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Immunohistochemistry at rescue in the cancers of unknown primary origin

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Abstract
Aims: To identify and explore the role of immune histochemical markers in the diagnosis of malignancies of unknown primary origin.
Background: Cancer of unknown primary (CUP) origin is a heterogenous group of cancers explained by the presence of metastatic disease with no identified primary tumor at presentation. These malignant neoplasms with unknown primary origin can be identified and classified by the use of a robust IHC panel which may permit development of tailored treatment algorithm with specific targeted agents.
Methods: In the current study, Histopathology and biopsy samples from 38 patients were included which were diagnosed as CUP and referred to IHC department, M. P. Shah medical college and hospital, Jamnagar from 2019 to 2023. CUP were diagnosed and classified of their histologic types on basis of hematoxylin and IHC slides.
Results: From 2019 to 2023, out of 4026 cases, CUP constitutes 0.99% (~1%). The age group (60-69) years have the highest cases 14/40 cases with equal distribution in both sexes. Lymph node represents (42.5%) which is the most common presenting site followed by liver (12.5%) and lung (10%). In 50% of the cases, the primary site was determined; whereas 37.5% cases were remained unknown for primary and 12.5% cases were given an only differential diagnosis. Adenocarcinoma (66.6%) was the most common subtype, followed by squamous cell carcinoma (13.15%), undifferentiated neoplasm (10.52%).
Conclusions: The most common presenting sites were lymph nodes, liver, lungs and others. CUP cases are uncommon (0.88%) and primary origin of these CUPs were identified in 50% cases. Sub typing of primary site were adenocarcinoma, squamous cell carcinoma and carcinoma with undifferentiated neoplasm.

Keywords: Cancer of unknown primary, Histopathology, Imuuno histochemistry

Introduction
With the ongoing additions of lineage-specific transcription factors, pathologists have varieties of relatively inexpensive Immunohistochemistry (IHC) ‘‘tools,’’ which more accurately identify CUP [1]. IHC provides diagnostic guidance in approximately 90% of undifferentiated malignant tumors but usually at the end of a fastidious and tedious algorithm based on both morphology and IHC. [Selves J]. In this current era of health care cost containment and targeted therapies, diagnostic accuracy is crucial, particularly with smaller sample sizes [1]. Among these situations, pathologists need to provide relatively quick diagnosis clinicians with a, IHC remains the gold standard at diagnosing CUP [1]. There is a significant change in the past two decades in the treatment approach to CUP patients [2]. The “one treatment fits all” approach and the empiric combination cytotoxic therapies have been taken off while the pivot point has been on improved methods to identify the primary tumor and give direct therapy to particular tumor type [2].
IHC is particularly valuable in diagnosing metastatic tumors, as it can be performed on paraffin-embedded tissue at a lower cost compared to advanced imaging studies and molecular genetic analysis. Histopathological examination coupled with clinical correlation continues to be the fundamental approach for morphologic diagnosis. IHC plays a crucial role by either supporting or ruling out potential differential diagnoses. It combines anatomical and immunologic techniques, relying on specific antigen-antibody reactions that are detectable through enzyme reactions with the antibodies in use. This combination of approaches enhances our ability to identify tissue components and aids in the accurate characterization of various diseases and conditions [3].

Biomarkers used in pathology for CUP are essential for diagnosing and determining the cancer type, subtype, and site, including the use of IHC and ancillary molecular tests [2]. With the emergence of more specific therapies, prognostic and predictive biomarkers have become essential for diagnosing and determining the cancer type, subtype, and site, including the use of IHC and ancillary molecular tests. However, it's worth noting that the best method for identifying the origin of CUP remains immunohistochemistry, which continues to be the gold standard, especially in countries with limited resources unable to afford other ancillary techniques. Timely diagnosis and appropriate treatment of patients with CUP are significant prognostic factors that can potentially improve survival rates [2, 4].

