Original Research Paper

Volume - 16 | Issue - 04 | April - 2026 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Pathology

INTEGRATED FINE-NEEDLE ASPIRATION CYTOLOGY, HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY FOR NECK SWELLINGS: A TISSUE-BASED DIAGNOSTIC ACCURACY STUDY FROM AN INDIAN TERTIARY CARE CENTRE.

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ABSTRACT Neck masses are frequent in clinical practice and encompass a wide diagnostic spectrum, from benign inflammatory processes to primary and metastatic malignancies. Rapid, tissue-based triage is crucial to patient management.[23,24] Fine-needle aspiration cytology (FNAC) is widely used as a first-line investigation, whereas histopathology and immunohistochemistry (IHC) provide definitive classification and biologically relevant subtyping.[1–7,12,18,22] We performed a retrospective–prospective observational diagnostic accuracy study of 800 neck masses evaluated by FNAC over two years in a tertiary care centre in North India; 353 had subsequent histopathology and formed the basis of performance analysis. IHC was applied in malignant and diagnostically challenging cases to refine lineage and site of origin. Lymph node swellings were the most frequent (487/800, 60.8%), followed by thyroid (161/800, 20.1%), salivary gland (93/800, 11.6%) and miscellaneous soft tissue/skin lesions (36/800, 4.5%). Tubercular lymphadenitis was the commonest specific lymph node diagnosis, reflecting the regional tuberculosis burden.[9–11] Overall FNAC–histopathology concordance was 97.4% (344/353). For broad categorisation into benign versus malignant/specific entities, FNAC showed a sensitivity of 98%, specificity of 87% and diagnostic accuracy of 92.16%. Discordant cases were largely confined to follicular-patterned thyroid lesions, rare lymphomas and selected salivary tumours with overlapping cytology.[12–18,20–22] IHC substantially improved diagnostic precision and clinical interpretability in malignant lesions by confirming T- and B-cell lineage in lymphomas, establishing primary thyroid origin via PAX8, TTF-1 and thyroglobulin, and subclassifying salivary gland and cutaneous tumours using CK7, p63/p40, EMA, S100, PLAG1, BCL2 and related markers.[12,15,18–22] These data support an integrated FNAC–histopathology–IHC pathway as a clinically useful diagnostic strategy for neck masses in high tuberculosis-burden, resource-constrained settings. This approach delivers rapid minimally invasive triage while preserving the ability to generate biologically and clinically meaningful classifications that guide treatment.

KEYWORDS : Neck Swelling; Neck Mass; FNAC; Histopathology; Immunohistochemistry; Diagnostic Accuracy; Tuberculosis; Clinical Pathway

INTRODUCTION

Neck masses are a common reason for referral to otolaryngology and oncology services, with a broad differential diagnosis including reactive and infective lymphadenopathy, granulomatous disease (particularly tuberculosis), primary thyroid and salivary gland neoplasms and metastatic malignancies.[23,24] The clinical challenge is to distinguish benign from malignant and specific treatable entities rapidly enough to guide appropriate imaging, surgery, oncological therapy and infectious disease management.

Fine-needle aspiration cytology (FNAC) is established as a first-line diagnostic test for palpable and image-guided neck masses because it is minimally invasive, inexpensive and provides rapid, tissue-based information.[1–4] Multiple series have demonstrated its utility in head and neck lesions and superficial lymphadenopathy, particularly in resource-limited settings.[1–8] FNAC is embedded in clinical algorithms for the evaluation of lymphadenopathy, thyroid nodules and salivary gland lesions and is included in practice guidelines for adult neck masses.[16,18,23,24] However, cytology alone has recognised limitations in entities where architectural features or invasion are crucial (e.g. follicular thyroid lesions, some lymphomas and complex salivary tumours).[5–7,12–15,18–22]

Histopathological examination of biopsy or resection specimens remains the definitive diagnostic standard, allowing combined architectural and cytological assessment.[5,6,12,18] Immuno-histochemistry (IHC) adds further clinical value by confirming lineage, distinguishing primary from metastatic tumours, enabling lymphoma classification and supporting risk stratification and biomarker-based treatment decisions.[12,15,18–22]

In tuberculosis-endemic regions such as India, distinguishing tubercular lymphadenitis from lymphoma or metastatic carcinoma is a critical clinical and public-health priority.[9–11] FNAC with Ziehl–Neelsen staining is an established tool for diagnosing tuberculous lymphadenitis, often obviating the need for excisional biopsy.[9–11] At the same time, rising head and neck cancer incidence demands early diagnostic stratification and timely referral.[16,23,24]

