College (RDGMC), a 750-bed referral and teaching hospital in Ujjain, Madhya Pradesh, India. The hospital serves the semi-urban and rural population of Ujjain city and the Palwa region.

Patients with a clinical presentation considered suggestive of TBLA by clinicians at CRGH were included in the study, and were prospectively enrolled from in-and outpatient departments between April 2018 and February 2020. Exclusion criteria were insufficient material for investigation on FNAC or receiving anti-tuberculous treatment at the time of enrollment. Informed written consent was obtained from all participants. None of the invasive procedures were performed for research purposes only. Verbal and written consent was obtained prior to HIV testing. The study was approved by the Regional Committee for Medical Research Ethics in Norway, REK Helse-Vest (2014/46/REK vest) and by the ethical committee in India (IEC 10/2018).

2.2 | Data collection

Detailed demographic information, clinical history and examination of all recruited patients was recorded by using a pre-designed questionnaire. Patients were interviewed in Hindi or in their local language. The presence, nature and duration of constitutional and local symptoms were specifically inquired.

Site, size and appearance of lymph nodes were recorded, blood samples were collected and radiological investigations including chest X-ray and/or ultrasound were performed for all patients. In some cases, computed tomography was available.

All patients were followed up regularly until the completion of treatment. At follow-up, clinical parameters for assessing response to treatment, including signs and symptoms, weight, and lymph node size were recorded. Patients not on anti-tuberculous treatment were followed up for 6 months after enrollment.

2.3 | Diagnostic samples and tests

Fine needle aspiration from lymph nodes was performed under sterile conditions, using a 21 G needle attached to a 10 mL disposable syringe. Specimen from lymph nodes were subjected to the routine diagnostic work-up; one of the smears was stained with May Grunwald-Giemsa for cytology, and one was stained with Ziehl-Neelsen (ZN) for demonstration of acid-fast bacilli (AFB). Criteria for a positive result ranged from 1–2 bacilli per 100 fields (100X) (weakly positive) to >9 bacilli per field (100X) (strongly positive).[13] The material from the syringe was used for Lowenstein-Jensen (LJ) culture, and GeneXpert MTB/RIF assay (Cepheid GeneXpert® System -Xpert). Sputum was also collected from most patients for Xpert, LJ culture and AFB microscopy.

On microscopic examination, the cytomorphological features for diagnosing TB were classified into four patterns according to necrosis and arrangement of cells; (1) granulomatous inflammation (presence of epithelioid cells with or without multinucleated cells) without necrosis, (2) granulomatous inflammation with necrosis, (3) only necrosis (without predominance of neutrophils) and (4) caseous necrosis with or without presence of lymphocytes.[2,14] The interpretation

TABLE 1 Criteria for categorization of patients into various categories of the composite reference standard

Bacteriologically confirmed TB case	Positive mycobacterial LJ culture and/or *M. tuberculosis* detected by the GeneXpert MTB/Rif assay
Clinically confirmed TB case	Clinical presumptive EPTB patient started on anti-tuberculous treatment (ATT) with a good response[a] at 2/3 months and/or at end of treatment.
Non-TB case	Patient started on ATT based on clinical presumptive EPTB but did not respond to treatment OR Improvement without ATT and/or response to specific non-tuberculous therapy OR Alternative diagnosis concluded by the clinician

[a]A good response to treatment was considered when a minimum of three of the following was recorded at 2/3 months and/or at end of treatment: (1) Improvement in systemic and local symptoms, (2) any weight gain, (3) reduction in lymph node size, (4) improvement in subjective score using VAS and EQ-5D.

Abbreviations: EPTB, Extrapulmonary tuberculosis; LJ culture, Lowenstein-Jensen culture.

was performed by a pathologist blinded for the categorization of patients into the TB and non-TB groups.

