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Correlation of Dark Specks with D-Dimer and IL-6 in Tuberculous and Non-Tuberculous Lymphadenitis

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Abstract: Tuberculous lymphadenitis diagnosis may be challenging when classical histopathological features are absent or equivocal. The objective of this study was to explore the correlation between dark specks observed in histopathology and circulating levels of D-dimer and IL-6 to distinguish TB lymphadenitis from non-TB cases. This research was an analytical observational study using a cross-sectional design. A total of 156 lymph node biopsy cases were analyzed in a cross-sectional study. Dark specks were evaluated by reexamination of hematoxylin-eosin-stained slides, while serum D-dimer and IL-6 levels were quantified by immunoturbidimetric and ELISA methods, respectively. Dark specks were identified in 51.9% of the samples and demonstrated a statistically significant association with tuberculous lymphadenitis ($p = 0.000008$). Cases exhibiting dark specks also showed markedly elevated serum levels of D-dimer and IL-6 ($p = 0.00027$ and $p = 0.0125$, respectively). A moderate positive correlation was noted between D-dimer and IL-6 concentrations ($\rho = 0.442$; $p < 0.001$). Receiver operating characteristic (ROC) curve analysis of the combined D-dimer and IL-6 score produced an area under the curve (AUC) of 0.987, signifying excellent diagnostic performance. It can be concluded that dark specks are significantly associated with tuberculous lymphadenitis and with higher circulating D-dimer and IL-6 levels, while the combined D-dimer–IL-6 score showed excellent discriminatory performance for differentiating tuberculous from non-tuberculous lymphadenitis.

Keywords: Tuberculous lymphadenitis; Dark specks; D-dimer; IL-6; Diagnostic biomarkers

INTRODUCTION

Lymphadenitis due to Mycobacterium tuberculosis continues to pose a substantial clinical challenge, particularly in regions with high TB prevalence. In 2024, an estimated 10.7 million people fell ill with tuberculosis worldwide, and tuberculosis caused about 1.23 million deaths, with Indonesia consistently among the highest burden countries globally.[1,2,3,4,5] Extrapulmonary tuberculosis accounts for a substantial minority of tuberculosis presentations, and lymph node tuberculosis is widely recognized as one of the most common forms of extrapulmonary disease, reported in the literature to comprise roughly one quarter up to more than half of extrapulmonary tuberculosis cases depending on setting and case mix.[1,2] While classical histopathological features, such as caseating granulomas and Langhans giant cells, typically aid in diagnosis, certain cases remain diagnostically challenging. When these hallmark features are absent or unclear, identifying alternative morphological indicators becomes essential.[1]

Dark specks, characterized by irregular, deeply pigmented granules on H&E slides, have recently drawn investigative attention. Their potential role as indicators of TB beyond mere histological findings is an area of growing interest. Nevertheless, studies correlating these features with systemic inflammatory markers are still limited.[2]

Understanding the systemic inflammatory milieu in tuberculous lymphadenitis has become increasingly important. Both D-dimer, a marker of fibrinolysis, and IL-6, a central mediator of inflammation, have been shown to rise significantly in infectious and inflammatory disorders, including active TB.[3,4] Nevertheless, the specific relationship between dark specks and systemic biomarkers such as D-dimer and IL-6 remains largely unexplored. A combined assessment of histopathological findings with systemic inflammatory markers may enhance diagnostic accuracy and help address the limitations of conventional histological criteria.[5]

Increased D-dimer concentrations observed in tuberculosis have been attributed to the widespread activation of coagulation pathways, reflecting an underlying prothrombotic state associated with the inflammatory process.[5,6], meanwhile, elevated IL-6 levels reflect the intensity of the underlying inflammatory response.[7] However, it remains unclear whether dark specks serve as histological surrogates for systemic activation. Accordingly, the relationship between tissue-level morphological features and circulating biomarkers warrants thorough investigation.[4,5,8]

Preliminary observations suggest that patients with dark specks may have elevated levels of D-dimer and IL-6 relative to those without such features. Nonetheless, in the absence of empirical validation, these assumptions remain speculative.[4,5] Given the diagnostic implications, a systematic comparison of dark specks and associated biomarker levels (D-dimer and IL-6) in tuberculous versus non-tuberculous lymphadenitis is both necessary and timely.[3,9,10]

Although histopathological examination remains central to the diagnosis of lymphadenitis, tuberculous lymphadenitis is frequently encountered in patterns in which classical features such as well-formed granulomas, overt caseous necrosis, or conspicuous Langhans-type giant cells are absent, focal, or obscured by extensive necrosis and inflammation. In such circumstances, a reproducible ancillary morphologic clue on routine hematoxylin and eosin sections would be clinically valuable, particularly if it can be linked to host response biology that extends beyond the tissue microenvironment. Dark specks, defined as fine pigmented granular structures within or adjacent to necrotic areas, have been described as a potentially helpful cytomorphologic feature in tuberculous lymphadenitis, including in children. However, the existing evidence remains limited and is largely descriptive, without systematic integration with circulating host response markers.[2] At the same time, tuberculous lymphadenitis and other forms of active tuberculosis are increasingly recognized to involve robust cytokine signaling at the site of infection and systemically, including IL-6-related pathways.[1,3,4,7,14,15,16,17,18] In parallel, inflammatory states in tuberculosis can engage coagulation and fibrinolysis pathways, supporting interest in D dimer as a marker that may capture an additional dimension of disease biology beyond histology alone.[5,6,10,11,20]

This study aims to investigate the relationship between the presence of dark specks on lymph node histopathology and circulating systemic inflammatory biomarkers, specifically D-dimer and interleukin-6 (IL-6), in patients with tuberculous and non-tuberculous lymphadenitis. Specifically, we sought to (1) determine the association between dark specks and microbiologically confirmed TB status, (2) compare serum D-dimer and IL-6 levels between cases with and without dark specks, and (3) assess the diagnostic utility of D-dimer and IL-6—individually and in combination- for discriminating tuberculous from non-tuberculous lymphadenitis.