In recent years, the diagnostic accuracy for identifying the primary site of undifferentiated neoplasms or tumors with uncertain origins has steadily improved thanks to the discovery of additional tissue-specific biomarkers [3]. The biology of Cancer of Unknown Primary (CUP) remains somewhat unclear, but there are common biological features shared among CUP tumors. These characteristics include early metastasis from a clinically undetectable primary tumor, an unpredictable metastatic pattern, aggressive biological behavior, and clinical characteristics that make CUP a challenging and unique entity in cancer diagnosis and management [5, 6]. Given that CUP is a common and challenging clinical problem, the role of IHC workup in CUP is crucial. It aids in classifying tumors according to their broad type, subtype, and, if possible, the site of origin [6]. This classification provides significant benefits in diagnosing and managing CUP patients [6]. Identifying patients with favorable disease through this approach is essential, as they may benefit from targeted treatments. It's worth noting that regional publications on this topic were not found in the literature, which underscores the importance of conducting this study, assessing the frequency, classification, and workup of CUP cases using available IHC markers from 2020 to 2023.

Materials and Methods

All the relatable clinical details and radiological reports of the patients in the current study were obtained from the request forms in records. Hematoxylin & eosin (H&E) stained slides from biopsy or tissue specimens and immune stained slide panels used in the workup were retrieved from the department of histopathology, M.P. Shah medical college archives. These slides were reviewed under light microscope. For this cross sectional study, a gradual approach was followed in the workup of CUP patients.

Following a biopsy, the initial task was to confirm the presence of cancer. This was achieved through a stepwise approach that involves using panels of immunohistochemistry (IHC) markers. Panels of markers were employed to identify the specific tumor type and subtype. Furthermore, organ-specific markers were used to recognize the likely site of origin for the tumor. This comprehensive approach assisted in accurately diagnosing and characterizing the cancer. In the current study, we used several different company biomarkers like dako, diagnostic biosystems, quartett and biocare medical.

Results

The current study covered 40 patients diagnosed with carcinoma of unknown primary out of 4326 cases presented to histopathology department, M. P. Shah medical college, during the study period. The greatest percentage (37.5%) was for the age group between 60-69 years. The age range of malignancy of unknown origin (MUO) patients was between 20-82 years, with mean age of 55 years [Table 1].

