Original Article

DOI: 10.7860/NJLM/2021/44787:2451

Pathology Section

A Combined Cytomorphological and Immunocytochemical Approach in the Diagnosis of Cancers of Unknown Primary Origin

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ABSTRACT

Introduction: Unknown Primary Cancers (UPCs) present a major diagnostic challenge to both clinicians and pathologists. Hence, there is a strong need to effectively diagnose these cases.

Aim: To study the cytological features of metastatic deposits from UPCs and the role of appropriate immunomarkers in identifying the primary site.

Materials and Methods: This was a prospective descriptive study conducted between December 2012 to December 2015 in the Department of Pathology in JIPMER, Puducherry, India. Patients presenting with suspicious nodal or other probable metastatic deposits were subjected to Fine Needle Aspiration Cytology (FNAC). Morphologic features were studied and provisional/differential diagnosis were made, following which whenever essential, Immunocytochemistry (ICC) using appropriate panels of immunomarkers was performed on smears or cell blocks. Only the cases with subsequent histological/clinico-radiological correlation were statistically analysed by Statistical Package for Social Sciences (SPSS version 16.0) software and the distribution of data was expressed in frequencies and percentages and the p-value <0.05 was considered as significant level.

Results: Out of total 224 cases of UPCs, 162 cases with subsequent follow-up were included in the study; 130 patients had histopathological follow-up, while 32 patients had only clinico-radiological follow-up. There was cytohistological concordance rate of 100% with a diagnostic accuracy of 95.4% for specific subtyping and a p-value of <0.0001. The maximum diagnostic accuracy for specific subtyping was noted with Squamous Cell Carcinomas (SCC), followed by Adenocarcinomas (ADC), miscellaneous diagnosis and lastly the Poorly Differentiated Carcinomas (PDC).

Conclusion: This study demonstrated that a combined cytomorphological/immunocytochemical approach is highly effective in predicting the cell of origin and also the possible primary site of Cancer of Unknown Primary (CUP).

Keywords: Fine needle aspiration cytology, Immunocytochemistry, Metastatic deposits

INTRODUCTION

The UPCs constitute a heterogeneous group of malignancies characterised by the presence of metastatic disease without a clinically identifiable primary site at routine work-up. They account for 3-5% of all cancers, although the incidence differs depending upon the criteria used, and the number of diagnostic procedures performed. According to the current hypothesis, the primary source of UPCs is microscopic which is clinically undetectable or involutes after giving rise to metastasis [1-4].

The FNAC is a safe and minimally invasive procedure for dealing with UPCs. UPCs are broadly categorised based on routine cytomorphological features into: (i) SCC; (ii) ADC; (iii) Neuroendocrine Carcinoma (NEC); and (iv) PDC based upon standard routine diagnostic terminologies used in light microscopy for the classification of malignancies [2]. Typing of undifferentiated tumours into specific subtypes is crucial for appropriate management of the patients. Routine cytological diagnosis alone cannot always provide a precise diagnosis since cytological features only give idea about the morphological type and cannot predict the tumour location. The same drawback also applies to histopathology of the metastatic site which cannot predict the primary site unless otherwise the biopsy is taken from the primary site after thorough diagnostic evaluation. Use of ancillary techniques such as cell blocks and ICC are of considerable help in increasing the diagnostic accuracy in such cases [5].

Considering the myriad possibilities of primary sources for undifferentiated tumours, based on the cytomorphological features, a pathologist needs to narrow down the possible primary sites and hence the panel of markers. By this combined cytomorphological and immunocytochemical approach, a cytopathologist can assist the clinician in identifying the precise primary site with further relevant tests. There has been a significant literature individually dealing with the various groups of UPCs, but not many authors have carried out a collective work on all possible sites of metastasis of unknown primary accessible to routine and ultrasound-guided FNAC. This prospective study was conducted to study the cytological features of metastatic deposits from UPCs and to evaluate the role of appropriate immunomarkers on cell blocks/smears in identifying the primary site.

MATERIALS AND METHODS

The present study was a prospective descriptive study, which was undertaken at the Department of Pathology in Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry, for a period of three years from December 2012 to December 2015.

