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Original Research Article

Metastatic carcinoma with unknown primary – does immunohistochemistry help to detect origin?

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Abstract: Carcinoma of unknown primary is a common clinical problem. They are often the first symptom of systemic malignancy. Hence, immunohistochemistry (IHC) is of importance in evaluating the primary origin. The aim was to detect the primary site of metastatic carcinoma and evaluate the role of IHC in diagnosing the same. Cases of metastatic carcinoma of unknown primary to lymph node, bone and parietis were collected. After initial histopathological examination on haematoxylin and eosin stained section, panel of immunohistochemistry were applied in search of most possible primaries. Forty eight cases of metastatic carcinoma of unknown primary to lymph node, bone, soft tissue and parietis were taken. After routine histopathological examination total seventeen antibodies were used in immunohistochemical test to detect the most possible primary site. Out of 48 cases, lung was primary site in 16 cases, gastrointestinal and pancreatico biliary tract in 12 cases, breast in 4 cases, thyroid in 5 cases, ovary in 8 cases and kidney in 3 cases. Immunohistochemistry has utility in detecting the primary site expeditiously, and thereby guide management and predict prognosis in a given case.
Keywords: Metastasis, immunohistochemistry, unknown primary

INTRODUCTION:

Carcinoma of unknown primary (CUP) origin defines metastatic tumour detected when the site of primary origin cannot be identified despite a detailed medical history, clinical examination and diagnostic work-up. Though most cancer patients come to clinical attention with their primary tumour, around 10-15% of cancer patients present with distant metastases, and in a proportion of these, the primary site cannot be identified at the time of treatment. It is a common clinical problem, representing one of the ten most frequent cancer diagnoses [1]. With the availability of sophisticated imaging techniques and targeted therapies in the treatment of cancer, the extent of workup in CUP remains a challenge and should be based on the clinical presentation, pathology, and the patient's ability to tolerate therapy. In our study, we have taken forty cases of metastatic carcinoma with unknown origin and with judicious use of different antibodies in immunohistochemical test on paraffin embedded tissue tried to find out most probable primary site of those tumours.

SUBJECTS AND METHODS:

Fifty five cases of metastatic carcinoma of unknown primary to lymph node, bone and parietis were collected from September, 2013 to August, 2014. Among the above cases, most of them (forty seven) presented as metastasis to different lymph nodes (cervical, supraclavicular, axillary or inguinal). Clinical evaluations did not help much in the determination of the site of origin. For histopathological evaluation, whole lymph nodes were taken out and in case of other sites, tru-cut/ incisional biopsy specimen were taken. Then after proper fixation in 10% neutral buffered formalin, fixed tissues were processed in automated tissue processor [1] and following that paraffin blocks were made. Histopathological examination on haematoxylin and eosin stain under light microscope was done initially and then subsequent

immunohistochemical test (IHC) with CK7 and CK20 were used in all the cases in search of most possible primaries. Depending on the positivity and negativity of the above two markers, further more antibodies were used along with radiological investigations to reach the final diagnosis. IHC was performed on formalin fixed paraffin sections in cases with unknown primary by applying the streptavidin–biotin–peroxidase-conjugate method in an automated stainer (Ventana Bench Mark XT, Ventana Medical Systems, Rosche) utilizing the manufacturer's protocol with prediluted ready-to-use antibodies (Dako, Glostrup, Denmark).Other IHC markers used subsequently were thyroid transcription factor1 (TTF1), Napsin A, epithelial membrane antigen (EMA), P^{63}, CDX2, carcinoembryonic antigen (CEA), CD10, chromogranin A (CGA), CD56, CA19-9, CA125, neuron specific enolase (NSE), gross cystic disease fluid protein (GCDFP15),estrogen receptor (ER) and progesterone receptor (PR).