Mycobacterial culture from lymph nodes was performed at the microbiology laboratory at RDGMC. The smears were incubated at 37 °C on Lowenstein-Jensen medium and cultured for 8 weeks. The Xpert assay was performed according to protocols by the WHO at RDGMC.[15]

Gram staining was performed by the pathologist in some cases. When a Mantoux test was performed, a skin reaction size of >15 mm at 48 h was considered positive.[16]

A random blood glucose or two-hour plasma concentration after oral glucose tolerance test of above 11.1 mmoL/L was considered diagnostic for diabetes mellitus (DM).[17]

2.4 | Patient categorization

A composite reference standard (CRS) was used to categorize the study participants into the bacteriologically confirmed TB, clinically confirmed TB and non-TB groups based on various diagnostic criteria (Table 1). The categorization was done at the end of follow-up and without the FNAC result included in the CRS.

2.5 | Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) for Windows, version 28. Chi square test was done to assess differences in categorical variables. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy was calculated using cross-tabulation. A p-value <0.05 was considered statistically significant.

3 | RESULTS

A total of 266 patients were included in the study. Based on the CRS, 158 (59%) were classified as TB patients, with 56 (21%) categorized as bacteriologically confirmed TB and 102 (38%) as clinically confirmed TB. The non-TB group consisted of 108 (41%) patients, serving as control group.

3.1 | Baseline characteristics

There was a significantly higher proportion of females in the TB group compared to the non-TB group (Table 2). The age distribution varied significantly between the groups with most of the TB patients (54%) being between 15–29 years. Few patients presented with HIV or DM comorbidity in both groups. Most of the patients in our cohort were underweight.

A significantly higher proportion of TB patients (85%) presented with systemic symptoms compared to 75% in the non-TB group. Regarding local signs, a significantly higher proportion of TB patients presented with lymph node enlargement in the posterior cervical triangle. No significant difference between the groups was observed in lymph node tenderness, discharge, or matted lymph node. The most common finding was unilateral lymph node enlargement, found in more than 90% in both groups.

The mortality in the cohort was low, 0.63% and 0.83% among TB and non-TB patients respectively.

3.2 | Cytologic findings and diagnostic validity of FNAC

On microscopic examination (Figure 1), the cytological findings compatible with TB were classified into four patterns according to necrosis and arrangement and composition of cells. The distribution of these four patterns according to TB category based on the CRS is shown in Table 3. The most common pattern was granulomatous inflammation with necrosis found in 59% of the TBLA cases, followed by necrosis only (21%). Granulomatous inflammation without necrosis (6%) was the least common finding. Non-TB cytomorphology was reported in 15% of CRS positive cases, with reactive patterns constituting 13% of these cases. In the bacteriologically confirmed TB group 54/56 (96%) of cases presented with cytomorphology considered tuberculous by the pathologist.

The sensitivity, specificity and 95% C.I. for the various patterns on FNAC compared to a CRS is shown in Table 4. Overall, FNAC correctly identified TB in 85% of TBLA cases, with a specificity of 66%.

TABLE 2 Demographic and clinical characteristics ($N = 266$)

	TB ($n = 158$) *n* (%)	Non-TB ($n = 108$) *n* (%)	*P*-value
Gender			
Male	53 (34)	60 (56)	<.001*
Female	105 (66)	48 (44)	
Age group, years			
<15	25 (16)	27 (25)	.003*
15–29	86 (54)	36 (33)	
30–44	31 (20)	23 (21)	
≥45	16 (10)	22 (20)	
HIV positive[a]	6 (4)	2 (2)	.480
DM comorbidity	7 (4)	9 (8)	.189
BMI[b,c]			
<18,5	98 (66)	66 (63)	.581
≥18,5	50 (34)	39 (37)	
Duration of symptoms			
≤2 months	108 (68)	84 (78)	.092
>2 months	50 (32)	24 (22)	
Systemic symptoms[d]			
Yes	134 (85)	81 (75)	.046*
No	24 (15)	27 (25)	
Local findings			
Unilateral	143 (91)	100 (93)	.552
Bilateral	15 (9)	8 (7)	
Matted	70 (44)	37 (34)	.101
Tender	48 (30)	29 (27)	.533
Discharge	7 (4)	3 (3)	.487
Site			
Anterior triangle of neck	57 (36)	53 (49)	.043*
Posterior triangle of neck	73 (46)	39 (36)	
Outside neck	23 (15)	14 (13)	.719
Death (any)	1 (.63)	1 (.90)	

*Statistically significant.
[a]Results available from 264 patients (157 in TB group and 107 in non-TB group).
[b]BMI < 18.5 kg/m^2 considered underweight.
[c]BMI results available for 253 patients (148 in TB group and 105 in non-TB group).
[d]Any of the following: fever, loss of appetite, weight loss, night sweats, fatigue.
Abbreviations: BMI, Body Mass Index; DM, Diabetes Mellitus; HIV, Human Immunodeficiency Virus.