MATERIAL AND METHOD

Study Design and Setting

A cross-sectional analytical study was conducted to explore the correlation between histopathological dark specks and serum levels of D-dimer and interleukin-6 (IL-6) in patients diagnosed with tuberculous and non-tuberculous lymphadenitis. This study was conducted at the Department of Anatomical Pathology and the Clinical Pathology Laboratory of Pasar Minggu General Hospital, Tarakan General Hospital, and Naura Medika Clinic and Lab from January 2024 to December 2024.

Participants and Sampling

The study population comprised 156 patients who underwent lymph node biopsy and received a histopathological diagnosis of lymphadenitis during the study period. Participants were recruited consecutively, with inclusion based on the following criteria: (1) availability of adequate hematoxylin-eosin (H&E) stained slides for histopathological reevaluation; (2) complete clinical and laboratory data, including serum levels of D-dimer and interleukin-6 (IL-6); and (3) confirmed diagnosis of tuberculosis through culture, polymerase chain reaction (PCR), or GeneXpert MTB/RIF assay. Exclusion criteria included the presence of

systemic conditions known to significantly alter inflammatory biomarkers, such as malignancy, autoimmune diseases, or sepsis, or inadequate histological material for assessing dark specks.

Operational Definitions

Dark specks were operationally defined as irregular, intensely pigmented granules measuring less than 10 µm, observed within or adjacent to necrotic areas at ×400 magnification on hematoxylin-eosin (H&E)-stained slides. The presence of dark specks was confirmed when at least three such granules were identified within a single high-power field. Serum D-dimer concentrations were expressed in nanograms per milliliter (ng/mL) fibrinogen equivalent units (FEU) and measured using immunoturbidimetric assay techniques. Interleukin-6 (IL-6) levels, reported in picograms per milliliter (pg/mL), were quantified using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' protocols. Tuberculosis (TB) status was classified as positive or negative based on microbiological confirmation by culture, polymerase chain reaction (PCR), or GeneXpert MTB/RIF assay.

Data Collection Procedures

Archived hematoxylin–two board-certified anatomical pathologists independently re-evaluated eosin (H&E) slides to determine the presence or absence of dark specks. For discrepant interpretations, a consensus review was conducted through a joint re-examination at a multi-head microscope, during which both pathologists discussed their findings until a single agreed-upon classification (presence vs. absence of dark specks) was reached; no third reviewer was involved. Corresponding serum D-dimer and interleukin-6 (IL-6) values were retrieved from clinical laboratory records. All observations were recorded using a standardized data collection form and subsequently entered into a digital dataset for statistical analysis. Descriptive statistics were calculated for D-dimer and interleukin-6 (IL-6) values, stratified by tuberculosis (TB) status and the presence of dark specks. Measures of central tendency and dispersion, including means, medians, and standard deviations, were computed as appropriate. Inferential analyses included the Chi-square test to examine the association between dark specks and TB status. Due to the non-normal distribution, the Mann–Whitney U test was employed to compare D-dimer and IL-6 levels between cases with and without dark specks.

The Spearman rank correlation coefficient was used to assess the relationship between D-dimer and IL- 6 concentrations. Diagnostic accuracy was evaluated using receiver operating characteristic (ROC) curve analysis, with the area under the curve (AUC) calculated for D-dimer, IL-6, and their combination. A composite diagnostic score was derived by averaging the normalized D-dimer and IL-6 values. Statistical significance was defined as $p < 0.05$ with a 95% confidence interval.

RESULT

The Participant Characteristics

A total of 156 patients with lymphadenitis were included in the study, comprising 84 with tuberculous lymphadenitis (TB) and 72 with non-tuberculous lymphadenitis (non-TB). Histopathological reevaluation revealed the presence of dark specks in 81 patients (51.9%), while the remaining 75 patients (48.1%) exhibited no identifiable dark specks on hematoxylin-eosin–stained sections.

Descriptive Statistics

The mean serum D-dimer level in TB patients was 829.40 ng/mL (standard deviation [SD]: 184.83), significantly higher than that observed in non-TB patients, which averaged 393.81 ng/mL (SD: 146.68). Likewise, the mean IL-6 level in TB patients was elevated at 27.82 pg/mL (SD: 9.12), compared to 15.22 pg/mL (SD: 7.68) in the non- TB group. The median values for both biomarkers demonstrated a similar trend, as presented in Table 1.

Table 1. Mean, Median, and Standard Deviation of D-dimer and IL-6 Levels by TB Status

Variable	TB (n=84)	Non-TB (n=72)
D-dimer, mean (SD) (ng/mL)	829.40 (184.83)	393.81 (146.68)
D-dimer, median (ng/mL)	814.56	400.66
IL-6, mean (SD) (pg/mL)	27.82 (9.12)	15.22 (7.68)
IL-6, median (pg/mL)	28.27	14.86

Association Between Dark Specks and Tuberculosis Status

Chi-square analysis revealed a statistically significant association between the presence of dark specks and TB status ($\chi^2 = 19.92$, $p = 0.000008$). Among patients exhibiting dark specks, 58 (71.6%) were diagnosed with tuberculous lymphadenitis, whereas only 26 (34.7%) of those without dark specks had TB. This finding suggests a strong association between histological dark specks and microbiologically confirmed tuberculosis.

Comparison of D-Dimer and IL-6 Levels Based on Dark Specks

The Mann–Whitney U test demonstrated a significant difference in D-dimer levels between patients with and without dark specks ($U = 4065.0$, $p = 0.00027$). Similarly, IL-6 levels were significantly higher in patients with dark specks than in those without ($U = 3742.0$, $p = 0.0125$). These findings indicate that dark specks are associated with elevated levels of both D-dimer and IL-6. Detailed comparisons are presented in Table 2.