RESULTS:

At first sixty two cases of metastatic malignant tumour with unknown primary to lymph node, soft tissue, bone and parietis were collected from September, 2013 to August, 2014. Then histopathological examination and subsequent immunohistochemical test (IHC) with cytokeratin (AE1/ AE3) showed that seven cases were cytokeratin negative. Further IHC revealed that two cases were metastatic amelanotic malignant melanoma; three cases were metastatic malignant germ cell tumour and two cases were synovial sarcoma metastatic to lymph node. These seven cases were excluded from study. Remaining 55 cases were taken to find out the primary sites. Among those 55 cases, most of them (forty seven) presented as metastasis to different lymph nodes (cervical, supraclavicular, axillary or inguinal). Mean age of the cohort was 48.7 years. Male: Female ratio was 1.75:1 (Male -35, Female –20). All the cases presented with metastasis in single anatomic region. The anatomic locations were as follows: cervical lymph node – 11, supraclavicular lymph node – 21, axillary lymph node – 4, inguinal lymph node –11 and others (bone and parietis)– 8. After applying IHC, CK7+/CK20- cases were 28, CK7+/ CK20+ cases were 9, CK7-/CK20+ cases were 4 and CK7-/CK20- cases were 14.

Out of 28 CK7+/CK20- cases, metastatic adenocarcinoma of lung were 9 (TTF1 and Napsin A positive) (16.4%) (fig 1, 2, 3 and 4), 5 cases were metastatic papillary carcinoma of thyroid (TTF1 positive and Napsin A negative) (9.1%), 4 cases were metastatic ductal carcinoma of breast (ER, PR and GCDFP15 positive) (7.3%), 3 cases were metastatic small cell neuroendocrine carcinoma of lung (TTF1, CD56 and CGA positive) and 7 cases were metastatic ovarian adenocarcinoma of serous or endometrioid type (EMA and CA125 positive and vimentin and CEA negative) (12.7%).

Out of 9 CK7+/ CK20+ cases, 5 cases were metastatic adenocarcinoma of stomach (CDX2 positive)(9.1%) and 4 cases were metastatic adenocarcinoma of pancreatico biliary tract (CA19-9 positive)(7.3%). Out of 4 CK7-/ CK20+ cases, all were metastatic colorectal carcinoma (CEA and CDX2 positive) (7.3%) (Fig 5, 6, 7 and 8).

Out of 14 CK7-/ CK20- cases, 3 cases were metastatic squamous cell carcinoma of upper aero-digestive tract (P^{63} positive and all cases presented as metastasis to cervical lymph node) (5.5%), one case was metastatic small cell neuroendocrine carcinoma of lung (TTF1, CD56 and CGA positive), 2 cases were metastatic renal cell carcinoma (Vimentin and CD10 positive).Two cases were metastatic neuroendocrine carcinoma (CD56 and CGA positive) and 6 cases were metastatic poorly differentiated carcinoma where primary sites could not be identified. So in total 8 cases primary sites were not found (14.5%).

Total 4 cases were diagnosed as metastatic small cell neuroendocrine carcinoma of lung (7.3%). The distribution of tumour types after application of IHC is depicted in Table 1 and distribution of metastatic site and related primary tumour has been shown in Table 2.

Table 1: Numbers of different types of carcinoma

Tumour type	Number of Cases (n = 55)
Adenocarcinoma of lung	9
Small cell neuroendocrine carcinoma lung	4
Squamous cell carcinoma of upper aero-digestive tract	3
Ductal carcinoma of breast	4
Papillary carcinoma thyroid	5
Colorectal carcinoma	4
Adenocarcinoma of stomach	5
Adenocarcinoma pancreatico biliary tract	4
Ovarian adenocarcinoma	7
Renal cell carcinoma	2
Metastatic carcinoma where primary sites not found	8

Table 2: Distribution of different types of carcinoma in respect to different lymph node groups

Tumour type	Cervical LN	Supraclavicular LN	Axillary LN	Inguinal LN	Bone (Femur)	Parietis
Adenocarcinoma of lung	3	6	0	0	0	0
Small cell neuroendocrine carcinoma lung	0	4	0	0	0	0
Squamous cell carcinoma of upper aero-digestive tract	3	0	0	0	0	0
Ductal carcinoma of breast	0	0	4	0	0	0
Ovarian adenocarcinoma	0	0	0	7	0	0
Adenocarcinoma of stomach	0	5	0	0	0	0
Adenocarcinoma pancreatico biliary tract	0	2	0	0	0	2
Papillary carcinoma thyroid	5	0	0	0	0	0
Renal cell carcinoma	0	1	0	0	1	0
Colorectal carcinoma	0	2	0	2	0	0
Metastatic carcinoma where primary sites not found	0	1	0	2	2	3