3.3 | Correlation between cytomorphologic patterns and various TB diagnostic tests

The correlation between cytomorphologic patterns and a positive ZN stain, Xpert and culture result is shown in Table 5. Samples containing necrotic material showed a higher correlation of ZN, culture and Xpert positivity compared to granulomatous inflammation without necrosis. ZN was positive in 31 samples, where only one was considered non-TB according to the CRS (condition improved without anti-tuberculous treatment), thus showing an excellent specificity, however, the sensitivity was only 19%. The sensitivity of Xpert and culture compared to a CRS was 34% and 27% respectively. For cases with a positive ZN stain, 84% and 77% were also positive with Xpert and culture respectively. Interestingly, one case described as having a reactive pattern on FNAC had a positive Xpert result, and one case with cytology suggestive of actinomycosis was both Xpert and culture positive. Gram staining was positive in two cases, both categorized as non-TB cases as they did not receive anti-tuberculous treatment.

We further analyzed the impact of various risk factors, clinical findings, and symptoms on FNAC cytomorphology among TB patients. The comorbidities DM, HIV and low body weight did not significantly impact the cytomorphology. Similarly, no difference was found in TB pathology between genders. When stratifying for age groups, a significantly higher proportion of patients in the oldest age group presented

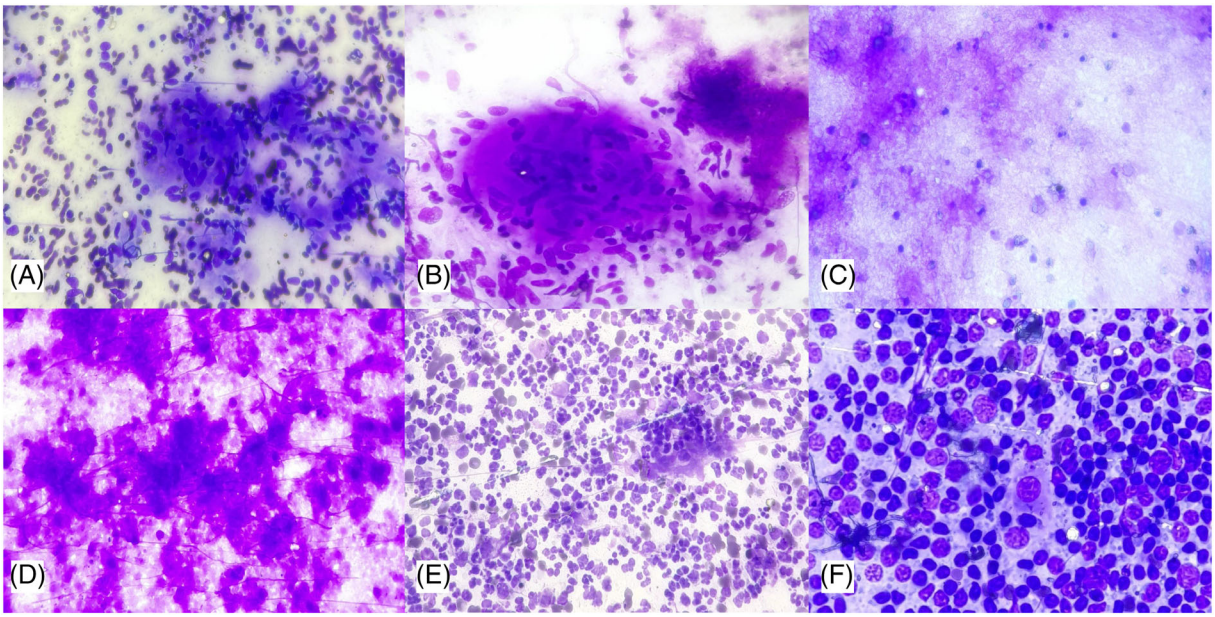

FIGURE 1 Cytomorphologic patterns. All samples stained with May Grunwald Giemsa stain. Figure 1A: Epithelioid cells without necrosis (X40). Figure 1B: Epithelioid cells with necrotic background (X40). Figure 1C: Necrosis with scanty neutrophils (X40). Figure 1D: Caseous necrosis with presence of lymphocytes (X40). Figure 1E: Non-TB abscess, showing abundant polymorphs in extensive necrotic background with nuclear debris (X40). Figure 1F: Reactive inflammation, showing mixed lymphoid populations (X40). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Cytomorphologic patterns in patient category according to a composite reference standard

FNAC pattern	TB cases ($n = 158$)			Non-TB cases ($n = 108$) *n (%)*
	Bacteriologically confirmed TB ($n = 56$) *n (%)*	Clinically confirmed TB ($n = 102$) *n (%)*	All TB cases ($n = 158$) *n (%)*	
Granulomatous inflammation without necrosis	2 (3.6)	7 (6.9)	9 (5.7)	2 (1.9)[a]
Granulomatous inflammation with necrosis	29 (51.8)	64 (62.7)	93 (58.9)	16 (14.8)[b]
Necrosis without predominance of neutrophils	15 (26.8)	3 (2.9)	18 (11.4)	14 (13.0)[c]