Table 2. Comparison of biomarker levels according to the presence of dark specks

Biomarker	With	Biomarker	With
D-dimer (ng/mL)	Higher	Lower	0.00027
IL-6 (pg/mL)	Higher	Lower	0.0125

A moderate positive correlation was found between serum D-dimer and IL-6 levels among all participants, as indicated by Spearman's rank correlation coefficient ($\rho = 0.442$, $p = 7.7 \times 10^{-9}$). Given that TB-associated inflammation can dysregulate the coagulation cascade and heighten cytokine release, it is plausible that D-dimer and IL-6 are not only individually associated with TB status but also interrelated through shared pathophysiological pathways. Therefore, in addition to evaluating each biomarker in relation to tuberculous versus non-tuberculous lymphadenitis, we also examined the correlation between D-dimer and IL-6 to better characterize their joint behavior in this setting. This result suggests that increased D-dimer concentrations were significantly associated with elevated IL-6 levels.

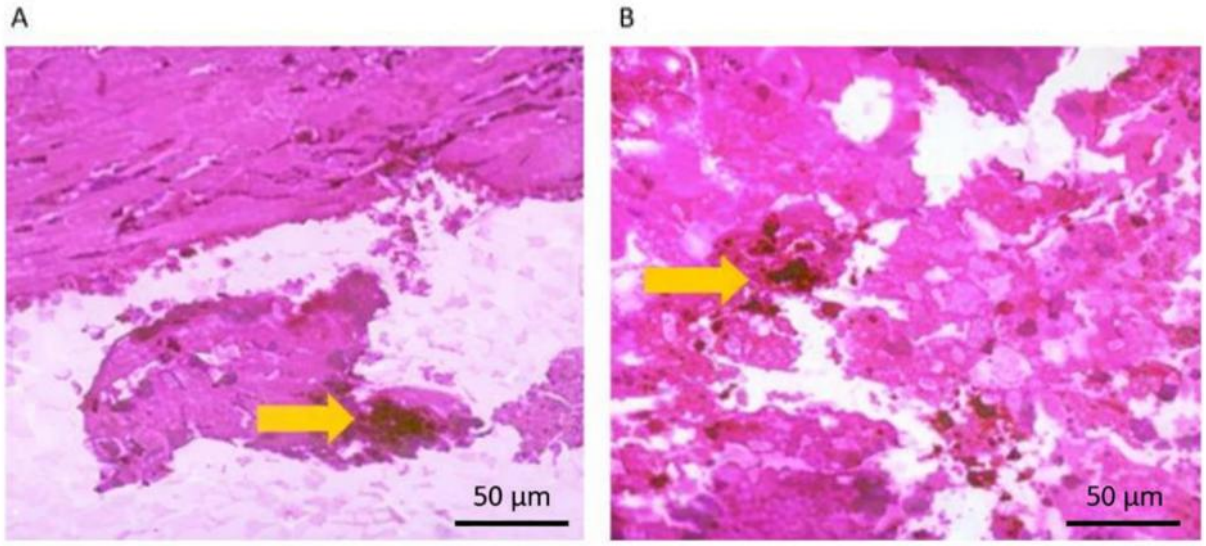

Figure 1. A. Histopathological section of a lymph node biopsy from a case of non-tuberculous lymphadenitis, stained with hematoxylin-eosin (HE), viewed at 40× magnification. Dark specks (indicated by yellow arrows) appear sparsely distributed within areas of necrosis.
B. Histopathological section of a lymph node biopsy from a case of tuberculous lymphadenitis, stained with hematoxylin-eosin (HE), viewed at 100× magnification. Numerous dark specks (highlighted by yellow arrows) are densely clustered within extensive necrotic regions.

Correlation of individual biomarkers with TB status

To further explore the relationship between each inflammatory marker and tuberculosis status, additional Spearman correlation analyses were performed, with TB lymphadenitis coded as 1 and non-TB lymphadenitis as 0. D-dimer levels showed a strong positive correlation with TB status ($\rho = 0.832$, $p < 0.001$), whereas IL-6 levels showed a moderate positive correlation ($\rho = 0.605$, $p < 0.001$). These findings are in line

with the group comparisons presented in Table 1, in which both D-dimer and IL-6 levels were significantly higher in TB than in non-TB lymphadenitis.

Diagnostic Accuracy of Combined D-Dimer and IL-6 for Tuberculosis

Receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic performance of the combined D-dimer and IL-6 score in differentiating tuberculous from non-tuberculous lymphadenitis. The area under the curve (AUC) was 0.987 (95% confidence interval: 0.972–0.999), indicating excellent diagnostic accuracy (Figure 2).

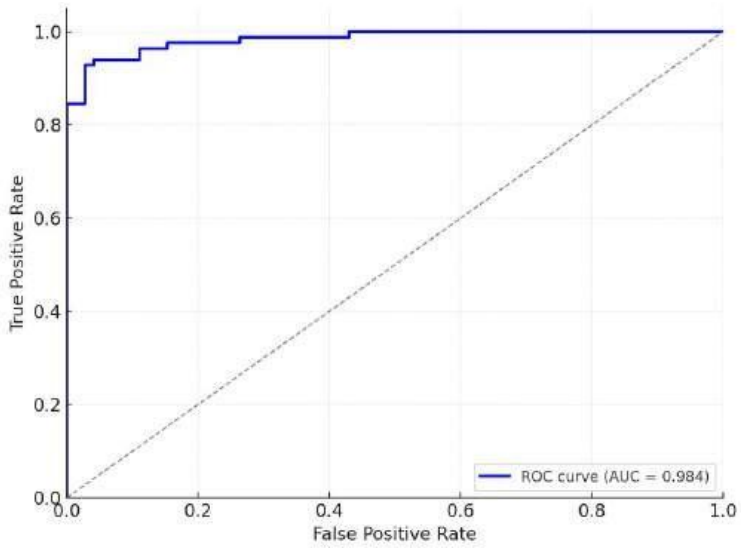

Figure 2. ROC curve depicting the diagnostic performance of the combined D-dimer and IL-6 score in distinguishing TB from non-TB lymphadenitis. The area under the curve (AUC) was 0.987 (95% CI: 0.972–0.999), reflecting excellent overall accuracy.