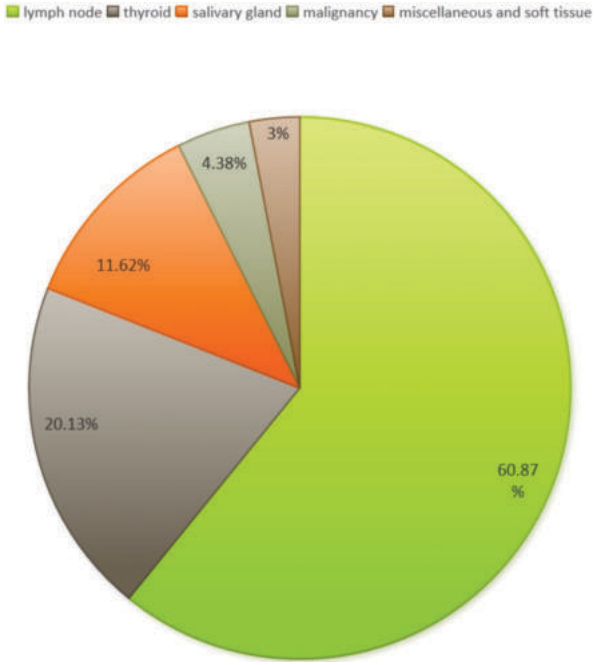

Figure 1 – Site-wise Distribution of Neck Masses

This study describes a large, single-centre series of neck masses evaluated with FNAC, histopathology and targeted IHC. The aims were: (i) to assess the diagnostic performance of FNAC against histopathology, (ii) to characterise patterns of concordance and discordance by site and lesion type, and (iii) to evaluate the incremental clinical value of IHC in malignant and challenging cases. The focus is on how an integrated FNAC–histopathology–IHC

pathway can function as a pragmatic clinical diagnostic strategy in a high tuberculosis-burden setting.

METHODS

Study Design and Setting

This retrospective–prospective observational study was conducted in the Department of Pathology, [GMC JALAUN, ORAI, ABVMU],North India, from January 2022 to December 2023. All FNAC procedures were performed as part of routine clinical care; the study represents a clinical audit of diagnostic performance with additional analysis of IHC usage, similar in spirit to other FNAC–histopathology correlation studies.[1–4,6,7]

The Institutional Ethics Committee of [GMC JALAUN, ORAI, ABVMU],North India approved the study (10/IEC/RMC/2023). The retrospective component used anonymised archival data with consent waived; prospective cases followed institutional consent policies. The study complied with the Declaration of Helsinki and national biomedical research guidelines.

Study Population

Inclusion Criteria

- Patients of any age and sex with clinically or radiologically detected neck masses undergoing FNAC during the study period.
- Adequate FNAC smears for cytological interpretation.

Exclusion Criteria

- Inadequate or haemorrhagic FNAC smears despite repeat sampling.
- Autolysed or suboptimal biopsy specimens precluding histo-pathological assessment.

A total of 800 consecutive FNACs of neck masses met inclusion criteria and were included in descriptive analyses. Histopathological follow-up (biopsy or resection) was available in 353 patients and formed the basis for performance evaluation.

FNAC procedure and Cytological Reporting.
FNAC was performed using 22–23-gauge needles and 10 mL syringes, following standard techniques for head and neck lesions.[1,2,5–7]

Lymph nodes, salivary glands and soft tissue masses: conventional aspiration technique.
Thyroid nodules: non-aspiration (capillary) technique to reduce haemorrhagic dilution.[12,16]

At least two passes were made for each lesion. Smears were air-dried for May–Grünwald–Giemsa and alcohol-fixed for Papanicolaou staining. Ziehl–Neelsen (ZN) staining was performed when granulomatous or necrotising lymphadenitis was suspected, in keeping with published approaches to tuberculous lymphadenitis. [9–11]

Cytology was reported by consultant pathologists using standard morphological criteria and categorised by site (lymph node, thyroid, salivary gland, miscellaneous soft tissue/skin). For analytic purposes, results were grouped as benign, inflammatory/infective, suspicious or malignant. For thyroid, diagnostic categories such as colloid goitre, thyroiditis, follicular neoplasm and papillary carcinoma were used in line with concepts underpinning the Bethesda System for Reporting Thyroid Cytopathology.[12–17]