Proper clearance from Institutional Ethics Committee was obtained prior to the study (IEC/SC/2012/5/180-dated 10.12.2012). Informed consent was obtained from all patients. Follow-up was done for a period of one year after the initial diagnosis.

Inclusion Criteria

Patients with clinically unsuspected or undetected primaries presenting with metastasis, diagnosed by FNAC of lymph nodes,

liver, lungs, skin and other sites with subsequent clinical, radiological and/or pathological follow-up were included in this study. Total of 162 patients who fulfilled the inclusion criteria recruited in the study, during the study period constituted the sample population.

Exclusion Criteria

Patients with similar clinical profile with undetected primaries but lost to further clinical or pathological follow-up were excluded from this study.

The FNAC was performed using the standard procedure or under ultrasound guidance in patients presenting to FNAC-outpatient department with suspicious nodal or other probable metastatic deposits. Multiple smears had been prepared and stained by both Papanicolaou (PAP) and May-Grünwald-Giemsa (MGG) stains. Wherever possible, diagnosis of possible primary was offered at routine smear examination on clinico-radiological correlation.

When routine cytomorphology with clinical correlation failed to identify the primary site, appropriate panel of immunomarkers comprising of Cytokeratin 7 and 20, Pan CK, Thyroid Transcription Factor 1 (TTF-1), Prostate Specific Antigen (PSA), CA 125, Carcinoembryonic Antigen (CEA), chromagranin, synaptophysin, CD56, Neuron Specific Enolase (NSE), vimentin, desmin, HMB45, S100, Melan A, Placental Alkaline Phosphatase (PLAP) were used on cell block sections/smears. The cell blocks were prepared by the plasma-thrombin method and the sections stained with, routine Haematoxylin and Eosin (H&E) and conventional special stains along with ICC with an appropriate panel of markers. Histological sections were also reviewed and the final histopathological diagnosis were given after assessing the histological patterns and cytological details. Cytological/immunocytochemical features and the cytological diagnosis were correlated with the histological features and diagnosis.

STATISTICAL ANALYSIS

The data was entered in MS Excel and the statistical analysis was done using SPSS version 16.0. Frequency and percentages were calculated and the results were tabulated.

RESULTS

During the study period, a total of 224 cases of UPC were diagnosed or suspected on FNAC, of which 162 cases which had a subsequent follow-up with histopathological and clinico-radiological correlation for a period of one year were included in the study. The age of the patients ranged from 1-85 years with maximum patients occurring in the age group 40-60 years. The male to female ratio was 4:1.

Site Distribution of UPC

Among the metastatic sites, cervical lymph nodes were the most common accounting for 63% (102/162 cases), followed by the supraclavicular group which accounted for 21% (34/162), inguinal nodes constituting 5% (8/162), liver and bone each accounted for 4% (7/162) of the cases. Other sites were involved in 3% (4/162) of cases; the sites included one each of axillary lymph node, retroperitoneal lymph node, scalp and omentum.

Cytological Diagnosis of UPC

The distribution of cytological diagnosis of UPC is shown in [Table/Fig-1]. ADC comprised the major category with 68 (42%) cases, followed by SCC with 65 (40.1%) cases.

Cytological diagnosis	n (162)	Percentage
Adenocarcinoma	68	42
Squamous cell carcinoma	65	40.1
Poorly differentiated carcinoma	10	6.2
Miscellaneous/other diagnosis	19	11.7

[Table/Fig-1]: Distribution of cytomorphological diagnosis in UPC.

Adenocarcinoma (ADC)

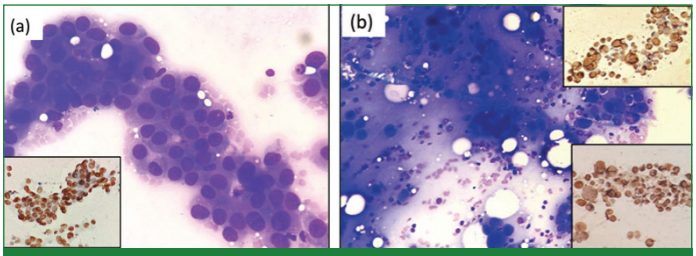
The distribution of ADC cases according to primary site is shown in [Table/Fig-2]. Lung accounted for 22.1% (15/68) followed by the thyroid which constituted 17.7% (12/68). In each of these cases clinico-pathological follow-up revealed the occult site by site directed investigations such as endoscopy, biopsy, tumour markers such as CEA, PSA and radiological examination.