Caseous necrosis with or without presence of lymphocytes	8 (14.3)	7 (6.9)	15 (9.5)	5 (4.6)[d]
Reactive inflammation	1 (1.8)	19 (18.6)	20 (12.7)	55 (50.9)
Other[e-f]	1 (1.8)	2 (2.0)	3 (1.9)[e]	16 (14.8)[f]

[a]Improved without ATT ($n = 1$), NTM ($n = 1$).
[b]Improved without ATT ($n = 8$), benign tumor ($n = 1$), other infections ($n = 6$), thyroiditis ($n = 1$).
[c]Improved without ATT ($n = 1$), leprosy ($n = 1$), acute abscess ($n = 12$).
[d]Improved without ATT ($n = 2$), acute abscess ($n = 1$), other infection ($n = 2$).
[e]Actinomycosis ($n = 1$), cystic lesion ($n = 1$), malignancy ($n = 1$).
[f]Acute abscess ($n = 2$), goiter ($n = 4$), malignancy ($n = 6$), benign tumor ($n = 2$), cystic lesion ($n = 2$).
Abbreviations: ATT, Anti-tuberculous treatment; FNAC, Fine needle aspiration cytology; NTM, nontuberculous mycobacteria.

with necrosis only, while a higher proportion of pediatric patients presented with a reactive pattern.

4 | DISCUSSION

This study showed that TBLA patients present with a wide spectrum of cytomorphologic findings on FNAC. Using a CRS, 36% of TB patients presented without granulomas, and this percentage increased to 45% in the bacteriologically confirmed TB group. The inclusion of non-granulomatous necrotic patterns, which were considered TB on FNAC, affected the performance of the test. If only patterns showing granulomatous inflammation were considered diagnostic for TBLA, the sensitivity and specificity of FNAC against a CRS was 65% and 83% respectively. However, when including the necrotic patterns suggestive of TB, the sensitivity of FNAC increased to 85%, while the specificity was reduced to 66%. In comparison, the sensitivity of Xpert, ZN staining and culture against a CRS was 34%, 19% and 27%

TABLE 4 Diagnostic validity (%) of FNAC according to cytomorphology compared to a composite reference standard

FNAC pattern[a]	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	Accuracy
Granulomatous inflammation without necrosis	6 (3–11)	98 (94–100)	82	41	43
Granulomatous inflammation with necrosis	59 (51–67)	85 (77–91)	86	58	69
Necrosis without predominance of neutrophils	11 (7–17)	87 (79–93)	57	40	42
Caseous necrosis with or without presence of lymphocytes	9 (5–15)	95 (90–98)	75	41	44
All TB patterns	***85 (79–91)***	***66 (56–75)***	***79***	***75***	***78***
Reactive inflammation	13 (8–19)	49 (39–59)	27	27	27

[a]Patterns, other than the one being evaluated, are added to non-TB cases. Prevalence set to 59% (CRS positive/whole cohort).
Abbreviations: CI, Confidence interval; FNAC, Fine needle aspiration cytology; NPV, Negative predictive value; PPV, Positive predictive value.

