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RESEARCH ARTICLE

Immunohistochemical Profiling of Metastatic Lymph Nodes to Identify Primary Tumor Sites: A Retrospective Study

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Abstract

Introduction: Metastatic lymphadenopathy is a common initial clinical presentation of many malignancies. Immunohistochemistry plays an important role in the determination of the primary site of the tumor. The objective of this study was to analyze the immunohistochemical profile of metastatic lymph nodes and to assess the common primary sites. **Materials and Methods:** This was a 4 year retrospective study of all the metastatic lymph nodes analyzed using immunohistochemistry. Regional lymph node excisions done as a part of treatment/staging were excluded from the study. The slides were retrieved and reviewed for morphology and IHC to identify the type of malignancy and the possible primary site. A comprehensive panel of IHC markers were used in most cases selected based on age, sex, clinical and imaging findings and morphology. **Results:** A total of 58 metastatic lymph nodes were reported. Cervical lymph nodes including supraclavicular were the most common group of lymph nodes involved in general (97%). A most probable primary site was suggested in 75.8% of cases, lung being the most common site (50%). In a smaller proportion of cases (12%), more than one possible primary was suggested and in 12% a primary site could not be assigned. The most frequently used immunohistochemical marker was CK7(86 %) and TTF1 was the most common site specific marker used (76%). **Conclusion:** Immunohistochemistry plays a pivotal role in the work up of metastatic lymph nodes . With an algorithmic approach and judicious use of markers the primary site may be ascertained in most cases, circumventing the need for additional biopsies and other investigations.

Keywords: Metastasis- Lymph node- Immunohistochemistry

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Introduction

It is well known that epithelial malignancies and melanomas metastasize most commonly to lymph nodes via the lymphatic pathway. Many sarcomas are also known to metastasize to lymph nodes. Many patients with primary malignancies at remote sites may present with lymph node enlargement especially of the cervical region. Initial evaluation of these patients include Fine needle aspiration cytology (FNAC) and excision. At times, metastatic lymph nodes are biopsied even when there is an obvious primary, in order to confirm the primary with the additional use of immune histochemical markers which point towards probable primary site.

Immunohistochemistry (IHC) has emerged as a powerful ancillary tool in the assessment of metastatic lymph nodes and a judiciously employed panel of antibodies will be able to identify or confirm the primary in most cases.

The objective of this study was to analyze the IHC profile of metastatic lymph nodes presenting to our institution and to assess the utility of IHC in suggesting the primary site. We also sought to identify the number and frequency of the IHC markers used in the work up of metastatic lymph nodes.

Materials and Methods

This was a 4 year retrospective study of all the metastatic lymph nodes analyzed using IHC. Regional

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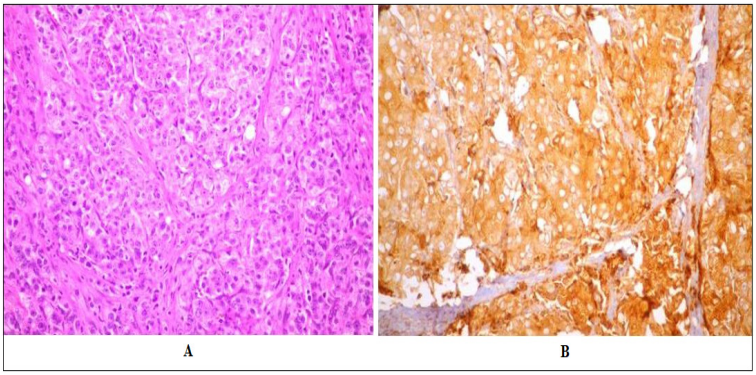

Figure 1. A. Morphology consistent with a metastatic adenocarcinoma in the lymph node in a male patient. H &E, 40x B. PSA immunostain positive in tumor cells, indicating prostate primary. PSA, x40.

lymph node excisions done as a part of treatment/staging were excluded from the study.The slides were retrieved and reviewed for morphology and IHC to identify the type of malignancy and the possible primary site. We used a comprehensive panel of IHC markers in most cases selected based on age, sex, clinical and imaging findings and morphology.

