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ORIGINAL RESEARCH

Application of Cytology and Immunocytochemistry in the Diagnosis of Metastatic Tumors

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ABSTRACT

Background: Metastatic tumors represent approximately 3–5% of all cancer diagnoses and commonly manifest at an older age with a slightly higher incidence in men.[1,2] Identifying the primary site of metastasis can be challenging, often requiring multidisciplinary approaches. Fine needle aspiration cytology (FNAC) has proven to be a rapid, cost-effective diagnostic modality for both primary and metastatic malignancies. Nevertheless, additional immunocytochemical (ICC) profiling is frequently needed to accurately determine tumor lineage and subtype.[3] **Methods:** We conducted a prospective study in the Department of Pathology at V.M.M.C. & Safdarjung Hospital, including 258 patients (213 men, 45 women; age range: 20–90 years) diagnosed with metastatic tumors. FNAC smears were initially assessed using cytomorphological criteria. Clinical information and radiological findings were then correlated to narrow differential diagnoses, and finally, ICC markers were applied in cases with ambiguous cytomorphological features. Data were analyzed using SPSS v17. A P-value of <0.05 was considered significant. **Results:** Cervical lymph nodes were the most common site of metastatic deposits (n=162; 62.79%), followed by submandibular swellings (n=46; 17.83%) and supraclavicular swellings (n=25; 6.59%). FNAC in combination with clinical details correctly identified 141/258 (54.65%) metastatic tumors—predominantly squamous cell carcinoma (SCC). An additional 13 cases (5.04%) were diagnosed after considering radiological findings. ICC evaluation was crucial for diagnosing 104 cases (40.31%) that were poorly differentiated on routine FNAC. Of these, p63 positivity was indicative of metastatic SCC in 48 cases (48.08%), while Muc-1 was positive in 40 adenocarcinoma cases (38.46%). A tailored panel of ICC markers (CK7, CK20, TTF-1, thyroglobulin, ER/PR, CEA, vimentin, CD10, PLAP) established final diagnoses in the remaining 14 cases. **Conclusion:** FNAC, supported by clinical and radiological correlation, is a powerful tool for diagnosing metastatic tumors. However, ICC significantly enhances diagnostic accuracy, especially in poorly differentiated malignancies. Integrating these modalities expedites definitive diagnosis, optimizes patient management, and facilitates targeted therapy.

Keywords: Metastatic tumors, FNAC, Immunocytochemistry, Squamous cell carcinoma, Adenocarcinoma

INTRODUCTION

Metastatic tumors, which account for 3% to 5% of all cancer cases, pose a significant diagnostic challenge in clinical practice. They can present at an advanced stage and often have an occult primary origin.[1] Determining the primary site is critical because targeted therapeutic decisions, as well as prognostic counseling, hinge on the accurate classification of the tumor. Unfortunately, a subset of metastatic neoplasms remains elusive, leading to a diagnostic category referred to as “carcinoma of unknown primary” (CUP).[2]

Fine needle aspiration cytology (FNAC) has long served as a cornerstone in the initial evaluation of suspicious lesions for several reasons. First, it is minimally invasive and highly cost-effective. Second, it provides a rapid preliminary diagnosis, often distinguishing carcinoma from other malignancies such as lymphoma or sarcoma. Third, it can be performed on superficial lymph node swellings (e.g., cervical, supraclavicular) as well as on deeper sites (e.g., liver, intra-abdominal lymph nodes) under radiological guidance.[3,4] However, one limitation of FNAC is that cytomorphological details alone may sometimes be insufficient to definitively categorize

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poorly differentiated or undifferentiated neoplasms. Ancillary studies, such as immunocytochemistry (ICC), are thus indispensable in refining the classification and origin of these tumors.[5]

Immunocytochemistry helps localize and identify tumor-specific antigens using monoclonal or polyclonal antibodies applied directly onto cytological smears or cell blocks.[6] Commonly used markers—such as p63 for squamous cell carcinoma (SCC), thyroid transcription factor-1 (TTF-1) for lung and thyroid primaries, Muc-1 for adenocarcinomas, and CD10 or vimentin for renal cell carcinoma—significantly enhance diagnostic precision.[6,7] For instance, p63 nuclear positivity is strongly linked to SCC, helping differentiate it from poorly differentiated adenocarcinoma or lymphoma.[8] Similarly, the demonstration of Muc-1 is often associated with epithelial tumors of glandular origin, thereby corroborating an adenocarcinoma lineage.[9]