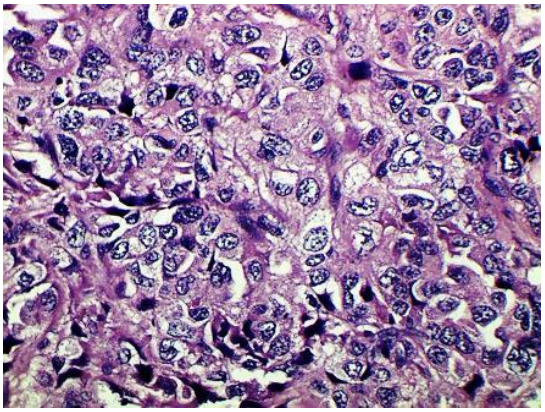

Fig-1: Metastatic carcinoma in supraclavicular lymph node

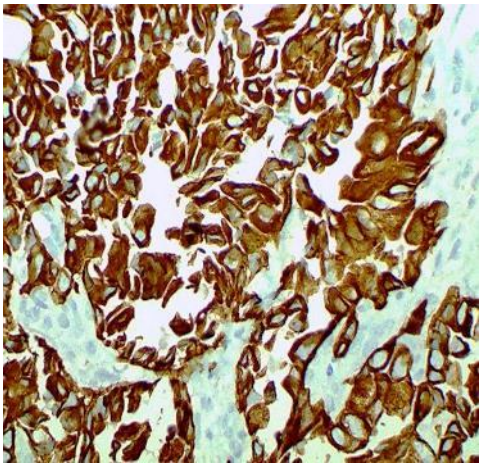

Fig-3: CK-7 showed diffuse cytoplasmic positivity.

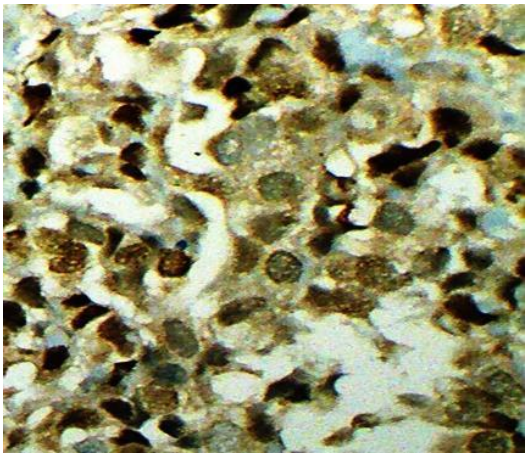

Fig-2: TTF-1 positivity noted in the tumour cells

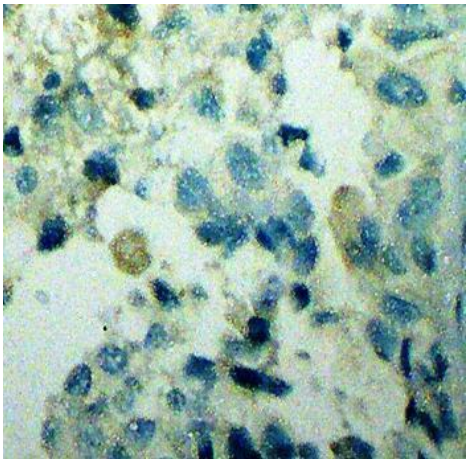

Fig-4: CK-20 staining was negative.

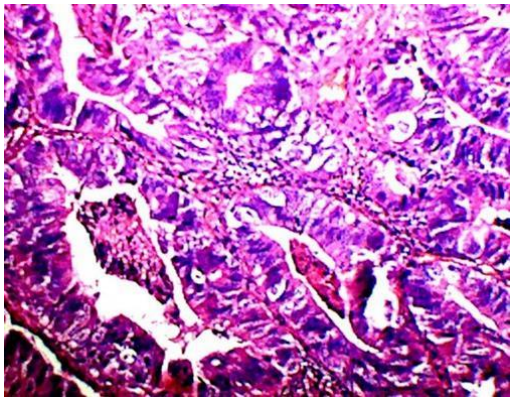

Fig-5: Metastatic adenocarcinoma in supraclavicular node with negative CK-7 staining