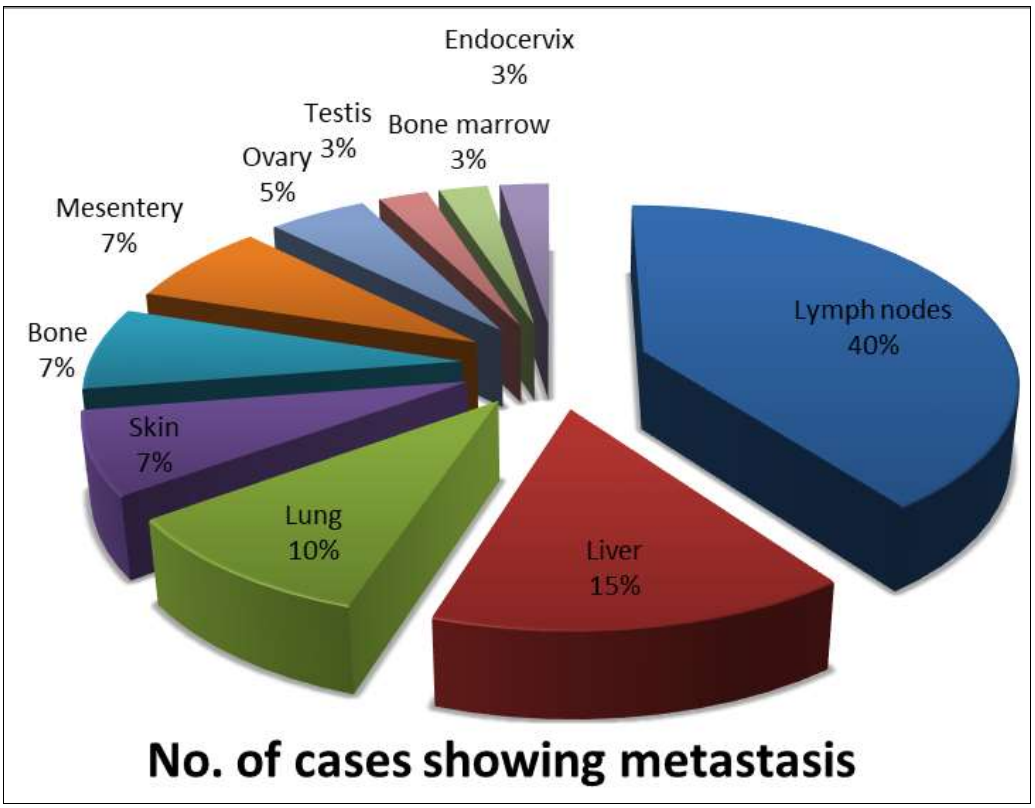

Fig 1: Site wise distribution in Malignancy of unknown origin (MUO) cases

Table 1: Age wise distribution in Malignancy of unknown origin (MUO) cases

Age group (years)	No. of cases diagnosed as secondary's	% of cases
<20	0	0
20-29	3	7.5%
30-39	3	7.5%
40-49	6	15%
50-59	6	15%
60-69	15	37.5%
70-79	4	10%
>80	3	7.5%

Females represented 21 and males 19 in this study. The most typical presenting sites were lymph nodes 16 (40%) after that liver 6 (15%), lung 4 (10%) and others 14 like a skin, bone, omentum, ovary, testis, bone marrow, endocervix [Figure 1].

Out of 16 metastatic CUP patients in the lymph nodes, cervical lymphnodes (7 /16) were the most common presenting site followed by axillary, supraclavicular, submandibular and mesenteric lymph nodes. The current study found 27 (67.5%) cases of adenocarcinoma, 5 (12.5%) cases of squamous cell carcinoma, 4 (10%) undifferentiated carcinoma, 3 (7.5%) carcinoma with neuroendocrine differentiation, and 1 (2.5%) germ cell tumor [Table 3].

Table 3: Histopathological variants in Malignancy of unknown origin (MUO) cases

Histopathological subtype	No. of cases	% of cases
Adenocarcinoma	27	67.5%
Squamous cell carcinoma	5	12.5%
Undifferentiated carcinoma	4	10%
Carcinoma with neuroendocrine differentiation	3	7.5%
Germ cell tumor	1	2.5%

This study exposed primary origin in 52.5% cases. On the contrary, 12.5% cases were reported with one differential diagnosis and in 35% CUP cases, primary origin was not determined. Few of the example cases workup is showed up in figure 2-4.

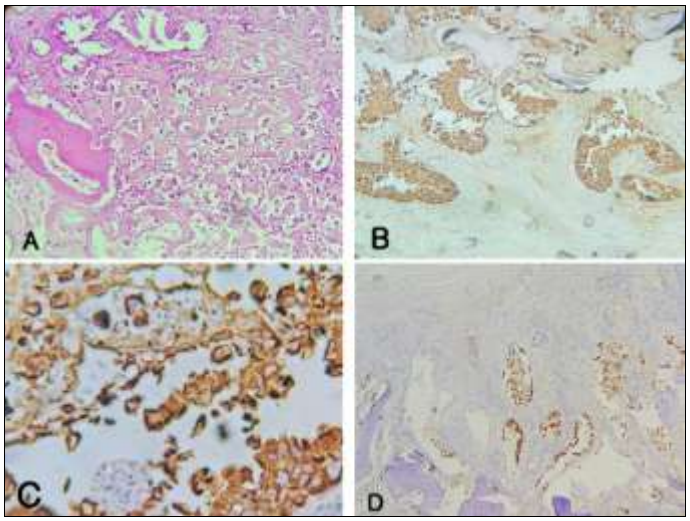

Fig 2: Case 1. Secondaries in bone from Lung adenocarcinoma. (A) H&E, (B & C) CK7+, (D) TTF-1 +.