Institutional Ethical Review Board approval was applied and obtained for the study.

Chi square test was used to calculate whether cervical lymph nodes were preferred sites for metastases from lung primary when compared to other lymph node sites.

The markers used included Pan-Cytokeratin (CK), CK 7, CK20, CK5/6, EMA, Vimentin, CD45, TTF1, Synoptophysin, CD56, Napsin, PSA, CD10, Mammaglobin, Chromogranin and WT1. An algorithmic approach was used in the selection of markers, subsequent panels being ordered depending on the positive markers in the initial panel.

Results

During the 4 year period from 2015 to 2019, IHC was performed on a total of 477 lymphnodes biopsies or excisions. Of these, 58 were metastatic lymph nodes. 302 were lymphomas and 117 were reactive lymph nodes in which lymphoma was a differential diagnosis. In two cases, IHC was done on cell block preparation from FNAC material.

Out of the total number of 58 cases with metastatic lymph nodes, 39 (67.2%) were men and 19 were women. There were 54 (94.1%) adults and 4 children. The mean age was 53.7 years; 56 years in males and 49 years in females. In both males and females, cervical lymph nodes including supraclavicular were the most common group of nodes that were biopsied (97% and 84% respectively). A chi square test was done to determine whether lung adenocarcinomas were more likely to metastasize to cervical lymph nodes rather than other nodes; however it was not found to be significant (p=0.3).

Among the histological subtypes adeno carcinoma was the most common type (46.55%), followed by neuroendocrine carcinomas (22.4%) and squamous cell carcinoma (6.89%). In men, adenocarcinomas constituted 35.89%, neuroendocrine carcinomas 30.76% whereas in women 68.42% were adeno carcinomas (p=0.01; Table 1).

A most probable primary site could be suggested in 44 (75.8%) of cases; in 7 (12%) cases more than one probable site was suggested and in 7 (12%) more cases the primary site could not be ascertained despite a battery of markers. Lung was the most common primary site (29 cases, 50%) followed by ovary (4 cases, 6.8%) (Table 2). 79.3% of the metastatic lung carcinomas occurred in men (p=0.05)

In poorly differentiated carcinoma (n=8), a definitive primary of lung was suggested in 1 case, multiple primaries were suggested in 4 cases, of which lung was included in 3, and was unknown in 3 cases (Table 3).

Among lung carcinomas, adenocarcinoma was the most common histologic type in general (62%), followed by neuro endocrine carcinoma (27.5%).

Among the IHC markers, CK7 (86%) was the most frequently used marker followed by TTF1 (76%) and CK20 (66%) (Table 4). On an average 8 markers were used per case and 6 for primary from the lung (Figure 1-3).

Table 1. Histologic Types of Metastatic Tumours

Histological Type	Total=58 (%)	Male=39 (%)	Female =19 (%)
Adenocarcinoma	27 (46.55)	14 (35.89)	13 (68.42)
Neuroendocrine carcinoma including small cell carcinoma	13 (22.4)	12 (30.76)	1 (5.26)
Squamous cell carcinoma	4 (6.89)	4 (10.25)	0
Nasopharyngeal Carcinoma	3 (5.17)	1 (2.56)	2 (10.52)
Neuroblastoma	1 (1.72)	1 (2.56)	
Adenosquamous carcinoma	1 (1.72)	1 (2.56)	
Poorly differentiated carcinoma	8 (13.79)	5 (12.82)	3 (15.78)
Rhabdomyosarcoma	1 (1.72)	1(2.56)	