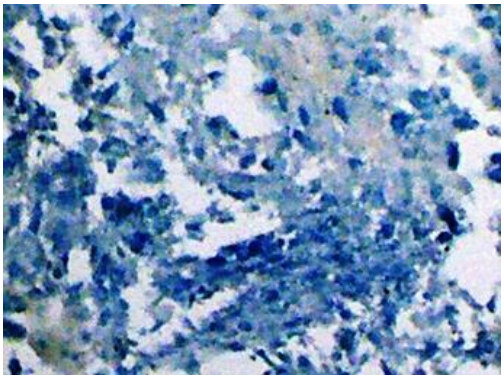

Fig-6: Metastatic adenocarcinoma in supraclavicular node with negative CK-7 staining

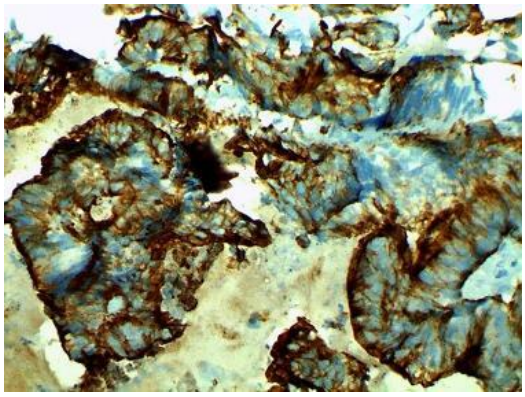

Fig-7: CK-20 showed diffuse cytoplasmic positivity.

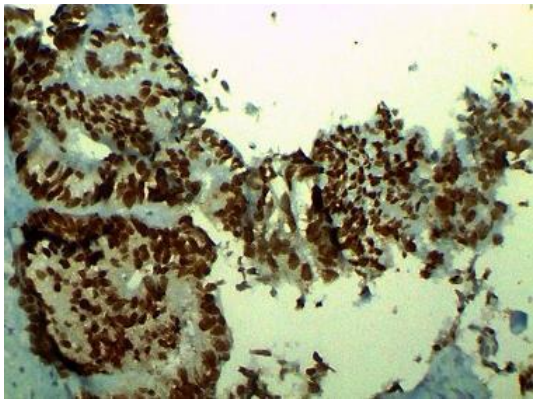

Fig-8: CDX-2 showed diffuse nuclear positivity

DISCUSSION:

Histopathology is the cornerstone in the diagnostic procedure of CUP. A good biopsy specimen is of great importance, especially in cases of poorly differentiated tumours, and for the application of special pathology techniques that can improve the diagnosis of chemo sensitive tumours which are subject to misdiagnosis. By definition, conventional light microscopy cannot identify the site of origin [2]. Identifying primary site is sometimes difficult for histopathologists particularly in case of metastatic adenocarcinoma. Interestingly, a correct diagnosis of only 48% was achieved by pathologists when they were shown 100 metastatic adenocarcinomas of known primary origin which were presented as unknowns with the provision of minimal essential clinical data. A higher accuracy was achieved for prostate, ovarian, and breast carcinomas, and a lower accuracy for the pancreatico biliary and upper gastrointestinal tract [3].

So IHC has great role in diagnosis of CUP. For that purpose, one should use ideal markers which are highly sensitive and specific. The nature of these proposed markers of primary site varies: some are nuclear proteins such as transcription factors, others are structural intermediate filaments; and yet others are specific cellular products, expressed on the surface or secreted. In assessing whether an antibody is positive or negative, it is therefore imperative to know where the staining is expected to be nuclear, cytoplasmic and/or membranous so that only true staining is accepted. Considering the location of staining, it is equally necessary to ensure that only tumour tissue is evaluated [4].

Cytokeratins (CKs) are the principal positive marker for carcinomas. There are 20 known subtypes of cytokeratin (CK) intermediate filaments, all of which have different molecular weights and levels of expression in different cell types and cancers. Monoclonal antibodies to specific CK subtypes have been used to help classify tumours according to their site of origin; the most commonly used CK stains in CUP adenocarcinoma cases are CK 7 and 20. CK 7 is expressed in upper gastrointestinal tract tumours, cholangiocarcinoma and tumours of pancreas, lung, ovary, endometrium and breast, whereas CK 20 is normally expressed in the lower gastrointestinal epithelium, urothelium, and Merkel cells [5].