DISCUSSION

The rationale for selecting dark specks, D-dimer, and IL-6 is grounded in the concept that tuberculous lymphadenitis reflects a convergence of localized tissue injury and systemic host response pathways. At the tissue level, Mycobacterium tuberculosis infection can create a necrosis-prone granulomatous microenvironment with intense cellular turnover and debris formation, and immunologic profiling studies in tuberculous lymphadenitis demonstrate distinctive cytokine and chemokine patterns at the site of infection and systemically.[1,17,18,19,21,22] Within this context, dark specks may represent a pragmatic morphologic correlate of necrosis-associated tissue breakdown and inflammatory burden that can persist even when canonical granulomatous architecture is subtle or fragmented, consistent with prior cytomorphologic observations of dark specks in tuberculous lymphadenitis.[2] At the systemic level, IL-6 is a key mediator of acute phase responses and sustained inflammatory signaling in active tuberculosis, and multiple clinical studies support its relevance to disease activity and outcomes, including associations with unfavorable treatment outcomes and severe radiologic phenotypes.[3,4,7,15,16] In parallel, chronic infection and inflammation can promote endothelial activation and immunothrombotic responses, leading to coagulation activation and secondary fibrinolysis, reflected by elevated D-dimer levels, which have been investigated in tuberculosis-related pleural disease and in hemostatic perturbations in pulmonary tuberculosis cohorts. [5,6,10,11,20] Because IL-6-mediated inflammation and fibrinolysis activation are biologically connected yet represent non-redundant axes of the host response, their concurrent assessment provides a mechanistic basis for improved diagnostic discrimination. It aligns with broader efforts to develop host biomarker-based strategies for the monitoring and evaluation of extrapulmonary tuberculosis.[23,24]

The findings of this study underscore that the presence of dark specks in lymph node biopsy specimens is meaningfully associated with elevated systemic inflammatory markers, specifically D-dimer and IL-6.[12,13] This observation introduces a potential novel histopathological marker that may aid in navigating the diagnostic challenges associated with tuberculous lymphadenitis 14–16). Although traditional histological criteria remain foundational, integrating ancillary morphological features could meaningfully improve diagnostic precision in equivocal presentations.

A significantly higher prevalence of dark specks was observed among TB-confirmed cases (71.6%) compared to non-TB cases (34.7%), suggesting that these structures are not merely incidental histological artifacts. Instead, dark specks appear to reflect, at least in part, the underlying inflammatory burden associated with mycobacterial infection. This interpretation is further supported by the concurrent elevation of systemic inflammatory markers, D-dimer, and IL-6, in patients exhibiting these histological features.[14,15]

Serum D-dimer and IL-6 levels exhibited distinct distributions when stratified by the presence or absence of dark specks. In certain cases, D-dimer concentrations in speck-positive samples were more than double those in speck-negative counterparts, suggesting a greater degree of coagulopathy and fibrinolytic activity among individuals with these histological deposits.[11]

Although IL-6 levels were less markedly elevated than D-dimer, they nonetheless followed a similar inflammatory trajectory. Notably, the moderate positive correlation between D-dimer and IL-6 (Spearman's $\rho = 0.442$, $p < 0.001$) highlights a synergistic systemic inflammatory response, suggesting an interrelated pathophysiological mechanism that merits further investigation. In addition to the inter-marker correlation, the strong positive correlation between D-dimer and TB status, and the moderate positive correlation between IL-6 and TB status further support the close association of both biomarkers with tuberculous rather than non-tuberculous lymphadenitis. These additional analyses reinforce the notion that D-dimer and IL-6 capture complementary aspects of TB-related coagulopathy and inflammation, thereby enhancing their usefulness as adjunctive diagnostic markers.

Receiver operating characteristic (ROC) curve analysis demonstrated that the combined D-dimer and IL-6 score yielded an area under the curve (AUC) of 0.987, indicating near-perfect accuracy in distinguishing tuberculous from non-tuberculous lymphadenitis. This high level of diagnostic performance likely reflects the underlying biological plausibility that tuberculosis triggers both dysregulation of the coagulation cascade and heightened cytokine-mediated inflammatory responses.[16] However, before these findings can be generalized, rigorous validation across diverse populations and clinical settings is necessary.

Several mechanisms may underlie the observed association between dark specks and systemic inflammatory markers. A plausible hypothesis suggests that these granules represent condensed remnants of necrotic cellular debris, iron accumulation, or apoptotic byproducts arising from heightened local immune activation.[8,20] Within such an inflammatory microenvironment, the spillover of proinflammatory mediators into the systemic circulation could plausibly account for the elevated serum levels of D-dimer and IL-6 observed in this study. However, the precise biological composition of dark specks remains speculative, as no direct ultrastructural or immunochemical characterization has yet been performed to confirm their molecular or cellular constituents.[8,16,17,20]

It is important to note that although dark specks demonstrated a strong association with TB status, they were not exclusively observed in TB-confirmed cases. Approximately 32.4% of patients with non-tuberculous lymphadenitis also exhibited dark specks, albeit at a lower frequency. This finding raises questions regarding the specificity of dark specks as a pathognomonic marker for tuberculosis. Potential explanations for this overlap may include the chronicity of inflammation, the presence of granulomatous reactions of non-tuberculous origin, or histological alterations resulting from prior treatment or tissue degeneration artifacts.[17,18,19]