Primary site	Total cases n=68 (%)	Cytological diagnosis	ICC marker profile
Lung	15 (22.1)	Adenocarcinoma	TTTF-1 positive CK7 positive CK20 negative
Thyroid	12 (17.7)	Papillary thyroid carcinoma (11) Follicular thyroid carcinoma (1)	
Prostate	4 (5.9)	Adenocarcinoma	PSA positive
Stomach	8 (11.8)	Adenocarcinoma	CK7>CK20
GE junction	3 (4.4)	Adenocarcinoma	CK>CK20
Colon	2 (2.9)	Adenocarcinoma	CK20 positive CK7 negative
Pancreas	2 (2.9)	Adenocarcinoma	
Ovary	4 (5.9)	Adenocarcinoma	CK7 positive CA 125 positive
Kidney	2 (2.9)	Renal cell carcinoma	CK positive CD10 positive Vimentin positive
Site unknown	16 (23.5)	Adenocarcinoma	

[Table/Fig-2]: Site-wise distribution of Adenocarcinoma (ADC) cases with clinicopathological follow-up.

Cytomorphological Characteristics of Adenocarcinoma (ADC) Subsets

The ADC distribution is tabulated in [Table/Fig-2]. In few cases of lung, ADCs predominant acinar pattern with positive immune profile for TTF-1 was observed [Table/Fig-3a]. In case of primary gastrointestinal tumours (stomach, colon and pancreas), ADC cells are usually arranged in clusters or glandular pattern in a background of mucin [Table/Fig-3b]. Prostatic ADC usually showed well-defined glandular pattern with minimal nuclear pleomorphism and positivity for PSA was observed in this study [Table/Fig-4a,b]). In cases of renal cell carcinoma, cells had abundant finely granular to clear vacuolated cytoplasm [Table/Fig-4c-e].



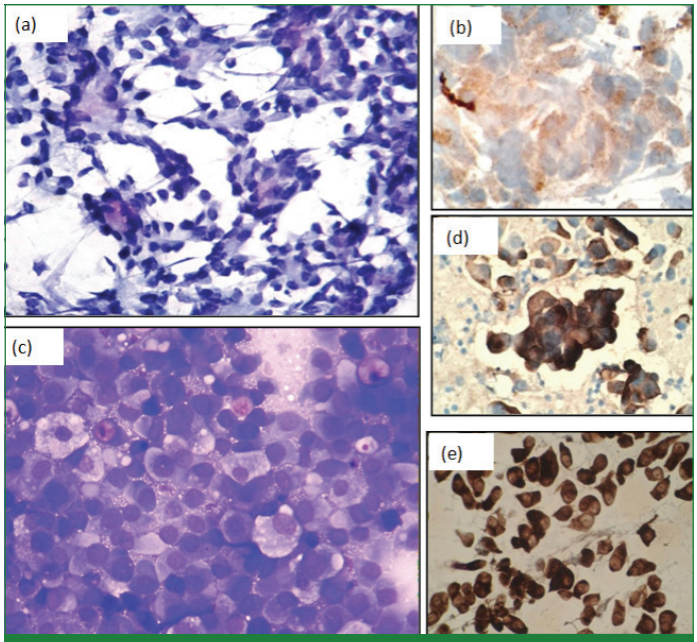
[Table/Fig-3]: a) Metastatic pulmonary ADC with malignant cells arranged in acinar pattern with inset showing TTF-1 positivity (MGGx400; ICCX400); b) Metastatic diffuse gastric ADC showing loose clusters and discrete malignant cells trapped in a pool of mucin (MGGx400). Inset CK7 (top) and CK20 positivity (ICCx400).