TABLE 5 Correlation between cytomorphologic pattern, ZN stain, Xpert and LJ culture result, *n*/*N* (%)

Cytomorphologic pattern	ZN positive *n* = 31	Xpert positive *n* = 53	Culture positive *n* = 42
Granulomatous inflammation without necrosis (*N* = 11)	0 (–)	1 (9)	2 (18)
Granulomatous inflammation with necrosis (*N* = 109)	22 (20)[b]	28 (26)	24 (22)
Necrosis without predominance of neutrophils (*N* = 32)	7 (22)[c]	14 (44)	11 (34)
Caseous necrosis with or without presence of lymphocytes (*N* = 20)	2 (10)[d]	8 (40)	4 (20)
Reactive inflammation (*N* = 75)	0 (–)	1 (1)	0 (–)
Other[a] (*N* = 19)	0 (–)	1 (5)[e]	1 (5)[e]
Comparison of TB diagnostic tests			
ZN positive (*N* = 31)	-	26 (84)	24 (77)
Culture positive (*N* = 42)	24 (57)	39 (93)	-
Xpert positive (*N* = 53)	26 (49)	-	39 (74)
CRS positive (*N* = 158)	30 (19)	53 (34)	42 (27)

[a]Actinomycosis (*n* = 1), cystic lesion (*n* = 3), malignancy (*n* = 7), acute abscess (*n* = 2), goiter (*n* = 4), benign tumor (*n* = 2).
[b]AFB grading: Weakly positive (*n* = 17), Moderately positive (*n* = 5).
[c]AFB grading: Weakly positive (*n* = 7).
[d]AFB grading: Weakly positive (*n* = 1), moderately positive (*n* = 1).
[e]Actinomycosis (*n* = 1).
Abbreviations: AFB, Acid-fast bacilli; CRS, Composite reference standard; LJ, Lowenstein-Jensen; Xpert, GeneXpert; ZN, Ziehl-Neelsen.
Note: AFB grading: Weakly positive: 1–2 bacilli per 100 fields (100X), Moderately positive: 1–9 bacilli per 100 fields (100X).

respectively. This illustrates how cytomorphologic patterns influence the performance of FNAC, and the significance of non-granulomatous patterns in increasing test sensitivity.

The important finding of the study is that about one-third of tuberculous lymphadenitis patients presented with non-granulomatous inflammation, thus suggesting that the pathologist should not only look for typical features of TB as they would appear on histology, but rather appreciate the complex presentation of TB. The cytomorphology in TB lesions is dynamic with varied cellular composition depending on the stage of disease, and a single organ may have various stages of TB lesions. It is challenging to understand and diagnose the disease from a small amount of material withdrawn from a single prick site and in relative absence of architectural pattern. In a high TB endemic setting, the major differential diagnosis to TBLA are acute infections, nontuberculous mycobacterial (NTM) infections, and malignancies, with very different treatment options.[18] By using FNAC, an invasive biopsy procedure for diagnostic purposes could be avoided, thus sparing the patient for complications and scarring. However, the features of granulomas on FNAC may be missing or atypical, resulting in reduced sensitivity. Moreover, non-specific findings resulting in a low specificity may potentially lead to a delayed diagnosis of an underlying condition.