Among the systemic markers assessed, D-dimer and IL-6 exhibited greater diagnostic precision. In particular, D-dimer levels exceeding 800 ng/mL were largely confined to TB cases, suggesting its value as a proxy for the heightened coagulopathic and inflammatory responses characteristic of active mycobacterial infection.10 Although IL-6 is traditionally regarded as a non-specific inflammatory cytokine, it nonetheless exhibited significantly elevated levels in TB cases, suggesting that, when interpreted within the appropriate clinical and histopathological context, it may hold diagnostic relevance.[14,16,21,22,23,24,25]

Despite the strong statistical associations observed in this study, the cross-sectional design precludes causal inference. The temporal relationship, whether dark specks precede, result from, or merely coexist with systemic inflammation, remains undetermined. Additionally, the potential influence of comorbidities, subclinical infections, or other unmeasured confounding factors on D-dimer and IL-6 levels cannot be entirely ruled out. To mitigate these issues, this study applied strict inclusion and exclusion criteria, excluding patients with major conditions known to affect inflammatory and hemostatic markers markedly, and required microbiological confirmation to classify TB status. This study also used a standardized operational definition of dark specks and independent readings by two anatomical pathologists with consensus review to reduce histopathological misclassification.

Nevertheless, several limitations remain. The study was confined to a limited number of centers, lacked longitudinal follow-up, and did not formally quantify interobserver variability in dark speck assessment. Future research should therefore include larger multi-center cohorts, prospective longitudinal designs to clarify temporal relationships, formal assessment of inter-rater reliability in dark speck identification, and ultrastructural or immunohistochemical characterization of dark specks to elucidate their biological nature. Such studies would help validate and refine the use of dark specks, D-dimer, and IL-6 as adjunctive diagnostic tools in routine practice.

Nonetheless, these findings open promising avenues for practical application. In resource-limited settings where access to advanced microbiological diagnostics is limited, a combined approach using simple histopathological assessment (e.g., identification of dark specks) alongside readily available laboratory markers (D-dimer and IL-6) may provide valuable diagnostic support. Such a combination could serve as a risk-stratification tool, helping clinicians prioritize patients for empirical antituberculosis therapy while awaiting definitive microbiological confirmation.

CONCLUSION

A moderate positive correlation between D dimer and IL-6 supports their concurrent elevation in this setting, and the combined D dimer and IL-6 score shows excellent discriminatory performance for differentiating tuberculous from non-tuberculous lymphadenitis. Overall, these findings align with the study objective by indicating that dark speck assessment, together with D-dimer and IL-6 measurements, can serve as a practical adjunct to routine histopathology for distinguishing TB lymphadenitis from non-TB cases, particularly when histologic features are equivocal.

ETHICAL CONSIDERATIONS

This study was approved by the Ethics Committee of Naura Medika Clinic and Laboratory (Approval No: 02/I/ethic/2024). All procedures were conducted in accordance with institutional ethical standards and the principles of the Declaration of Helsinki. Patient confidentiality was strictly upheld, and no additional interventions or risks were introduced to the participants beyond standard clinical care.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study. They have no financial relationships with any organizations or companies that could inappropriately influence or bias the content of this article. All authors affirm that they have no relevant financial or non-financial interests to disclose.

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dark spects

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Dark Spects, D-Dimer, and IL-6 Levels in Tuberculous and Non-Tuberculous Lymphadenitis

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ABSTRACT

Background: Tuberculous lymphadenitis (TB) continues to pose diagnostic challenges, particularly in cases lacking classical histopathological features.

Objectives: This study aimed to evaluate the association between histopathological dark spects and serum D-dimer and IL-6 levels, and to assess the diagnostic potential of their combination in distinguishing TB from non-TB lymphadenitis.

Methods: A cross-sectional study was conducted on 156 lymph node biopsy cases. Dark spects were assessed through hematoxylin-eosin slide reevaluation, while D-dimer and IL-6 were measured using immunoturbidimetric and ELISA assays.

Results: Dark spects were observed in 51.9% of cases and showed a significant association with TB status ($p = 0.000008$). Patients with dark spects had significantly higher D-dimer and IL-6 levels ($p = 0.00027$ and $p = 0.0125$, respectively). A moderate positive correlation was found between D-dimer and IL-6 ($\rho = 0.442$; $p < 0.001$). ROC curve analysis of the combined D-dimer and IL-6 score yielded an AUC of 0.987, indicating excellent diagnostic accuracy. **Conclusion:** These findings support the utility of dark spects and systemic biomarkers as supplementary diagnostic tools for TB lymphadenitis, particularly in resource-limited healthcare settings.

INTRODUCTION

Lymphadenitis caused by *Mycobacterium tuberculosis* represent a considerable clinical burden, especially in countries with a high incidence of tuberculosis (TB). Although the diagnosis of tuberculous lymphadenitis is often facilitated by classical histopathological findings such as caseating granulomas and Langhans giant cells, a subset of cases still presents significant diagnostic dilemmas. In situations where the histological hallmarks are either absent or ambiguous, the search for additional morphological markers becomes critical.(1)

Dark spects, irregularly shaped, intensely pigmented granules observed in hematoxylin-eosin-stained slides have begun to attract investigative attention. From this perspective, it is intriguing to explore whether these dark spects could serve not merely as morphological curiosities but as meaningful indicators of tuberculous infection. Systematic exploration linking dark spects with systemic inflammatory markers remains conspicuously limited.(2)

It becomes crucial to examine the systemic inflammatory response accompanying tuberculous lymphadenitis. D-Dimer, a fibrin degradation product, and Interleukin-6 (IL-6), a potent pro-inflammatory cytokine, have both been recognized as elevated in various infectious and inflammatory states, including active TB.(3,4) However, it is worth noting that the specific

correlation between dark spects and systemic biomarkers such as D-Dimer and IL-6 has scarcely been delineated. The synergistic evaluation of histopathological features alongside systemic markers may enrich diagnostic strategies, potentially bridging the gaps left by traditional histopathological criteria.diga(5)