Squamous Cell Carcinoma (SCC)

Site-wise distribution of SCC is shown in [Table/Fig-5]. The use of triple endoscopic procedures, head and neck imaging greatly aided in the diagnosis of primary site. The tumours cells are usually in sheets or scattered and have abundant cytoplasm with hyperchromatic nuclei in MGG stain and orangeophilic cytoplasm with pyknotic nuclei in PAP stain. Individual cell keratinisation is noted in well-differentiated tumours. Necrosis was also noted.

Poorly Differentiated Carcinomas

Cytological diagnosis of PDCs was offered in 10 cases where it was not possible to type the malignancy based on cytology. The primary



[Table/Fig-4]: a) Metastatic prostatic ADC showing a glandular pattern and lack of striking nuclear pleomorphism (Papx400); b) PSA positivity (ICCx400); c) Metastatic renal cell carcinoma with cells showing moderate to abundant, finely granular to vacuolated/clear cytoplasm and fairly monomorphic round to ovoid nuclei (MGGx400); d and e) cells exhibiting Vimentin and CD-10 positivity (ICCx400).

Site	Number of cases (n=65)	Percentage
Hypopharynx	11	16.9
Tonsil	6	9.2
Oral cavity	8	12.3
Base of tongue	4	6.15
Larynx	6	9.2
Esophagus	6	9.2
Lung	1	1.5
Nasopharynx	1	1.5
Site unknown	22	33.8

[Table/Fig-5]: Site-wise distribution of biopsy-proven cases of Squamous Cell Carcinoma (SCC).

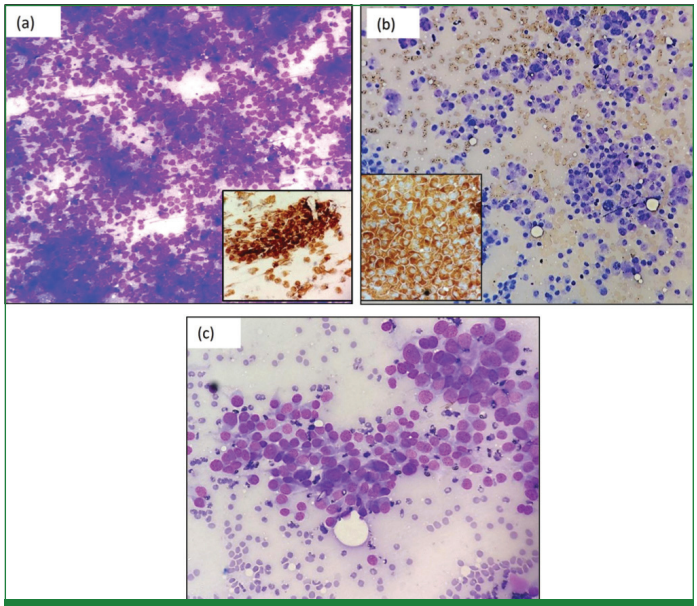
site could be located in five cases which included two cases of lung primary, and one case each of tongue, supraglottis and ovary. In the remaining five cases, primary site could not be located even after extensive investigations during the follow-up period.