The presence of necrosis in the absence of granulomatous inflammation in a high TB burden setting represents a challenge for the pathologist to rule out TB. Classically, caseous necrosis has been considered TB, however, a more atypical necrotic pattern, resembling abscess has been described, especially in immunocompromised.[19] In our study we proposed that the two necrotic patterns; necrosis without predominance of neutrophils and caseous necrosis with or without presence of lymphocytes could suggest TB. A high proportion of TB cases present with only necrosis in our study (21%), which is similar to other studies from high burden settings, as shown in Table 6 (23–51%).[2,19–26] Variations in the proportions of necrotic patterns could be explained by differences in the prevalence of immunocompromised included in the studies and the reference standard used. When combining the necrotic patterns with or without granulomatous inflammation across the included studies, the significance of necrosis on FNAC is further emphasized, as it is found in 71% to 83% of TB cases. Interestingly, in our cohort, 13% of TBLA patients presented with a reactive pattern on FNAC, including one case with a positive Xpert result. These patients were not considered TB by the pathologist and would not have started anti-tuberculous treatment based on FNAC alone. Similar observations have been described in other studies from TB endemic settings[12,27] and could be explained by the relatively small amount of material on FNAC leading to the granulomas present in the lymph node not being sampled. Despite challenges in sampling, FNAC remains a valuable diagnostic tool in a high TB burden setting. Our study, in concordance with literature, highlights the importance of considering TBLA also in the absence of granulomas on FNAC.

TABLE 6 Studies analyzing cytomorphology in tuberculous lymphadenitis patients from a high TB burden setting

Study (year)	Gr. Inflammation without necrosis. *n/N* (%)	Gr. Inflammation with necrosis. *n/N* (%)	Necrosis only. *n/N* (%)
Nayak et al. (2004)[a] [19]	7/42 (17)	14/42 (33)	21/42 (50)
Mittal et al. (2010)[b] [20]	6/36 (17)	17/36 (47)	13/36 (36)
Chand et al. (2013)[b] [21]	156/550 (28)	120/550 (22)	274/550 (50)
Hemalatha et al. (2014)[b] [2]	29/150 (19)	84/150 (56)	34/150 (23[d])
Masilamani et al. (2015)[b] [22]	40/212 (19)	102/212 (48)	70/212 (33)
Venkatraman et al. (2017)[b] [23]	22/132 (17)	73/132 (55)	37/132 (28)
Dasgupta et al. (2017)[b] [24]	63/257 (24)	64/257 (25)	130/257 (51)
Gupta et al. (2017)[25]	18/69 (26)	34/69 (49)	17/69 (25)
Mitra et al. (2017)[b] [26]	53/180 (29)	72/180 (41)	55/180 (30)
Present study (2023)[c]	9/158 (6)	93/158 (59)	33/158 (21[e])

[a]TB case based on positive ZN stain, histological features of caseation and granulomas or positive culture. Twenty-one HIV positive patients.
[b]TB case based on cytology.
[c]TB case based on a CRS incl. culture, Xpert, AFB, histology and response to anti-tuberculous treatment. Eight HIV positive patients.
[d]In addition, pattern with numerous macrophages (2%).
[e]In addition, reactive pattern (13%) and other patterns (2%).
Abbreviations: AFB, Acid-fast bacilli; CRS, Composite reference standard; Gr. Inflammation, Granulomatous inflammation; HIV, Human Immunodeficiency Virus; ZN, Ziehl-Neelsen.

Even in the presence of granulomatous inflammation, it can be difficult to differentiate TB from other diseases causing granulomas on FNAC, e.g., sarcoidosis, leprosy and fungal infections.[28] In our study, granulomatous inflammation was present in the majority, 102/158 (65%), of TB cases. When analyzing the diagnostic accuracy of the various morphologic patterns, granulomatous inflammation with necrosis showed a higher sensitivity (59%) than the other patterns, and a specificity of 85%. Still, even this pattern, generally considered highly suggestive of TB in a high endemic setting, yields a false positive rate of 15% in our cohort. According to clinical data for these false positive patients ($n = 16$), additional investigations revealed thyroiditis and a benign tumor in two of the cases, while the remaining cases improved without treatment or had acute infections. For non-TB cases presenting with granulomatous inflammation without necrosis on FNAC, one was diagnosed with NTM infection on culture and one improved without anti-tuberculous treatment. If a decision to treat was based solely on a FNAC result, these patients would have received anti-tuberculous treatment for at least 6 months causing unnecessary costs and morbidity. Overall, cytomorphological findings alone are not sufficient for an accurate TBLA diagnosis highlighting the importance of confirmatory tests for TB, even in a high endemic setting.