D-Dimer elevation in TB has been postulated to arise from widespread activation of the coagulation cascade (5,6), while increased IL-6 levels mirror the magnitude of the underlying inflammatory milieu.(7) Nevertheless, it remains uncertain whether the presence of dark spects reflects a histological surrogate of this systemic activation. Thus, the interplay between tissue-level morphology and blood-based biomarkers deserves systematic scrutiny.(4,5,8)

Based on preliminary observations, it can be surmised that patients with dark spects may exhibit higher levels of D-Dimer and IL-6 compared to those without such findings. However, without empirical data, such assumptions remain speculative.(4,5,8) Clearly, a structured investigation comparing dark spects, D-Dimer, and IL-6 levels between tuberculous and non-tuberculous lymphadenitis cases is warranted.(3,9,10)

Essentially, two principal questions arise: firstly, whether the presence of dark spects correlates significantly with elevated D-Dimer and IL-6 levels; and secondly, whether these parameters, individually or in combination, could aid in distinguishing TB from non-TB lymphadenitis.(4,6,11) Unraveling these associations may have tangible diagnostic implications, especially in resource-limited settings where access to sophisticated microbiological tests is constrained.

To address these hypotheses, an observational cross-sectional study design has been employed. In this study, lymph node biopsy samples will be evaluated for the presence of dark spects by independent pathologists. Simultaneously, D-Dimer and IL-6 serum levels, obtained through immunoturbidimetric and ELISA methods respectively, will be analyzed. By comparing these parameters across TB-confirmed and non-TB groups, statistical analyses including Chi-Square tests, Mann-Whitney U tests, Spearman correlation, and ROC curve analysis will be conducted.

The findings of this study may not only validate dark spects as an adjunctive histological marker but may also highlight the clinical utility of combining tissue and blood-based parameters. Notably, it may offer a supplementary pathway for TB diagnosis, particularly in challenging cases lacking classical histological or microbiological confirmation.

METHODS

Study Design and Setting

A cross-sectional analytical study was conducted to investigate the association between the presence of histopathological dark spects and the levels of D-dimer and interleukin-6 (IL-6) in patients with tuberculous and non-tuberculous lymphadenitis. The study was carried out at the Department of Anatomical Pathology and the Clinical Pathology Laboratory of Pasar Minggu General Hospital, Tarakan General Hospital and Naura Medika Clinic and Lab , between January 2024 and December, 2024.

Participants and Sampling

The study population included 156 patients who underwent lymph node biopsy and were diagnosed with lymphadenitis based on histopathological examination during the study period. Participants were recruited consecutively, and inclusion was based on the following criteria: (1) availability of adequate hematoxylin-eosin (HE) stained slides for histopathological reassessment, (2) complete clinical and laboratory data, including D-dimer and IL-6 serum levels, and (3) confirmed TB diagnosis via culture, polymerase chain reaction (PCR), or GeneXpert MTB/RIF assay. Patients were excluded if they had systemic illnesses that could significantly affect inflammatory markers—such as malignancy, autoimmune disorders, or sepsis—or if the histological specimens were insufficient for dark spects evaluation.

Operational Definitions

Dark spects were defined as irregular, darkly pigmented granules (<10 μm) identified within or

around necrotic foci under ×400 magnification on HE-stained sections. Their presence was determined when at least three dark spects were visualized in a single high-power field. Serum D-dimer concentrations were measured in ng/mL fibrinogen equivalent units (FEU) using immunoturbidimetric assays. IL-6 levels (pg/mL) were quantified using enzyme-linked immunosorbent assay (ELISA) kits following manufacturer protocols. TB status was categorized as positive or negative based on microbiological confirmation.

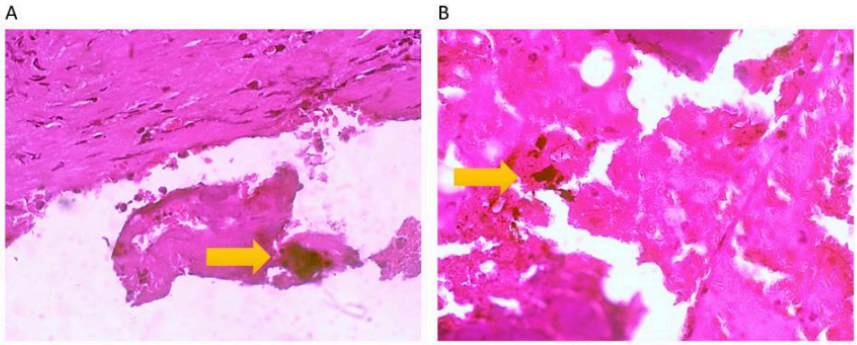

Figure 1. Histopathological section of a lymph node biopsy from a non-tuberculous lymphadenitis case, stained with hematoxylin-eosin (HE) at 40× magnification. Dark spects (indicated by yellow arrows) are sparsely distributed within necrotic areas. B. Histopathological section of a lymph node biopsy from a tuberculous lymphadenitis case, stained with hematoxylin-eosin (HE) at 100× magnification. Numerous dark spects (highlighted by yellow arrows) are clustered within extensive necrotic foci.

Data Collection Procedures

Archived HE slides were independently re-evaluated by two certified anatomical pathologists to determine the presence or absence of dark spects. Discrepancies were resolved by consensus. Corresponding D-dimer and IL-6 values were obtained from clinical laboratory records. All data were recorded in a standardized observation sheet and transferred to a digital dataset for analysis.