Miscellaneous Diagnosis

The group of cases with miscellaneous diagnosis is listed in [Table/Fig-6]. Nine metastatic neuroendocrine tumours were diagnosed on FNAC chiefly on cytomorphological features in conjunction with ICC. Among them, there were three metastatic NECs [Table/Fig-7a] diagnosed on FNAC. There were four cases of metastatic small cell carcinoma which showed scattered cells with scant cytoplasm with distinctive nuclear features of 'salt and pepper' chromatin, nuclear moulding. Streaking artefact and karyorrhectic debris were noted in the background. The cells exhibited positive expression for neuroendocrine markers like CD56, NSE, chromagranin and synaptophysin. In the only case of carcinoid [Table/Fig-7b], metastatic to liver, ICC with synaptophysin and chromogranin was done due to a strong cytomorphological indication. There was an interesting case of metastatic medullary thyroid carcinoma [Table/Fig-7c] presenting as a CUP. Nuclear scan detected a suspicious focus in the thyroid, further confirmed by an elevated serum calcitonin level. A diagnosis of nasopharyngeal carcinoma was made in three cases of CUP presenting in cervical lymph nodes. ICC was done which showed the pleomorphic cells to be PAN-CK positive. Subsequent biopsy from the nasopharynx confirmed the diagnosis in all three cases. There were two cases of metastatic sarcomatoid carcinoma presenting as CUP, one each in the cervical lymph nodes and scalp, respectively. Metastatic rhabdomyosarcoma [Table/Fig-8a] presenting as CUP in the inguinal lymph node was observed in this study. The characteristic cytomorphology with presence of rhabdomyoblasts and desmin positivity confirmed the diagnosis. Two cases of metastatic mixed germ cell tumours [Table/Fig-8b] were documented in this study. Both of them presented with supraclavicular lymph node metastasis. The presence of tigroid background is characteristic of seminomatous component. Both these cases had positive staining with PLAP, vimentin and Epithelial Membrane Antigen (EMA). In addition to these markers, the second case showed focal positivity for Alpha Fetoprotein (AFP).

Cytological diagnosis	N=19	Site of primary	ICC marker profile
Neuroendocrine carcinomas	3	Unknown (n=2) Lung (n=1)	Synaptophysin+ Chromagranin+
Carcinoid	1	Unknown (n=1)	Synaptophysin+ Chromagranin+
Small cell carcinoma	4	Unknown (n=2) Lung (n=2)	CD 56+ Synaptophysin+
Medullary thyroid carcinoma	1	Thyroid (n=1)	-
Nasopharyngeal carcinoma	3	Nasopharynx (n=3)	PANCK +
Germ cell tumours	2	Testes (n=2)	PLAP+ Vimentin+
Sarcomatoid carcinoma	2	Vault growth (n=1) Unknown (n=1)	CK+ Vimentin+
Rhabdomyosarcoma	1	Testes (n=1)	Desmin+
Transitional cell carcinoma	1	Bladder (n=1)	-
Melanoma	1	Unknown (n=1)	HMB45+ S100 +

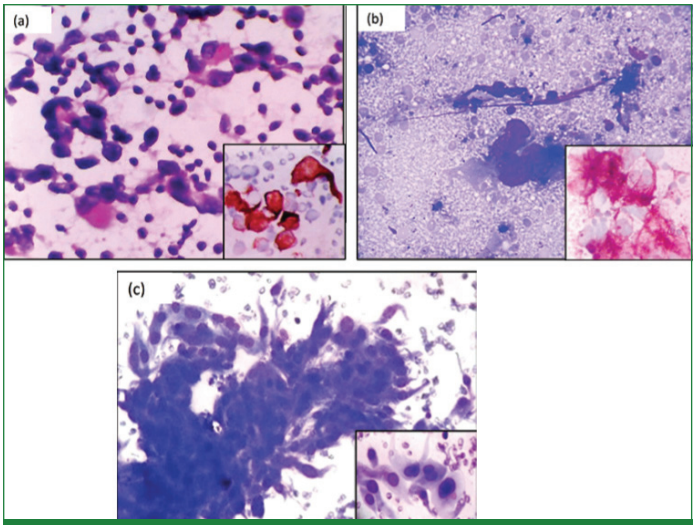
[Table/Fig-6]: Characterisation of cases with miscellaneous diagnosis with clinicopathological follow-up.



[Table/Fig-7]: a) Metastatic Neuroendocrine Carcinoma (NEC) (MGGx200): Inset: chromagranin positivity; b) Metastatic carcinoid with discrete, monomorphic, plasmacytoid neoplastic cells having moderate amount of cytoplasm and round nucleus with stippled chromatin (MGGx400): Inset: Synaptophysin positivity (ICCx400); c) Metastatic medullary thyroid cancer showing plasmacytoid cells of variable size exhibiting scanty to moderate amount of cytoplasm, stippled chromatin and inconspicuous nucleoli (MGGx400).