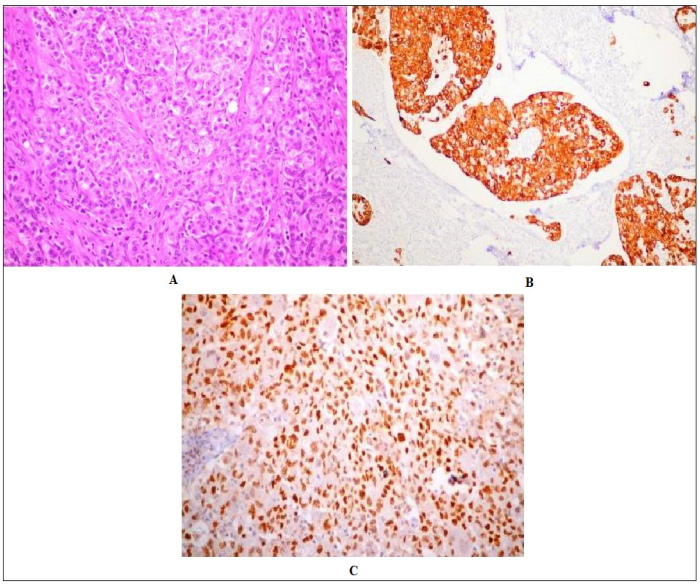

Figure 2. A. Morphology consistent with a metastatic adenocarcinoma in the lymph node. H &E, 40x. B. Tumor cells showing positive staining for CK7 immunostain. CK7, 40x. C.Tumor cells showing positive staining in nuclei for TTF1, indicating lung primary. TTF1, 40x.

Of the 8 cases in which a primary site could not be ascertained on IHC, 4 showed positivity for neuro endocrine markers, 2 showed squamous differentiation and 2 were poorly differentiated on morphology and showed cytokeratin positivity. On an average 12.12 markers were used for the 8 cases in which the primary could not be ascertained.

Supraclavicular lymph nodes were a frequent site of metastases (43.1%). In 50% of cases metastases were found in other group of cervical lymph nodes.

Discussion

This is a retrospective study of metastatic lymph nodes from patients who initially presented with malignant lymphadenopathy. At the time of biopsy of the lymph nodes, the primary sites were not yet established through imaging or other means. This study sought to assess the utility of IHC in assigning the site of primary in these patients.

In our study population, the majority (67.2%) were males and adults (94.1%). This reflects the general distribution of cancers among the Indian population [1].

Among the histological subtypes, we found adenocarcinomas to be the most common, followed by neuroendocrine carcinomas. A large study of fine needle aspiration (FNA) in metastatic lymph nodes showed squamous cell carcinoma to be the most common morphological subtype followed by adenocarcinoma [2]. Our study did not show a high prevalence of squamous cell carcinoma; which may be attributed to the fact that a FNA diagnosis of metastatic squamous cell carcinoma is usually not followed by biopsy or IHC especially when a primary is found in the head and neck region. Another study from Turkey showed, Melanoma to be the most frequent malignancy found in metastatic lymph nodes [3]. Interestingly, we did not have a single case of Melanoma in our study.

Adeno carcinomas are gland forming tumours, originate in many sites such as lung, gastrointestinal tract,

Table 2. Most Probable Primary Sites Suggested after IHC

Primary site (n=58)	Number (percentage)	Males (n=39)	Females
Lung	29 (50)	23	6
Ovary	4 (6.8)		4
Prostate	1 (1.7)	1	
Breast	1 (1.7)		1
Nasopharynx	3 (5)	1	2
Gastrointestinal tract	1 (1.7)		1
Chest wall-Rhabdomyosarcoma	1 (1.7)	1	
Mediastinal neuroblastoma	1 (1.7)	1	
Urinary bladder	1 (1.7)	1	
Posterior Mediastinal NEC	1 (1.7)	1	
Multiple sites	7 (12)	4	3
Unknown site	8 (13.79)	6	2