Statistical Analysis

Descriptive statistics were computed for D-dimer and IL-6 values, stratified by TB status and the presence of dark spects. Means, medians, and standard deviations were calculated as appropriate. Inferential analyses included the Chi-square test to assess the association between dark spects and TB status. The Mann–Whitney U test was used to compare D-dimer and IL-6 levels between patients with and without dark spects due to the non-normal distribution of the data.

The Spearman correlation coefficient was calculated to evaluate the relationship between D-dimer and IL-6 levels. To assess the diagnostic performance of D-dimer and IL-6 levels in distinguishing TB from non-TB lymphadenitis, receiver operating characteristic (ROC) curve analysis was performed, and the area under the curve (AUC) was calculated. A combined score was derived by averaging normalized D-dimer and IL-6 values. Statistical significance was defined as $p < 0.05$ with a 95% confidence interval.

Ethical statement

This study was approved by the Ethical Board of Naura medika Clinic and Lab, No : 02/IV/ethic/2025. All procedures were performed in accordance with ethical standards. Patient confidentiality was strictly maintained, and no additional interventions or risks were imposed on participants.

RESULTS

Participant Characteristics

A total of 156 patients with lymphadenitis were included in this study, comprising 84 cases of tuberculous lymphadenitis (TB) and 72 cases of non-tuberculous lymphadenitis (non-TB). Dark spects were identified in 81 patients (51.9%), while 75 patients (48.1%) had no dark spects detected on histopathological examination.

Descriptive Statistics

The mean serum D-dimer level among TB patients was 829.40 ng/mL (standard deviation [SD]: 184.83), notably higher than the 393.81 ng/mL (SD: 146.68) observed in non-TB patients. Similarly, the mean IL-6 level was elevated in TB patients (27.82 pg/mL, SD: 9.12) compared to non-TB patients (15.22 pg/mL, SD: 7.68). The median values followed a comparable pattern (Table 1).

Table 1. Mean, Median, and Standard Deviation of D-dimer and IL-6 Levels by TB Status

Variable	TB (n=84)	Non-TB (n=72)
D-dimer, mean (SD) (ng/mL)	829.40 (184.83)	393.81 (146.68)
D-dimer, median (ng/mL)	814.56	400.66
IL-6, mean (SD) (pg/mL)	27.82 (9.12)	15.22 (7.68)
IL-6, median (pg/mL)	28.27	14.86

Association Between Dark Spects and Tuberculosis Status

Chi-square analysis demonstrated a significant association between the presence of dark spects and TB status ($\chi^2 = 19.92$, $p = 0.000008$). Among patients with dark spects, 58 (71.6%) were diagnosed with TB, while among those without dark spects, only 26 (34.7%) had TB.

Comparison of D-Dimer and IL-6 Levels Based on Dark Spects

The Mann–Whitney U test revealed significant differences in D-dimer levels between patients with and without dark spects ($U = 4065.0$, $p = 0.00027$). Likewise, IL-6 levels differed significantly based on the presence of dark spects ($U = 3742.0$, $p = 0.0125$). Patients with dark spects exhibited higher levels of both biomarkers compared to those without dark spects (Table 2).

Table 2. Comparison of Biomarker Levels Based on the Presence of Dark Spects

Biomarker	With Dark Spects	Without Dark Spects	p-value
D-dimer (ng/mL)	Higher	Lower	0.00027
IL-6 (pg/mL)	Higher	Lower	0.0125

Correlation Between D-Dimer and IL-6

A moderate positive correlation was observed between serum D-dimer and IL-6 levels across all participants (Spearman's $\rho = 0.442$, $p = 7.7 \times 10^{-9}$). This finding indicates that higher D-dimer concentrations were associated with elevated IL-6 levels (Figure 1).

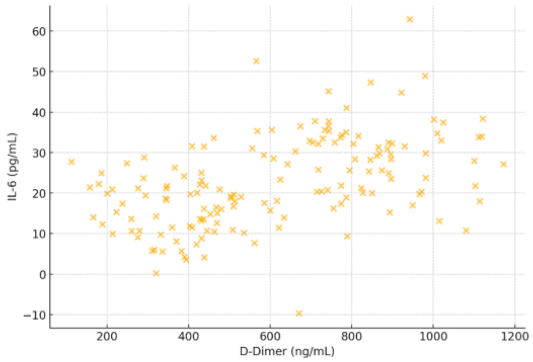

Figure 2. Scatter plot demonstrating the correlation between D-dimer and IL-6 levels (Spearman's ρ = 0.442, p < 0.001).

Diagnostic Accuracy of Combined D-Dimer and IL-6 for Tuberculosis

ROC curve analysis demonstrated diagnostic performance of the combined D-dimer and IL-6 score in distinguishing TB from non-TB lymphadenitis. The area under the curve (AUC) was calculated as 0.987 (95% CI: 0.972–0.999), indicating very high diagnostic accuracy (Figure 2).

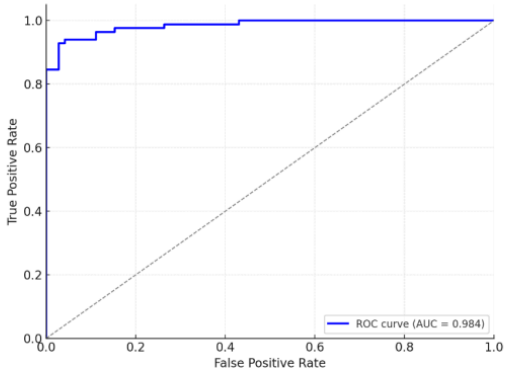

Figure 3. Receiver operating characteristic (ROC) curve for combined D-dimer and IL-6 score to predict TB lymphadenitis (AUC = 0.987).