Integrating ICC findings with clinical history and radiological investigations further refines the diagnostic approach. Studies have shown that a coordinated effort by clinicians, radiologists, and pathologists significantly increases the yield of definitive diagnoses of metastatic tumors with unknown primaries.[7,10] In particular, the emphasis on tissue-specific or organ-specific markers—such as thyroglobulin for thyroid carcinoma or ER/PR for breast carcinoma—facilitates subtyping and guides personalized treatment strategies.

This study aimed to evaluate the role of FNAC and ICC, along with clinical and radiological correlation, in diagnosing metastatic tumors of varying morphological presentations. We present our findings from 258 cases of suspected metastatic lesions, emphasizing how a stepwise algorithmic approach incorporating cytomorphology, imaging, and tailored ICC panels can accurately pinpoint the primary site. By delineating our comprehensive strategy, we hope to provide a practical framework that can be adopted in other pathology services for efficient workup of metastatic tumors.

MATERIALS AND METHODS

Study Design and Setting

A prospective observational study was conducted in the Department of Pathology at V.M.M.C. & Safdarjung Hospital. Approval was obtained from the Institutional Ethics Committee prior to study initiation. Informed consent was secured from all participants.

Study Population

A total of 258 consecutive patients with a clinical and/or radiological suspicion of metastatic malignancy were included. The cohort comprised 213 males and 45 females, ranging in age from 20 to 90 years. Each patient underwent a thorough clinical evaluation, which included physical examination and relevant imaging studies (e.g., ultrasound, CT, or MRI).

FNAC Procedure

Fine needle aspiration was performed on accessible swellings (most commonly cervical, submandibular, supraclavicular, axillary, and chest wall). Deeper sites such as intra-abdominal lymph nodes or liver lesions were aspirated under radiological guidance. A 23-gauge needle was used, and multiple smears were prepared. Smears were fixed in 95% ethanol for Papanicolaou staining and partially air-dried for Giemsa staining.

Cytomorphological Assessment

Two experienced cytopathologists independently reviewed all FNAC slides. They documented cellularity, architecture, nuclear and cytoplasmic features, background elements (inflammatory cells, necrosis), and any diagnostic clues suggestive of metastatic disease. Discrepancies were resolved by consensus discussion.

Immunocytochemistry (ICC)

ICC was performed on either direct smears or cell block preparations in cases where the cytomorphological impression was equivocal or when a specific lineage determination was critical. A panel of markers (p63, Muc-1, CK7, CK20, TTF-1, ER, PR, thyroglobulin, CEA, vimentin, CD10, and PLAP) was employed based on the differential diagnosis suggested by cytomorphology and clinical/radiological correlation. Antigen-antibody detection was conducted using an avidin-biotin or polymer-based system, with appropriate positive and negative controls.

Data Analysis

Clinical, radiological, and laboratory data were compiled, along with FNAC and ICC results, in a standardized proforma. Statistical analysis was done using SPSS software version 17 (SPSS Inc., Chicago, IL, USA). Categorical variables were compared using chi-square or Fisher's exact tests. A P-value <0.05 was deemed statistically significant.

Outcome Measures

The primary outcome was to establish a definitive diagnosis of metastatic tumor subtype. Secondary outcomes included evaluating the diagnostic utility of FNAC alone versus FNAC plus ICC, as well as correlating final diagnoses with sites of metastatic involvement.

RESULTS

Overall Study Population

Our study analyzed 258 patients with suspected metastatic tumors. There was a distinct male predominance, with 213 men (82.56%) and 45 women (17.44%). The average age was 65 years, ranging

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from 20 to 90 years. Most patients presented with painless swelling in the cervical region, although some had more generalized symptoms such as weight loss, anorexia, or fatigue.