For salivary gland lesions, cytological diagnoses were rendered with awareness of the risk-of-malignancy categories and terminology suggested in the Milan System for Reporting Salivary Gland Cytopathology, although formal scoring was not always applied. [18–22]

Histopathology

Biopsy and surgical specimens were fixed in 10% neutral buffered formalin, processed conventionally and stained with haematoxylin and eosin. Neck dissections and lymph node biopsies were evaluated for architectural pattern, granulomas, necrosis and involvement by lymphoma or metastasis.[5–8,9–11] Thyroid and salivary gland specimens were classified according to standard histological criteria consistent with Bethesda and Milan-based cytological interpretations.[12–17,18–22] Histopathology was treated as the diagnostic reference standard.

Immunohistochemistry

IHC was performed on formalin-fixed paraffin-embedded sections in: All malignant cases, and selected diagnostically challenging cases with ambiguous morphology.

Standard antigen retrieval and detection systems were used, with appropriate positive and negative controls, according to established practice.[12,15] Panels were chosen based on clinical and morphological differentials:
Lymphoma: CD3, CD20, CD5, CD23, CD10, BCL2, Ki-67, ±CD34, CD117.[12,15]
Thyroid tumours: PAX8, TTF-1, thyroglobulin, CK7/CK20, calcitonin, CEA.[12–17]
Salivary gland tumours: CK7, p63, p40, EMA, S100, PLAG1, Ki-67, p16.[18–22]

Cutaneous and soft tissue lesions / metastases: CK5/6, p63/p40, BerEP4 (EpCAM), BCL2, CD10, S100, Melan-A and others as required.[5,6,15]

At least two pathologists interpreted the IHC profiles, integrating them with morphology and clinical data.

Statistical Analysis

Data were entered into spreadsheets and analysed using standard statistical software. For the 353 cases with histopathology, 2×2 tables were constructed to calculate:
Sensitivity and specificity of FNAC.
Overall diagnostic accuracy.

for broad benign versus malignant/specific pathologic categorisation. [1–4,6,7] Concordance between FNAC and histopathology was calculated by site (lymph node, thyroid, salivary gland, miscellaneous). Ninety-five percent confidence intervals (CIs) were generated where appropriate. The Chi-square test was used to explore patterns of discordance; $p < 0.05$ was considered statistically significant.

RESULTS

Demographic and Anatomical Distribution
Of 800 FNACs of neck masses, 416 patients (52%) were male and 384 (48%) female (M:F 1.08:1). The most affected age group was 21–30 years, followed by 31–40 years, similar to other Indian and South Asian series of head and neck lesions.[1–4,16]
Anatomical distribution was:
Lymph- nodes: 487/800 (60.8%).
Thyroid: 161/800 (20.1%).
Salivary glands: 93/800 (11.6%).
Miscellaneous soft tissue/skin: 36/800 (4.5%).

Table 2. FNAC–Histopathology Concordance by Site (n = 353 with HPE)

Site	Cases with HPE	Concordant with FNAC	Discordant with FNAC	Concordance (%)
Lymph node	155	153	2	98.7
Thyroid	109	103	6	94.5
Salivary gland	59	58	1	98.3
Miscellaneous	30	30	0	100
Total	353	344	9	97.4

HPE: Histopathological examination.

Lymph node pathology clearly dominated the clinical workload, consistent with previous FNAC-based studies of superficial lymphadenopathy and head and neck masses.[1–8]
Cytological spectrum by site
Lymph nodes (n = 487)
Cytological Diagnoses:
Tubercular lymphadenitis: 213 (26.6%).
Reactive lymphadenitis: 200 (25.0%).
Non-specific lymphadenitis: 67 (8.3%).
Lymphoma: 7 (0.9%).
Metastatic carcinoma: 9 (1.1%).

Tubercular lymphadenitis was the most frequent specific diagnosis, reflecting high regional tuberculosis prevalence, as reported in other FNAC series using ZN staining and clinical correlation.[9–11]

Thyroid (n = 161)
Colloid/multinodular goitre: 121 (15.1%).
Thyroiditis (including Hashimoto's thyroiditis): 24 (3.0%).

Developmental cystic lesions: 9 (1.1%).
Benign neoplasms (follicular/Hürthle cell): 7 (0.9%).
Malignant lesions (papillary carcinoma thyroid): 3 (0.3%).