DISCUSSION

In this study's findings, it becomes apparent that the presence of dark spects within lymph node biopsy samples carries a meaningful association with systemic inflammatory markers, such as D-dimer and IL-6.(12,13) This observation provides a novel histopathological anchor in the challenging diagnostic landscape of tuberculous lymphadenitis.(14–16) While classical histological features remain the cornerstone, the subtle integration of additional morphological clues could substantially refine diagnostic confidence, particularly in borderline or ambiguous cases.

There is significantly higher prevalence of dark spects among TB-confirmed cases (71.6%) compared to non-TB cases (34.7%). Dark spects cannot be dismissed as incidental artifacts.

Rather, they seem to mirror, at least partially, the underlying inflammatory burden associated with mycobacterial infection. This alignment was further strengthened by the concomitant elevation of D-dimer and IL-6 levels among patients exhibiting these histological findings.(17,18)

Serum D-dimer and IL-6 levels demonstrated distinct patterns when stratified by the presence or absence of dark spects. In some cases, D-dimer concentrations exceeded twice the values observed in spect-negative samples, suggesting that coagulopathy and fibrinolytic activity might be more profound in individuals displaying these dark deposits.(11) IL-6, albeit less dramatically elevated, still paralleled this inflammatory trend. Importantly, the correlation between D-dimer and IL-6 (Spearman's $\rho = 0.442$, $p < 0.001$) underscores a synergistic systemic response that warrants closer scrutiny.

At the ROC curve analysis, the diagnostic performance of the combined D-dimer and IL-6 score achieved an AUC of 0.987, nearly perfect in discriminating TB from non-TB lymphadenitis. Undoubtedly, the robustness of the model reflects the biological plausibility that TB induces both coagulation cascade perturbations and cytokine storms.(19) Nevertheless, external validation across broader, more heterogeneous populations would be essential before universal adoption.

Several mechanisms could underlie the relationship between dark spects and systemic inflammatory markers. One plausible hypothesis posits that these granules represent condensed remnants of necrotic cellular debris, iron deposits, or even apoptotic byproducts resulting from intense local immune activation.(8,20) In such a microenvironment, the spillover of inflammatory mediators into the systemic circulation would logically explain the observed biochemical elevations. However, direct ultrastructural or immunochemical validation of the composition of dark spects remains absent, leaving the precise biological nature speculative at best.(5,8)

It is worth noting that while dark spects correlated strongly with TB status, they were not exclusively confined to TB cases. Approximately 32.4% of non-TB lymphadenitis patients also demonstrated dark spects, albeit at a lower frequency. Thus, the specificity of dark spects as an exclusive TB marker might be questioned. Factors such as chronicity, granulomatous inflammation of non-tuberculous etiology, or prior treatment artifacts could feasibly contribute to this overlap.

The systemic markers D-dimer and IL-6 showed better discriminatory power. In particular, D-dimer elevation beyond 800 ng/mL was predominantly observed in TB patients, reinforcing its potential role as a surrogate of disease activity.(10) IL-6, traditionally viewed as a non-specific inflammatory cytokine, nonetheless displayed significant differences aligned with TB presence, suggesting that within the right context, it may still offer diagnostic value.(20)

Despite the strong statistical associations demonstrated herein, causality cannot be inferred from this cross-sectional design. Temporal dynamics — whether dark spects precede systemic inflammation or merely coexist — remain unresolved. The possibility that comorbid conditions, subclinical infections, or unmeasured confounders might have influenced D-dimer and IL-6 levels cannot be fully excluded.

These results open several avenues for practical application. In resource-limited settings, where access to advanced microbiological testing is constrained, the combination of simple histological evaluation (dark spects) and routine laboratory markers (D-dimer, IL-6) could enhance diagnostic algorithms. This combination might serve as a risk stratification tool, aiding clinicians in deciding which patients warrant empirical anti-tuberculosis therapy while awaiting definitive microbiological confirmation.

CONCLUSION

Dark spects emerge not merely as visual oddities but as potential sentinels of systemic inflammation in tuberculous lymphadenitis. The significant associations with D-dimer and IL-6, the moderate positive correlation between these biomarkers, and the near-perfect diagnostic performance of their combination reinforce the idea that integrating tissue and blood-based assessments could elevate diagnostic precision. Moving forward, multi-center studies, functional characterization of dark spects, and longitudinal outcome analyses will be pivotal to cement their

role in clinical practice.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this study. No financial relationships with companies whose products or services may be related to the subject matter of this article were reported. authors should make a conflict-of-interest disclosure statement or a declaration that they do not have any conflicts of interest.

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DATA AVAILABILITY

The datasets generated and analyzed during the current study are not publicly available due to patient confidentiality but are available from the corresponding author upon reasonable request and with permission from the ethical committee.

SUPPLEMENTAL DATA

No supplemental data are provided for this manuscript. All essential data are included in the main text, tables, and figures.

AUTHOR CONTRIBUTIONS

R.A.D. conceptualized the study, led histopathological reevaluation, and wrote the manuscript. H.M. and R.S.H. contributed to biomarker data acquisition and statistical analysis. E.P. coordinated sample collection and data management. F.I. and J.C. participated in slide interpretation and manuscript revision. All authors read and approved the final version of the manuscript.

DECLARATION OF USING AI IN THE WRITING PROCESS

Artificial intelligence (AI) tools, including large language models, were used to assist with language editing, grammar checking, and formatting of this manuscript. The intellectual content, data interpretation, and conclusions were entirely the responsibility of the authors.

LIST OF ABBREVIATIONS

TB: Tuberculosis; HE: Hematoxylin and Eosin; IL-6: Interleukin-6; D-dimer: Fibrin Degradation Product; ROC: Receiver Operating Characteristic; AUC: Area Under Curve; SD: Standard Deviation; ELISA: Enzyme-Linked Immunosorbent Assay; FEU: Fibrinogen Equivalent Units; PCR: Polymerase Chain Reaction; MTB/RIF: Mycobacterium tuberculosis/Rifampicin

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