Thyroid transcription factor-1 is a useful tool to identify lung and thyroid as the primary site of tumour origin. In our cohort, the positivity rate for TTF-1 was 23.6% (13 out of 55). Most small-cell carcinomas (around 90%) and large-cell neuroendocrine carcinomas are positive for TTF1, whether they originate in lung or elsewhere [6]. Gross cystic disease fluid protein 15 is a marker of apocrine differentiation that is specifically expressed in breast carcinomas; expression is detected

in 62%–72% of cases [7]. Estrogen receptor (ER) is a nuclear stain and its expression is restricted to carcinomas of the breast, ovary and elsewhere in the gynaecological tract[8]. CA-125 was characterised as a membrane glycoprotein in ovarian carcinoma cells nearly 20 years ago. It is expressed in around 61% adenocarcinoma of ovary [9]. Carcinoembryonic antigen (CEA) is recognised principally as a serological marker for the follow-up of patients with colon cancer. CEA has a membranous and cytoplasmic distribution. It is mostly expressed in carcinoma of colon (almost 100%), stomach (67-80%), pancreas (50-92%) and mucinous carcinoma of ovary (67-80%) [8]. CA19-9 has been used as a serum marker for the diagnosis and follow-up of gastrointestinal and especially pancreatic cancer, but it is also expressed in benign pancreatic disease including chronic pancreatitis [10]. CDX2, like TTF1, is a home box protein transcription factor: it is named for its homology with the Drosophila gene caudal. CDX2 is normally expressed in intestinal epithelial cells from the proximal duodenum to distal rectum, where it plays an important role in epithelial differentiation and maintenance. Positive staining is therefore nuclear: cytoplasmic staining may occur but should be disregarded. Over 90% of colonic adenocarcinomas show CDX2 staining which is strong and diffuse, that is, present in most cells [11].

In routine diagnostic practice, immunohistochemical markers are generally used not in isolation but as part of a larger panel. However, while the individual markers described above have been investigated in depth, the utility of comprehensive antibody panels (other than cytokeratins) in predicting the primary site of adenocarcinomas has been addressed by surprisingly few primary studies and review articles.

In 1997, Perry et al. investigated 68 consecutive biopsies from brain metastases where the primary site was known. Spread to the brain commonly occurs with lung, breast and gastrointestinal adenocarcinomas, and with renal cancers. The helpful markers were: GCDFP-15, ER and CK7 for breast; CK20 for gastrointestinal and CK7 for lung. GCDFP-15 and ER were relatively specific but insensitive markers, whereas CK7 and CK20 scored highly on both parameters. CAM 5.2, wide-spectrum keratins and progesterone receptor (PR) were unhelpful [12].

Brown et al. studied 128 metastatic adenocarcinomas from five sites of origin (breast, colon, lung, ovary and upper gastrointestinal tract) in 1998. They tested eight markers and selected four: CEA, CA199, CA125 and BCA225 (breast cancer antigen 225). Using these, the primary site was correctly predicted in 66% [13].

In our study, we found lung to be the most common primary site (23.6%) followed by gastrointestinal tract (stomach and colon) (16.4%). Thyroid transcription factor-1 is a useful tool to identify lung as the primary site of tumour origin. Bohinski et al. observed that none of the non-pulmonary sites showed a positive result with TTF-1 [14]. Hence, TTF-1 should be routinely included in the evaluation of metastases with unknown primary. For example, a presumptive diagnosis of ovarian primary was made in our study by CA125 positivity and CEA and vimentin negativity. Similarly, an unknown breast primary was diagnosed by positivity for ER, PR, GCDFP 15 and CK7. An unknown colonic primary was diagnosed by positivity for CK20, CDX2 and CEA and negativity for TTF-1. CEA positivity favours colonic primary and TTF-1 negativity excludes lung primary. Practically morphological features of the initial haematoxylin and eosin-stained sections will determine the starting point of the first round of IHC studies and then a protocol should be set to investigate each and every case. We could not identify primary sites in 14.5% cases. So we can say that IHC is diagnostic in rest 85.5% of cases.

Thus, IHC has utility in detecting the primary site expeditiously, and thereby guide management and predict prognosis in a given case.

CONCLUSION

Carcinoma with unknown primary is really a challenging field for clinicians as well as pathologists. Only Judicious use of routine histopathology and immunohistochemistry along with clinico-radiological correlation can solve the problem.

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