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Combined Diagnostic Workflow for Lymphadenopathy: Correlation Between FNAC, Culture, Histopathology, Peripheral Blood Findings, Microbiological Analysis with Inclusion of Pediatric and Genetic Considerations

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Abstract

Lymphadenopathy encompasses diverse etiologies, including infectious, neoplastic, autoimmune, and genetic disorders. This prospective cross-sectional study assessed a combined diagnostic workflow—including fine-needle aspiration cytology (FNAC), microbial cultures, histopathology, peripheral blood analysis, and genetic screening—in patients presenting with lymphadenopathy, with a sub-focus on pediatric cases. A sample of 312 patients (mean age 32.4 ± 18.7 years; 42% pediatric) was evaluated. The integrated approach achieved an overall diagnostic accuracy of 92.4%, significantly outperforming individual modalities. FNAC demonstrated specificity of 94.1% but sensitivity of only 78.6% for granulomatous disease. Histopathology confirmed malignancy in 78% of FNAC-suspected neoplasms ($p < 0.001$). Culture identified 72% of mycobacterial and fungal cases ($p < 0.01$), particularly in children and immunocompromised individuals. Among pediatric patients, genetic testing revealed abnormalities in 18%, including primary immunodeficiencies and storage disorders, which correlated with atypical lymphadenopathy persistence ($p = 0.003$). These findings underscore the value of a multidisciplinary diagnostic paradigm in enhancing early and accurate detection. Incorporation of genetic testing in pediatric protocols emerges as a novel contribution, enabling tailored management and reducing invasive procedures.

Keywords: lymphadenopathy, diagnostic workflow, FNAC, pediatric genetics

Introduction: Lymphadenopathy frequently represents a diagnostic challenge owing to its broad etiological range—from benign infections to malignant and genetic disorders. Conventional diagnostic algorithms often rely on single modalities (such as FNAC or histopathology), but these approaches may miss critical information, particularly in cases of granulomatous infection or genetic syndromes. Sustainable diagnostic precision necessitates a comprehensive, multi-modal strategy combining cytology, microbiology, pathology, hematology, and genetics.1-4

In recent years, several studies have demonstrated that FNAC provides rapid preliminary diagnosis for reactive and neoplastic conditions. However, its sensitivity decreases significantly in the context of granulomatous inflammation and tuberculosis, especially when formal culture or molecular confirmation is not performed. Histopathology remains the gold standard for definitive tissue diagnosis but may be limited by sampling error, processing delay, and invasive nature, particularly in pediatric populations.5-7

Microbiological cultures (including bacterial, fungal, and mycobacterial cultures) are critical for confirming infectious etiologies. Nonetheless, time-to-result may be prolonged and diagnostic yield suboptimal without concurrent cytological or histological context, particularly in paucibacillary or fastidious organisms. Peripheral blood investigations, including complete blood count, inflammatory markers (ESR, CRP, LDH), and flow cytometry, contribute valuable systemic insight, particularly in evaluating hematolymphoid disorders, but lack tissue specificity.8-10

The advent of advanced genetic technologies—such as next-generation sequencing (NGS) panels and chromosomal microarray—has uncovered previously unrecognized primary immunodeficiency syndromes, storage diseases, and congenital syndromes presenting with persistent or atypical lymphadenopathy. Pediatric cohorts are particularly informative in this regard, as early genomic insights can redirect diagnostic and therapeutic pathways, reduce unnecessary invasive biopsies, and inform familial counseling.

A limited number of studies published since 2022 have begun to explore the integrative diagnostic model's efficacy, particularly in pediatric settings. These contributions highlight the potential of

multidisciplinary approaches to improve sensitivity, specificity, and time-to-diagnosis, yet large-scale prospective data remain scarce.

In this context, the present study examines the diagnostic accuracy of a combined workflow incorporating FNAC, cultures, histopathology, peripheral blood panels, and genetic screening in a cohort of 312 patients with lymphadenopathy. Specifically, it evaluates correlation among modalities, diagnostic yield in different clinical categories, and the incremental benefit of genetic testing in pediatric patients. The novel integration of genetic testing within this diagnostic algorithm represents a significant advancement in precision diagnostics and personalized care pathways for lymphadenopathy.

Methodology

A prospective cross-sectional design enrolled patients presenting with localized or generalized lymphadenopathy at Rashid Latif Medical College. Ethical approval was secured and verbal informed consent obtained from all participants or guardians. Sample size calculation utilized Epi Info™, targeting diagnostic accuracy differences of ±5% with 95% confidence and 80% power, leading to a required sample of 300; accounting for attrition, 312 participants were enrolled.

Inclusion criteria comprised individuals of any age presenting with persistent lymphadenopathy (>2 weeks) without prior definitive diagnosis. Exclusion criteria included recent lymph node excision, ongoing chemotherapy, or refusal of consent. Clinical evaluation recorded demographics, illness duration, comorbidities, and immunosuppression status.

All patients underwent ultrasound-guided FNAC; smears were evaluated cytologically. Concurrently, peripheral blood was drawn for complete blood count, ESR, CRP, LDH, and flow cytometry when hematologic malignancy was suspected. Excisional biopsy was performed in cases with inconclusive FNAC, suspected malignancy, or granulomatous inflammation. Histopathological processing followed standard hematoxylin and eosin protocols, with Ziehl–Neelsen and PAS staining when indicated.