A single case of metastatic transitional cell carcinoma [Table/Fig-8c] presenting as an unknown primary was documented in this study. FNAC was done from the inguinal and cervical nodes and diagnosis of "metastatic deposit suspicious of urothelial carcinoma" was offered. Later, the biopsy confirmed the diagnosis of urothelial carcinoma.

A single case of metastatic malignant melanoma presented as an unknown primary in this study. The smears showed discohesive cells with multinucleate and binucleate forms exhibiting prominent nucleoli and occasional intracytoplasmic melanin pigment. On subsequent clinico-pathological follow-up, the biopsy of the lymph

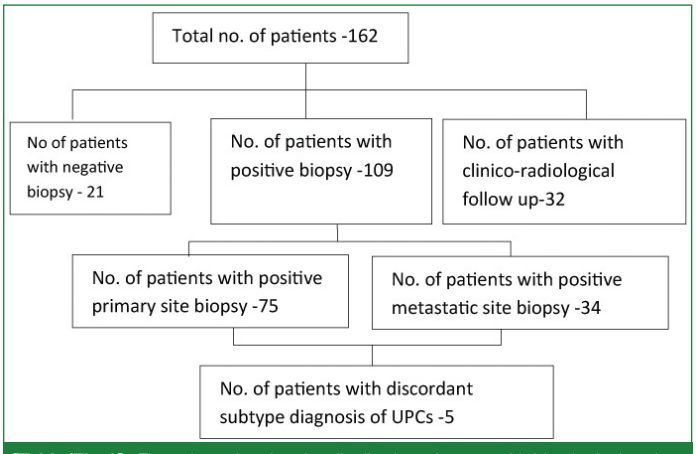


[Table/Fig-8]: a) Metastatic rhabdomyosarcoma showing a mixture of loosely arranged small round cells admixed with classic rhabdomyoblasts (Pap x400): Inset. shows desmin positivity (ICCx400); b) Metastatic germ cell tumour showing the characteristic tigroid background of seminomatous component (MGGx400): Inset shows PLAP positivity ((ICCx400); c) Metastatic urothelial carcinoma shows cells resembling bland metaplastic squamous cells (MGGx200): Inset shows cercariform cells (MGGx400).

node and IHC confirmed the diagnosis. However, the primary site could not be located.

Histopathological Correlation

Of the 162 cases of UPCs included in the study, biopsy of the primary site was available in 75 patients while biopsy of metastatic lymph nodes was available in another 34 patients [Table/Fig-9]. A concordant cytological diagnosis was obtained in all cases, except five cases which included four cases of PDCs which were diagnosed as SCC on biopsy and one case of undifferentiated nasopharyngeal carcinoma misdiagnosed as SCC on cytology. The observed concordance rate for diagnosis of malignancy was 100% with a diagnostic accuracy of 95.4% for specific subtyping and a p-value of <0.0001. Biopsy of suspicious primary sites in 21 cases which were negative for malignancy were excluded from the statistical correlation as they do not precisely represent the true negative group, considering the fact that the true primary site has not been sampled or remained occult.



[Table/Fig-9]: Flow chart showing the distribution of cases with histological and clinico-radiological follow-up.

The cases which persisted as unknown primary even after detailed clinico-radiological work-up and negative biopsy of suspicious sites are shown in [Table/Fig-10]. SCC comprised the majority of persistent unknown primary cases.

DISCUSSION

The UPCs constitutes about 3-5% of solid tumours referred to the oncology department. The scenario of an unknown primary can be very disturbing for both the patient and the treating clinician. This is to a large extent provoked due to the uncertainity in treatment and the automatic assumption of a grim prognosis. It's noteworthy that the entity of CUP actually represents a group of diseases with widely divergent prognosis with certain favourable subsets [1,3,6,7].

Cytological diagnosis	n=50	Percentage
Squamous cell carcinoma	22	44
Adenocarcinoma	16	32
Poorly differentiated carcinoma	5	10
Others	7	14

[Table/Fig-10]: Cases which persisted as unknown primary, even after a detailed clinicoradiological work-up.

It is thus essential to conduct a consistent and thorough diagnostic evaluation to determine if the patients fall into any one of the favourable subgroups and this approach enables the physicians to optimise the treatment regimen for each patient [8,9].