Anatomical Distribution of Metastases

Cervical lymph nodes were the most frequent site of metastatic deposits (162/258; 62.79%). Other commonly involved sites included submandibular swellings (46/258; 17.83%), supraclavicular areas (25/258; 6.59%), and axillary lymph nodes (11/258; 4.26%). A small proportion of patients presented with chest wall swellings (3/258; 1.16%) or intra-abdominal lymph node involvement (2/258; 0.78%). Nine patients (3.49%) had metastases in various other locations such as the liver (4), bone (3), parotid (1), and scalp (1).

Diagnostic Yield of FNAC and Correlative Methods

Table 1 outlines the diagnostic correlation based on the method employed.

Method of Detection	SCC	ADC	Other Metastatic Tumor	Total	P value
1. FNAC with Clinical Detail	138 (97.87%)	3 (2.13%)	0 (0.00%)	141 (100%)	
2. FNAC, Clinical Detail & Radiological Investigation	13 (100.00%)	0 (0.00%)	0 (0.00%)	13 (100%)	<0.0005
3. FNAC, Clinical Detail, Radiological & ICC	50 (48.08%)	40 (38.46%)	14 (13.46%)	104 (100%)	
Total	201 (77.91%)	43 (16.67%)	14 (5.43%)	258 (100%)	

- **FNAC + Clinical Details (Method 1):** This combination alone yielded definitive diagnoses in 141/258 (54.65%) cases. Among these, 138 were metastatic squamous cell carcinoma (SCC) and 3 were metastatic adenocarcinoma (ADC).
- **FNAC + Clinical Details + Radiological Findings (Method 2):** An additional 13 cases (5.04%) were diagnosed by incorporating relevant imaging data into the diagnostic process. All 13 cases were confirmed as SCC.
- **FNAC + Clinical Details + Radiological Findings + ICC (Method 3):** In 104/258 (40.31%) cases, ICC played a pivotal role. Poorly differentiated tumors that could not be definitively classified by cytomorphology alone were accurately categorized as SCC (n=50), ADC (n=40), or other metastatic tumors (n=14).

Immunocytochemistry Profiling

A subset of 104 difficult-to-classify tumors underwent ICC. P63 positivity characterized 48 cases of SCC, signifying nuclear reactivity consistent with squamous differentiation. Muc-1 positivity was identified in 40 poorly differentiated adenocarcinomas. The remaining 14 cases showed specific marker profiles leading to identification of metastatic thyroid carcinomas (papillary and follicular), mucoepidermoid carcinoma, lobular breast carcinoma, small cell lung carcinoma, renal cell carcinoma, and seminomas.

Table 2 summarizes the immunocytochemical marker expression for these "other" 14 metastatic tumors:

Other Metastatic Tumor	Frequency	Immunocytochemistry
Papillary carcinoma of thyroid	4	CK7, Thyroglobulin, TTF-1 (+); CK20 (−)
Follicular carcinoma of thyroid	2	CK7, Thyroglobulin, TTF-1 (+); CK20 (−)
Mucoepidermoid carcinoma	3	CK7 (+), CK20 (−)
Lobular carcinoma of breast	1	CK7, CEA, ER, PR (+); CK20 (−)
Small cell carcinoma of lung	1	CK7, TTF-1 (+); CK20 (−)
Renal cell carcinoma	1	CK7, Vimentin, CD10 (+); CK20 (−)
Seminoma of testis	2	PLAP (+), EMA (−)