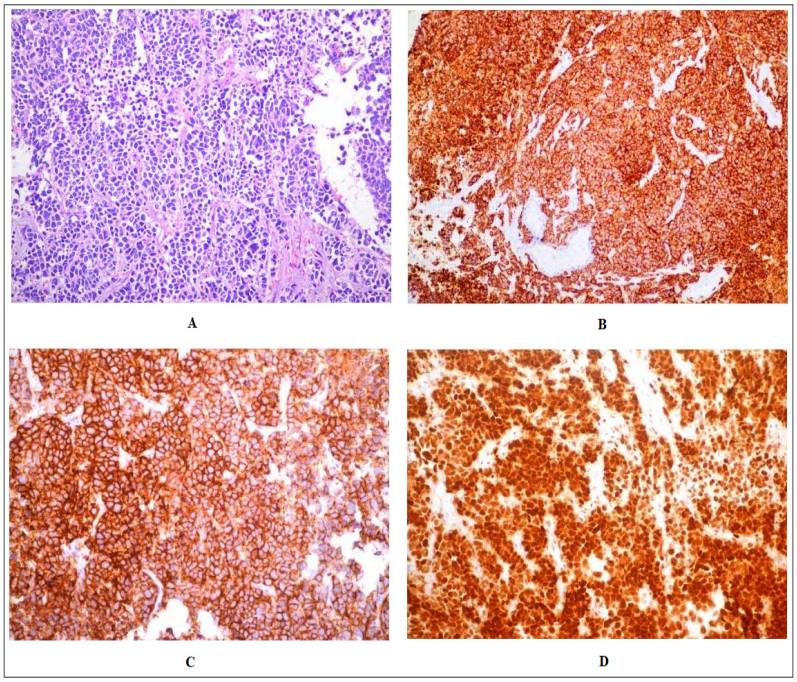

Figure 3. A; Metastatic carcinoma in lymph node with neuroendocrine morphology. H &E, 40x. B and 3C; Synaptophysin and CD56 positivity in tumor cells, indicating neuroendocrine differentiation. Synaptophysin and CD56, x40. D; Tumor cells showing positive staining in nuclei for TTF1 indicating lung primary. TTF1, 40x. P=0.42 (SCL vs others) 0.26 (All Cervical vs others)

breast (in females especially) and prostate (in males). When an algorithmic approach is employed, the primary sites can be narrowed in a majority cases in both males and females [4-6]. In the present study, we were able to suggest a probable primary in 75.8% of cases among which lung primary constituted 50%.

The cervical group of lymph nodes are most likely to be involved in metastases from head and neck cancers [7, 8]. Although metastases from distant sites to cervical lymph nodes, are not as common but not unknown [9]. In general these tumors metastasize to supraclavicular lymph nodes. The current study included only lymph nodes in which IHC was done to establish the primary, and is therefore not truly reflective of the common primaries that metastasize to lymph nodes. If in a head and neck cancer, where the primary is already located and a FNAC of the lymph node shows metastatic carcinoma, further biopsy and IHC may not be done, as they may not add additional information or influence therapeutic decisions.

IHC is an invaluable tool which is inexpensive and widely available that helps us narrow down the probable primary in metastatic malignant neoplasms. Patients in whom a site can be ascribed have better outcomes than those in whom the primary site is unknown. Some of the most useful markers are those which are lineage restricted such as TTF1, GATA 3, PAX 8 and CDX2 [5].

Table 3. Comparison of Lymph Node Groups and Primary Sites

Histological Type (n=58)	Supraclavicular (n=25)	Other cervical (n=29)	Inguinal (n=3)	Axillary (n=1)
Lung (n=29)	14	14	0	1
Ovary (n=4)	3		1	0
Prostate (n=1)	1			
Breast (n=1)	1			
Nasopharynx (n=3)		3		
GIT (n=1)			1	0
Chest wall-RMS(n=1)		1		
Mediastinal NB (n=1)		1		
Urinary bladder (n=1)	1			
Posterior Mediastinal NEC (n=1)		1		
Multiple sites (n=7)	2	5		
Unknown primary (n=8)	3	4	1	

Table 4. Frequency of IHC Markers Used

Marker	Number (%)
CK	31 (53)
CK 7	51 (86)
CK5/6	30 (51)
TTF1	45 (76)
WT1	9 (15)
Napsin	8 (14)
CK20	39 (66)
P63	32 (54)
Synaptophysin	19 (32)
CEA	6 (10)
CD10	5 (8)
CD56	16 (27)
Chromogranin	16 (27)
PSA	10 (17)

Patient sex, age and incidence rates determine the pre IHC probability in most cases and should determine the choice of markers and the algorithmic approach. In the current series of cases, an algorithmic approach was employed keeping in mind the factors listed above. For instance, PSA may be included in the panel in male patients (for prostate) and Mammaglobin (for breast) in female patients. TTF1 continues to be a common marker used as lung adenocarcinoma is a common malignancy that is encountered.