Sites of Metastasis

Analysis of the frequency of metastatic malignancies showed the lymph nodes to be the most common site of metastasis with maximum number of aspirations from the cervical lymph nodes (63%). In similar studies conducted by Wilkinson AR et al., and Alam K et al., cervical lymph nodes were the most common group affected by metastatic malignancies [10,11].

Cytological Diagnosis

In the current study, ADC was the most common cytological diagnosis followed by SCC. A comprehensive review of literature showed varying proportions of metastatic malignancies presenting as CUP, with some authors observing SCC as the predominant diagnosis, while others documenting ADC as the major diagnosis [12-15].

Locating the Primary Site of ADC by Combined Approach

Of the cytomorphological findings assessed in this study, the presence of glandular cells with mucin in primary gastrointestinal carcinomas, clear to finely vacuolated cells in renal cell carcinoma, glandular arrangement without striking nuclear pleomorphism in prostatic ADC were striking diagnostic clues.

Primary tumours from the gastrointestinal tract have certain common cytological features. FNAC reveals a moderate to high cellularity with cells arranged in clusters, sheets and glandular pattern. Individual cells show vesicular nucleus with prominent nucleoli and scant cytoplasm. Presence of signet ring cells usually points to a gastric or pancreatic primary, though signet ring cells may be present in a wide variety of ADCs originating in other sites. Imaging and panel of immunomarkers such as CK7 and CK20 may be used to localise the primary site. Cytological findings of ADC subsets and the use of immunomarkers have been documented in the literature [16,17].

There have been only a few studies discussing the cytological features of metastatic prostatic ADC. In one published series, Gong Y et al., described the presence of cells having an abundant cytoplasm and bland oval nuclei, arranged in papillary fronds and sheets, with some showing peripheral nuclear palisading [18]. ICC with PSA and Prostate Specific Acid Phosphatase (PSAP) is essential to arrive at a definitive diagnosis.

Several studies have reported similar cytomorphological findings in localising the primary site of ADC. The importance of ICC in localising the primary site has been emphasised by few authors [16,17].

Squamous Cell Carcinoma (SCC)

The cytomorphological characteristic of well-differentiated SCC is straightforward. In the current study, there was difficulty in diagnosing cases which were poorly differentiated, associated with necrosis and cystic change. These pitfalls in diagnosing metastatic SCC have been well documented in the literature. The primary sites were predominantly located in the head and neck region [19].

Poorly Differentiated Carcinoma (PDC)

Metastasis from PDC poses a genuine difficulty in the detection of unknown primary. There has been a significant confusion in the diagnosis of this group of lesions, as precise cytomorphological diagnosis is not possible. Even in the present study, this category presented a grey area in diagnosis. This heterogeneous group includes not only undifferentiated carcinomatous tumours, but also tumours which present with overlapping morphological features. Bahrami A et al., has also emphasised the application of abroad panel of immunomarkers, based upon the clinical profile of the patients in the diagnosis of undifferentiated tumours [20].

Miscellaneous Diagnosis

Nine cases of metastatic neuroendocrine tumours were encountered in the present study. The cytomorphological features of NECs included uniform population of cells, delicate "salt and pepper chromatin", nuclear molding and grooving and absence of necrosis. Architectural patterns such as an organoid arrangement, rosettes may also give 'clue' to the diagnosis. Similar cytological findings of neuroendocrine tumours were reported by Gupta RK et al., in his study of metastatic neuroendocrine tumours in liver [21].

The occurrence of metastatic nasopharyngeal carcinoma has been documented in the literature. The cytomorphology usually consists of cells with clusters of large undifferentiated cells which contain large nucleus with or without prominent nucleoli and moderate amount of cytoplasm. The cells are admixed with lymphocytes in the background. Mohanty SK et al., evaluated 15 cases of metastatic nasopharyngeal carcinoma to lymph nodes and described similar cytological features [22].