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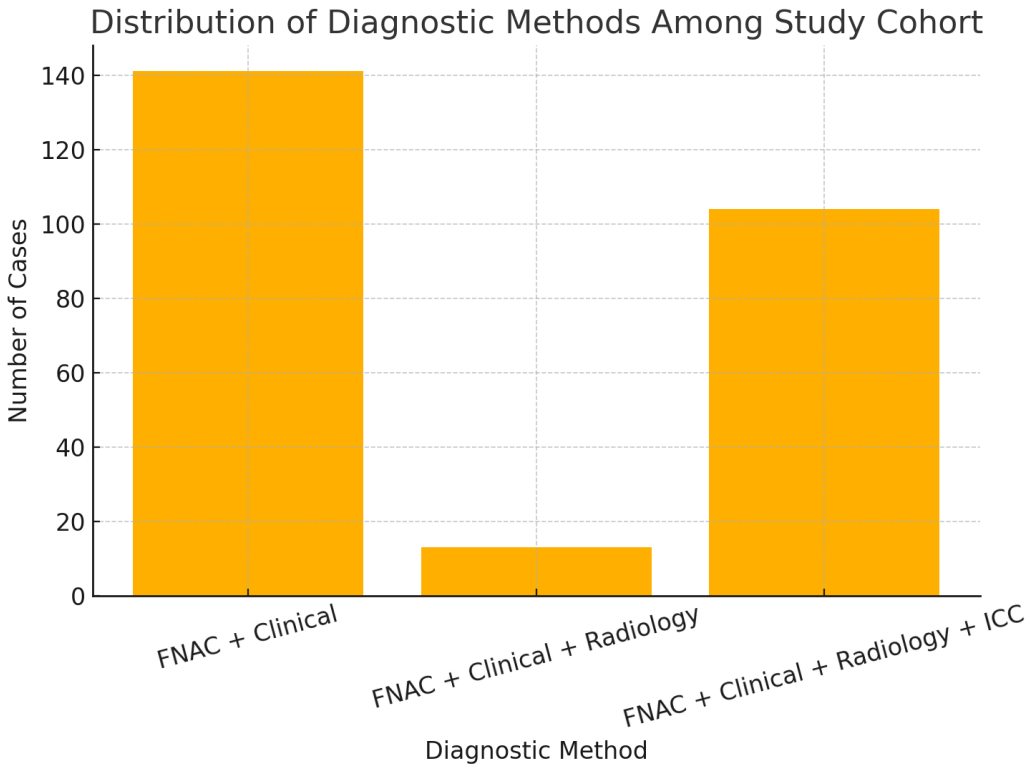

Figure 1Distribution of Diagnostic Methods Among Study Cohort

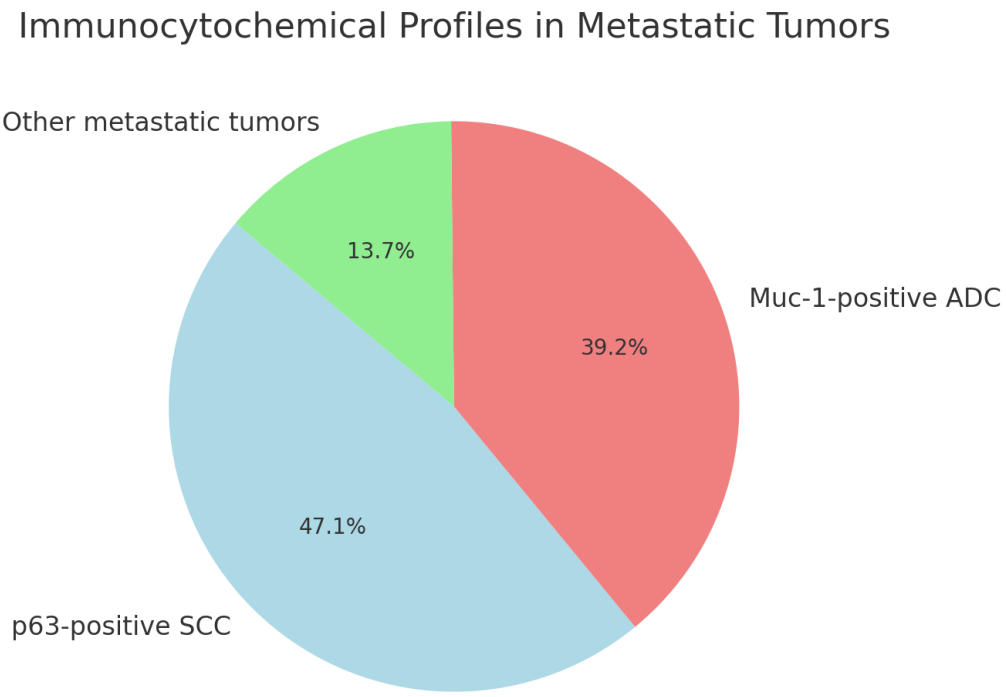

Figure 2Immunocytochemical Profiles in Metastatic Tumors

Overall, SCC was the most common metastatic lesion (201/258; 77.91%), followed by ADC (43/258; 16.67%), and other metastatic tumors (14/258; 5.43%). Integrating clinical, radiological, and immunocytochemical data significantly refined diagnostic accuracy, particularly in poorly differentiated neoplasms.