Benign nodular disease and thyroiditis predominated, in keeping with Bethesda-based thyroid cytology series with histological follow-up.[12–17]

Salivary glands (n = 93)
Pleomorphic adenoma: 33.
Benign lymphoepithelial lesion: 18.
Basal cell adenoma: 4.
Sialadenitis (acute/chronic/granulomatous): 38.
Malignant tumours: 5
Mucoepidermoid carcinoma: 3.
Carcinoma ex pleomorphic adenoma: 2.

This distribution, with pleomorphic adenoma and inflammatory lesions predominating, is comparable to Milan System-based studies of salivary gland FNAC.[18–22]

Miscellaneous soft tissue/skin (n = 36)
Lipoma: 10.
Epidermal inclusion cyst: 14.
Benign adnexal tumour: 1.
Malignancies: 11

Squamous cell carcinoma: 6.
Basal cell carcinoma: 1.
Malignant melanoma: 2.
Metastatic epithelial tumours: 2.

FNAC–histopathology Concordance
Histopathological follow-up was available in 353/800 cases:
Lymph nodes: 155.
Thyroid: 109.
Salivary glands: 59.
Miscellaneous soft tissue/skin: 30.

Concordance between FNAC and histopathology for major diagnostic categories was:
Lymph nodes: 153/155 (98.7%)
Thyroid: 103/109 (94.5%).
Salivary glands: 58/59 (98.3%).
Miscellaneous: 30/30 (100%).

Overall, 344/353 cases (97.4%) were concordant, and 9/353 (2.6%) were discordant, comparable to published FNAC–histology correlation rates.[1–4,6,7,18–22]

Discordant cases
Lymph nodes (2 cases):
- One cytologically diagnosed as lymphoma was reported as reactive follicular hyperplasia on biopsy.
- One reported as reactive lymphadenitis on FNAC showed follicular hyperplasia on histology.

Thyroid (6 cases):
- Four reported as colloid goitre on FNAC were diagnosed as follicular adenoma on histology.
- Two cytologically categorised as "follicular neoplasm" proved to be follicular carcinoma (capsular/vascular invasion) on resection, illustrating the known limitation of FNAC in follicular-patterned lesions.[12–17]

Salivary gland (1 case)
One mucoepidermoid carcinoma showed overlapping cytological features with other clear-cell lesions and required IHC for definitive classification, similar to the challenges highlighted in Milan System-based series. [18-22]

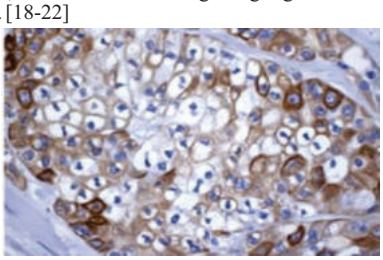

Figure 2 – Immunohistochemistry in Malignant Lesion - CK 7 Expression in Mucoepidermoid Carcinoma with Clear Cell Changes.

No discordance occurred in the miscellaneous soft tissue/skin group.
Diagnostic performance of FNAC
For broad benign versus malignant/specific pathologic categorisation, using histopathology as reference (n = 353):
Sensitivity: 98%.
Specificity: 87%.
Overall diagnostic accuracy: 92.16%.

These values are comparable to FNAC performance reported in other large series of superficial lymphadenopathy, thyroid and salivary gland lesions.[1–8,12–22]

Contribution of immunohistochemistry to clinical diagnosis
IHC was performed in all malignant cases and selected equivocal lesions. Clinically relevant contributions included:

Lymphomas: T- and B-cell markers (CD3, CD20, CD5, CD23, CD10), BCL2 and Ki-67 facilitated lineage confirmation and proliferation assessment, supporting distinction between reactive hyperplasia and low-grade lymphoma and enabling more accurate classification, in line with contemporary lymphoma diagnostic practice.[12,15]

Thyroid carcinomas: PAX8 and TTF-1 nuclear positivity with thyroglobulin cytoplasmic staining confirmed primary thyroid origin in papillary carcinoma, excluding metastasis from lung or other primaries, consistent with the Bethesda-based thyroid literature. [12–17]

Salivary gland tumours: CK7, p63/p40, EMA, S100 and PLAG1 helped differentiate mucoepidermoid carcinoma and carcinoma ex pleomorphic adenoma from other clear-cell and metastatic lesions, echoing Milan System-informed series where IHC refines salivary tumour subtyping.[18–22]

Cutaneous and soft tissue tumours/metastases: BerEP4/EpCAM, BCL2 and CD10 supported basal cell carcinoma diagnosis, while S100 and Melan-A confirmed malignant melanoma, influencing excision margins and systemic work-up as described in broader dermatopathology literature.[5,6,15]

In the small group of discordant or borderline cases, IHC resolved the diagnosis in most, underscoring its role as a clinically impactful adjunct to morphology.