In the current study, TTF1 proved to be the most useful marker in assigning a lung primary in 50% of our cases. In these cases, proving lung origin in a metastatic site was beneficial as a lung biopsy could be avoided and also tissue was available for further studies such as EGFR mutation studies.

Despite an extended panel of markers, differentiation and site of primary cannot be established in some metastatic cancers. Carcinomas of unknown primary (CUP) are defined as those for which clinical, radiological and pathological evidence of primary origin are not present [4]. The reported incidence of CUP varies among various authors. It is impossible to workup every case completely (including autopsy) and in most situations pathologists encounter small biopsies to work up the metastatic site. In the current study in 13.7% of cases, the probable primary site could not be ascertained. One fourth of these cases showed neuroendocrine differentiation. An average of 12 markers were used for these cases.

With the advent of newer and better lineage specific markers, work up for unknown primaries will be further enhanced [10].

Many studies have shown that an algorithmic approach in the use of IHC markers would result in narrowing down the primary in most cases of metastases [10]. A combination of morphology with the knowledge of the common cancers in the population is useful in arriving at an algorithm. The best approach is to begin with the broad lineage markers like cytokeratin, CD45, Vimentin and S100 followed by CK7 and CK20 for epithelial malignancies. Neuroendocrine morphology would warrant the use of neuroendocrine markers such as synaptophysin and chromogranin. Site specific markers such as PSA, and TTF1 may then be employed to suggest the probable primary.

The strengths of this study are that it addresses an important clinical diagnostic challenge seen in the oncology setting, and adds to the existing knowledge of the utility of IHC in assigning a primary in most cases. IHC being a widely available ancillary tool is invaluable in the work up of metastatic cancers.

As already stated, the current study may be biased toward cases requiring IHC, as cases with obvious primaries (e.g., head and neck cancers diagnosed by FNAC) were less likely to undergo biopsy and IHC. In a population where head and neck squamous carcinomas are very common, IHC is not necessary to establish the primary when the primary is obvious on clinical examination and imaging. Also the sample size (n=58) is relatively small, limiting the generalizability of findings, especially for rare primaries, like rhabdomyosarcoma. Further, additional site specific markers like GATA3, NKX3.1 and PAX 8 were not used in this study, thus limiting the number of IHC markers used to assign the primary. Also, imaging data was not available for many cases, precluding a comprehensive analysis. Molecular testing was not done in any of our cases and therefore we were unable to integrate the same with the IHC findings.

In conclusion, a systematic approach using appropriate panel of IHC markers in concordance with histomorphology will help in identification of primary site of metastatic nodes. In the emerging era of molecular diagnostic methods, its high cost and inapplicability at every center emphasize the importance of routine pathologic examination and IHC. Judicious use of IHC with algorithmic approach will go a long way in establishing the primary in the majority of cases of metastatic nodes.

Declarations

Funding

No funding was obtained for this study.

Clinical trial registration

Not applicable.

Conflicts of interest/Competing interests

Authors declare that they have no conflicts of interest.

Availability of data and material

The data sets used and/or analyzed during the current study are available from the corresponding authors per reasonable request.

Authors' contributions

Dr AA contributed to the conception, design, and final drafting of the manuscript. Dr ET contributed to data collection and the primary drafting of the manuscript. Dr AA supervised the study. Both authors approved the final version for submission.

Ethics approval

This study was approved by the Ethic Committee of the St John's National Academy of Health Sciences.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Originality Declaration for Figures

All figures included in this manuscript are original and have been created by the authors specifically for the purposes of this study. No previously published or copyrighted images have been used. The authors confirm that all graphical elements, illustrations, and visual materials were generated from the data obtained in the course of this research or designed uniquely for this manuscript.

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Nil

Declaration on generative AI and AI-assisted technologies in the writing process

Not used AI.

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