DISCUSSION

Our study underscores the critical role of FNAC and immunocytochemistry in diagnosing metastatic tumors, highlighting that a combined, multi-step approach is often required to identify the primary tumor accurately. In our series of 258 patients, initial FNAC coupled with basic clinical information successfully diagnosed more than half of the cases as metastatic squamous cell carcinoma (SCC). This high

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yield can be partly attributed to the characteristic cytomorphological features of SCC—keratinization, intercellular bridges, and abundant necrosis—which remain relatively well-preserved in many metastatic deposits.[3]

However, a substantial subset of cases (5.04%) necessitated radiological imaging—typically ultrasound, CT, or MRI—to further localize potential primaries before a definitive diagnosis could be offered. Imaging helped identify primary lesions in regions such as the head and neck, lungs, breast, or abdomen in scenarios where clinical examination was inconclusive.[7]

Despite these advancements, a notable 40.31% of our patients presented with poorly differentiated or undifferentiated tumors where neither FNAC features nor routine radiological evaluations were sufficient. For these cases, immunocytochemistry emerged as a pivotal tool to decode lineage and origin. The nuclear positivity for p63 in metastatic SCC aligns with the observations by Wu et al., who demonstrated that p63 remains an excellent discriminator of squamous phenotype, particularly in cases that lack overt squamous differentiation on routine smears. Similarly, the positivity of Muc-1 in adenocarcinomas correlates with data from Seog-Yun Park et al. and Chen et al., suggesting that Muc-1 expression in epithelial neoplasms, especially those originating from glandular tissues in the lung, breast, or gastrointestinal tract, can be critical in differential diagnosis.[10,11]

We also identified a small but important group of metastases from various primaries such as thyroid, breast, lung, kidney, and testis. Thyroid carcinomas, specifically papillary and follicular subtypes, showed classic co-expression of CK7, thyroglobulin, and TTF-1, while lacking CK20. Seminomas, on the other hand, exhibited positivity for PLAP with negative epithelial markers, a finding corroborated by Khadim et al., who emphasized the importance of using ICC to categorize non-epithelial tumors accurately.[12] The morphological heterogeneity seen in these metastatic lesions underscores the value of a targeted ICC panel, carefully selected based on clinical suspicion and cytomorphological hints.

Our findings parallel those of Sinha et al. and Didolkar et al., both of whom stressed the necessity of integrating clinical history, imaging, and cytopathological data in achieving optimal diagnostic accuracy.[4,6] Notably, while Didolkar et al. reported a predominance of metastatic adenocarcinoma, our series identified SCC as the most frequent metastatic histotype.[6] This discrepancy highlights the importance of demographic factors, referral patterns, and underlying cancer prevalence in determining the most common metastatic subtypes.

In essence, the present study reaffirms the synergistic potency of FNAC, clinical-radiological correlation, and immunocytochemical analysis in investigating metastatic tumors. By pinpointing the specific origin of poorly differentiated lesions, ICC not only refines diagnosis but also broadens therapeutic possibilities, enabling site-directed therapies such as targeted molecular agents or hormonal interventions. As precision oncology continues to evolve, the incorporation of ancillary immunoprofiling in metastatic workups will increasingly shape personalized treatment strategies and potentially improve patient outcomes.

CONCLUSION

In conclusion, our study demonstrates that FNAC, in conjunction with radiological correlation and immunocytochemical profiling, constitutes a powerful triad for the diagnosis of metastatic tumors. While morphological cues on FNAC are often sufficient for well- or moderately differentiated metastatic carcinomas, a significant fraction of poorly differentiated lesions necessitates targeted ICC panels to accurately determine tumor lineage. This integrated approach ensures prompt, accurate diagnoses, facilitating appropriate and timely therapy. Given the rising complexity of oncology, especially in cases of unknown primary tumors, robust cytopathological investigations paired with advanced immunocytochemical techniques will remain indispensable in modern cancer diagnostics.

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