DISCUSSION

This large, single-centre study demonstrates that FNAC, when integrated with histopathology and targeted IHC, provides a high-performance, clinically useful diagnostic pathway for neck masses in a high tuberculosis-burden setting. The overall FNAC–histopathology concordance of 97.4% and diagnostic accuracy of 92.16% are comparable to or better than many published FNAC series for head and neck lesions, lymphadenopathy, thyroid nodules and salivary gland masses.[1–8,12–22]

Clinical Implications in a High Tuberculosis-burden Environment
The predominance of lymph node pathology and the high proportion of tubercular lymphadenitis mirror the continuing tuberculosis burden reported in Indian and regional series.[9–11] For clinicians confronted with cervical lymphadenopathy, FNAC combined with ZN staining offers rapid differentiation between reactive, tubercular and malignant nodes, allowing timely initiation of anti-tubercular therapy or oncologic work-up.[9–11] This has direct implications for patient outcomes and resource allocation in low- and middle-income health systems.

Thyroid and Salivary Lesions: Strengths and Limitations
For thyroid nodules, FNAC showed high concordance for colloid goitre, thyroiditis and classical papillary carcinoma, in line with Bethesda-based experience and meta-analyses.[12–17] As expected, discordance clustered in follicular-patterned lesions, where cytology cannot assess capsular or vascular invasion. This limitation underlies the need for diagnostic lobectomy or excision in indeterminate Bethesda categories and is well recognised in the thyroid cytology literature.[12–15]

Salivary gland FNAC showed excellent performance for pleomorphic adenoma, sialadenitis and many malignant tumours, echoing Milan

System-based data.[18–22] The few challenging cases involved clear-cell tumours, which often require integration of IHC profiles with morphology and clinical findings. Accurate classification is clinically important because treatment options and follow-up strategies differ substantially between, for example, mucoepidermoid carcinoma, carcinoma ex pleomorphic adenoma and metastatic lesions.[18–22]

Role of IHC in Enhancing Clinical Relevance

The added value of IHC in this study lies in its ability to transform histomorphological impressions into clinically actionable tumour classifications and biomarker profiles.[12,15,18–22] In lymphomas, reliable T/B lineage assignment and Ki-67 proliferation indexing are essential for modern prognostic stratification and therapy choice.[12,15] In thyroid and salivary malignancies, confirming primary site and tumour subtype affects decisions on surgery, radioactive iodine therapy and systemic treatments, which is consistent with the expectations of contemporary, pathology-driven clinical guidelines.[12–17,18–22,23,24]

Strengths and Limitations

Strengths include the relatively large cohort, representation of all major neck mass categories and systematic cyto–histopathological correlation with targeted IHC. The design reflects real-world clinical practice in a high patient-volume, resource-constrained public hospital, enhancing generalisability within similar health systems.

Limitations include the single-centre nature and incomplete histological follow-up (353/800), which may introduce verification bias, as patients with more suspicious FNAC results are more likely to undergo surgery.[1–4] Clinical outcome data (e.g. recurrence, survival) were not systematically captured, limiting direct prognostic analyses. There was also no external central review, although local double-checking of difficult cases partially mitigated this.

Future Directions

Future work could prospectively link this diagnostic pathway with treatment decisions and clinical outcomes, allowing evaluation of how early FNAC–IHC triage impacts time to treatment, stage at diagnosis and survival, as emphasised by neck mass guidelines and contemporary clinical research frameworks.[16,23,24] Development of minimal, cost-effective IHC panels optimised for common neck malignancies in TB-endemic regions would be particularly valuable, balancing biological insight with resource constraints.

CONCLUSION

An integrated tissue-based diagnostic pathway combining FNAC, histopathology and targeted IHC provides high diagnostic accuracy for neck masses and directly supports clinical decision-making in a high tuberculosis-burden, resource-limited setting. FNAC offers rapid, minimally invasive triage; histopathology supplies definitive architectural context; and IHC delivers lineage- and site-specific information essential for modern oncologic and infectious disease management. This pragmatic model can be adapted in similar healthcare environments globally.

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