Two metastatic germ cells tumours with the characteristic tigroid background were documented in this study. Diagnosis of metastatic mixed germ cell tumours on cytology is challenging because of their rarity and varied morphological characteristics, particularly when they present as unknown primary at unexpected sites. They are an important differential diagnosis for metastatic undifferentiated carcinomas in young adult males presenting with cervical lymphadenopathy. Characteristic cytopathological features in an appropriate clinical scenario, helps in clinching the accurate diagnosis. Seminomas have discohesive cells with vacuolated cytoplasm, prominent nucleoli in a background of lymphocytes. Germ cell tumours are often misdiagnosed as undifferentiated carcinoma; therefore a high index of suspicion is required for their correct diagnosis. Gupta R et al., studied 88 cases of extra gonadal germ cell tumours and reported similar cytomorphological findings [23]. Correlation with the tumour markers like PLAP, AFP and β-hCG is useful. Sub-classification of germ cell tumours is essential for definitive treatment.

One case of metastatic transitional cell carcinoma with characteristic cytomorphological features was reported in the present study. Presence of Cercariform Cells (CCs) in smears is considered as a strong 'diagnostic clue' of urothelial origin. Renshaw AA and Madge R, broadened this definition and cells with shorter, broader, but flattened and often bulbous tail with vacuole and eccentric placed nucleus have been described as characteristic features of CCs. Although, CCs vaguely resemble squamous cells, their bland nature and lack of keratinisation should suggest urothelial carcinoma and facilitate appropriate clinical follow-up [24,25].

Metastatic malignant melanoma presenting as unknown primary is uncommon; however, cases have been reported in the literature [26]. The common hypothesis accepted for an unknown primary melanoma is the spontaneous involution of the primary cutaneous tumour. The cytological features of melanoma reported by Perry MD et al., include: (i) dissociated cells with well-defined cytoplasm; (ii) eccentric nuclei; (iii) prominent anisokaryosis; (iv) dense chromatin; (v) binucleate cells; (vi) prominent nucleoli; (vii) intranuclear inclusions; (viii) evidence of melanin pigment; and (ix) malignant cells with abundant cytoplasm with a dark staining paranuclear area [27].

The ICC is an essential ancillary investigation in the diagnosis of metastatic tumours of unknown origin. Few authors have emphasised the combined role of immunomarkers along with cytology and histopathological examination in localising the primary site. All these techniques used in combination with complete clinico-radiological investigations help in establishing the primary site for management of UPCs [28]. In the present study, cytohistological concordance rate of 100% with a diagnostic accuracy of 95.4% for specific subtyping was observed. In a similar study conducted by Mehdi G et al., concordance rate of 93.1% on cytohistological correlation was observed [29].

Recent developments in molecular research including gene expression profiling have greatly improved diagnosis and treatment strategies for CUP patients [30]. In developing countries like India, the combined approach with limited panel of markers is more cost effective in making therapeutic decisions. The present study emphasises the effective role of a combined immuno-cytomorphological approach in the preoperative diagnosis and management of UPCs.

Limitation(s)

The major limitation of this study is the long period of follow-up required to monitor the cases and confirm the primary site. Even after the follow-up period of one year, few cases persisted as unknown primary.

CONCLUSION(S)

A combined cytomorphological/immunocytochemical approach is highly useful in predicting the cell of origin and also the possible primary site of carcinoma/CUP. Attention to the details such as the age and sex of the patients and an awareness of common malignancies in different groups of patients along with the essential 'cytomorphological clues' assist in the selection of immunomarkers and narrowing down the panel. A cytopathologist can rely upon certain "strong morphological clues" in predicting the cell of origin of UPCs in malignancies such as neuroendocrine tumours, rhabdomyosarcoma, germ cell tumours, melanoma and medullary thyroid carcinoma. Even in cases where a biopsy is not possible, the prediction of the cell of origin by the combined cytomorphological/immunocytochemical approach helps the clinicians in deciding further investigations to detect the primary site.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Apr 24, 2020
- Manual Googling: Jul 11, 2020
- iThenticate Software: Dec 18, 2020 (6%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Apr 23, 2020**
Date of Peer Review: **May 28, 2020**
Date of Acceptance: **Jul 13, 2020**
Date of Publishing: **Jan 01, 2021**