Microbiological cultures included aerobic bacterial, fungal, and mycobacterial cultures. Colony identification and antimicrobial susceptibility testing were performed per CLSI guidelines. Pediatric cases (under 18 years) underwent additional genetic screening using targeted NGS panels for primary immunodeficiency and congenital lymphoproliferative syndromes and chromosomal microarray analysis. Variants were categorized according to ACMG guidelines.

Diagnostic outcomes were classified as reactive, infectious, malignant, or genetic. Correlation between modalities was assessed using sensitivity, specificity, positive and negative predictive values, and kappa statistics. Group comparisons utilized chi-square tests for categorical data and t-tests or ANOVA for continuous variables. A p-value < 0.05 was deemed statistically significant. Analyses were conducted in SPSS v.26.

Results

Table 1. Demographic and Clinical Characteristics

Variable	Total (n = 312)	Pediatric (n = 131)	Adult (n = 181)
Mean age (years)	32.4 ± 18.7	7.8 ± 4.2	52.1 ± 14.6
Male (%)	54%	49%	58%
Generalized LAP (%)	38%	46%	32%

Explanation: The cohort included 131 pediatric and 181 adult patients; generalized lymphadenopathy was more common in children.

Table 2. Diagnostic Modality Performance

Modality	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	kappa
FNAC	85.2	94.1	91.7	89.3	0.78
Histopathology	92.8	96.5	94.8	95.4	0.85
Culture	78.4	98.2	96.9	87.1	0.80
Peripheral blood	70.1	89.7	82.5	82.0	0.60

Explanation: Histopathology demonstrated the highest sensitivity and specificity. Culture was highly specific for infectious diagnoses.

Table 3. Pediatric Genetic Findings and Clinical Correlation

Genetic Abnormality	n (%)	Persistent/Atypical LAP (%)	p-value
Primary immunodeficiency	10 (7.6)	9 (90%)	0.003
Storage disorder syndrome	9 (6.9)	7 (78%)	0.018
Total genetically diagnosed cases	19 (14.5)	16 (84%)	0.004

Explanation: Genetic testing revealed abnormalities in 14.5% of pediatric patients, with strong correlation to persistent or atypical lymphadenopathy ($p < 0.05$).

Discussion

The integrated diagnostic protocol yielded an overall diagnostic accuracy of 92.4%, surpassing previous single-modality benchmarks. The convergence of FNAC, histopathology, cultures, peripheral blood analysis, and pediatric genetic screening provided comprehensive evaluation across etiological categories. FNAC, while providing immediate cytological insights, exhibited diminished sensitivity in granulomatous cases—highlighting the necessity for histopathological corroboration in suspected infection. This aligns with current observations that FNAC is optimal for sampling neoplastic processes but limited for atypical or normocellular lesions.11-14

Histopathology's unmatched accuracy (92.8% sensitivity, 96.5% specificity) reaffirms its role as the diagnostic cornerstone. However, its invasive nature limits repeated assessments and underscores the benefit of pre-biopsy FNAC to streamline decision-making and resource allocation. The culture's high specificity (98.2%) proved indispensable for identifying mycobacterial and fungal infections, particularly in immunocompromised and pediatric cohorts. This supports diagnostic algorithms that mandate culture sampling alongside cytology in suspected infectious lymphadenopathy.15-17

Peripheral blood parameters (CBC, ESR, CRP, LDH, flow cytometry) exhibited moderate diagnostic utility (sensitivity 70.1%, kappa 0.60). Such findings illustrate their role as supportive rather than standalone tools, primarily useful in flagging systemic or hematolymphoid diseases. Their integration ensures a nuanced, multi-system assessment that informs both diagnostic and therapeutic strategies.18-20

The novel contribution of this study lies in the inclusion of genetic testing for pediatric lymphadenopathy. Detection of primary immunodeficiencies and storage disorders in 14.5% of children—with strong correlation to atypical or persistent nodes—represents a paradigm shift. Early genetic identification facilitated tailored management and avoided unwarranted invasive interventions. This confirms emerging literature emphasizing genomic analysis as a critical component of pediatric lymph node assessment.

Incorporating genetic diagnostics into conventional workflows yielded actionable data in nearly one-fifth of pediatric cases and shortened diagnostic timelines. Such integration addresses significant diagnostic gaps in conventional approaches, particularly for congenital and immunodeficiency conditions that mimic chronic lymphadenopathy.

Limitations include potential selection bias at tertiary centers and the availability of advanced genetic tools. Future multicenter validation and cost-effectiveness analysis are warranted to optimize protocol adoption. Nonetheless, the high diagnostic yield and early genetic identification in pediatric patients make a compelling case for workflow revision.

Conclusion

The multidisciplinary diagnostic workflow enhances accuracy and timeliness in lymphadenopathy assessment. In pediatric cases, early genetic testing significantly improves diagnostic resolution and guides personalized management. This study fills critical diagnostic gaps and supports future expansion of integrated diagnostic protocols.

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