In line with existing literature, we found a higher proportion of ZN positivity among samples with necrotic material.[14,29] ZN staining improved the diagnostic accuracy, as shown by a positive ZN stain among 9/31 (29%) of patients who did not have granulomatous inflammation on FNAC. Furthermore, ZN positivity can help distinguish TB from other non-tuberculous granulomatous conditions, and thus increase the specificity of cytomorphology interpretation. Interestingly, ZN staining identified an additional four and six patients which were negative with Xpert and culture respectively. This confirms the added value of ZN staining along with cytomorphology, molecular testing and culture in the diagnosis of TBLA.

In our cohort, we found a significantly higher proportion of females in the TB group, with a male to female ratio of 1:2. A predominance of females among TBLA patients has also been shown in other studies.[30,31] Also in line with existing literature,[31] we found a significantly higher proportion of TBLA in the younger age group of 15–29 years. Regarding cytomorphology, gender did not affect the findings on FNAC. When stratifying the cohort into age groups, we found a higher proportion of patients above 45 years of age presenting with a necrotic pattern, which could be explained by a weakened immune system in the elderly.

EPTB has been shown to be more prevalent among HIV coinfected and underweight, however, in our study there was no significant difference between the TB and non-TB group regarding these known risk factors. Perhaps surprisingly, the prevalence of DM was higher in the non-TB group, although not statistically significant. This contrasts with studies on pulmonary TB (PTB) where DM is known to increase the risk of developing the disease,[32] and screening for DM is currently recommended in the TB control programme in India.[33] As a patient with both PTB and EPTB would be defined as PTB, this might also result in a lower proportion of DM among EPTB patients in our cohort. Regarding cytomorphologic features, more DM patients presented with necrosis on FNAC, while other conditions related to a reduced immune system, namely low body weight and HIV, did not impact the patterns on FNAC. This must be interpreted with caution as the prevalence of HIV and DM is low in our cohort.

Diagnosing TBLA based on symptoms without laboratory confirmation is challenging as TB may mimic a number of other conditions, e.g., sarcoidosis, neoplasm and NTM. In literature, TBLA is generally considered to present with few systemic symptoms.[34,35] Interestingly,

we found a significantly higher proportion of TBLA cases presenting with systemic symptoms when compared to the non-TB group, while no difference was observed between TB and non-TB cases regarding local findings. As TBLA is widely diagnosed clinically in rural low-resource settings, the diagnostic criteria should improve.

The strengths of this study are the relatively high number of participants being prospectively enrolled in a TB endemic setting, a long follow-up, and the comparison of FNAC performance to a CRS consisting of a wide range of diagnostic tests and clinical response to treatment. By using a CRS, we wanted to compensate for the lack of a perfect gold standard in diagnosing TBLA. However, the limitation of a CRS is the risk of misclassification bias, especially in the clinically confirmed group where response to treatment is a major criterion. Keeping in mind that rifampicin, used in first-line TB treatment, is a rather broad-spectrum antibiotic, this could result in improvement among patients with other bacterial infections. Another limitation of the study is that the protocol did not guide the clinicians on what symptoms or clinical findings were compatible with TBLA, which could lead to a variation in pre-test probability of TB for the patients enrolled. On the other hand, we wanted the data to mimic real-life practice, providing valuable data on routine TB diagnostics and management in high TB burden and limited-resource settings.

In conclusion, we found that about one-third of TBLA patients presented without granulomas on FNA, highlighting the importance of considering TB in a wide spectrum of cytomorphology in a high TB burden setting. Our study supports the use of FNAC as a first-line investigation tool for diagnosing TBLA in a low-resource setting due to its relative simplicity and good sensitivity. However, the low specificity of FNAC, emphasizes the need for a second-tier confirmatory test with improved specificity.

AUTHOR CONTRIBUTIONS

OMBH performed data curation, formal analysis, drafted the manuscript, performed literature review and edited the manuscript. MK performed data collection, investigations, sampling and reviewed the manuscript. NA performed data collection, investigations and sampling. MRP developed the methodology, supervised and edited the manuscript. TM conceptualized the study, acquired funds for the study, developed the methodology, supervised and edited the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data generated and analyzed during the study is available in the article. Further details may be available from the corresponding author upon a reasonable request.

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