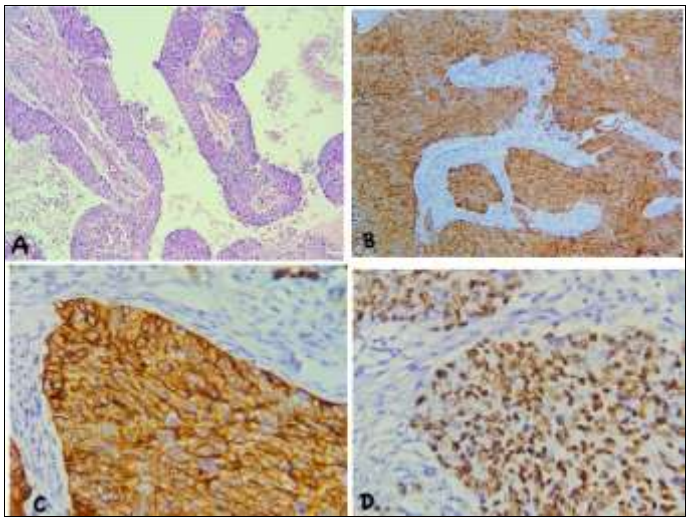

Fig 3: Case 2. Mesenteric deposits from high grade serous ovarian carcinoma. (A) H&E, (B) CK7+ C) p16+, (D) ER +

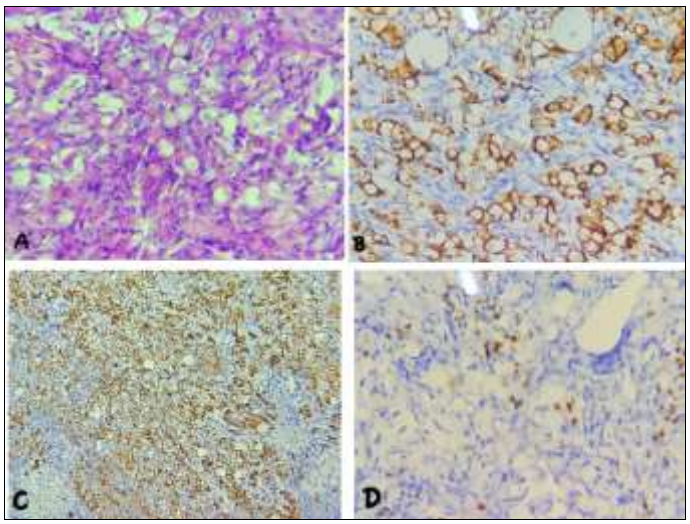

Fig 4: Case 3. Metastasis of gastric adenocarcinoma to bilateral ovaries (Krukenberg tumor). (A) H&E, (B) CK7+ C) CK20+, (D) CDX2+

Discussion

This research study was aimed to explore the role of all the available immunohistochemical markers in detecting malignancies of unknown primary origin. The present study covered 40 CUP cases out of 4026 cases diagnosed during the study period of 2019 to 2023, represents 0.99%, which is less than the other studies in which this ratio ranges between 3-5% [6, 7].

The study showed 0.27% in the year of 2019, which is increasing every year and it, became 2.17% in 2023. This rise in cases may be due to increase in the oncologist appreciation of the role of IHC in identifying CUP cases and the availability of drugs that target biomarkers detected by IHC helps the patients for management with directed therapy [8]. The present study shows that the age group 60-69 years has a high incidence (37.5%) of the study population, with slightly high ratio in females (51%) to males (49%). This finding is different from the Royal College of pathologists – cancer datasets reports, which shows median age 60 years with 53% males and 47% females in CUP cases [7].

The current study indicates that lymph nodes are the most common presenting sites (40%) followed by liver (15%), lung (10%), bone (7.5%). This observation was comparable with the study of O R Omar *et al*. study [6] and Zaun G *et al*. study [9] findings. Cervical lymph nodes are the most familiar among CUP lymph nodes followed by axillary and supraclavicular lymph nodes. IHC panels were run for these cervical CUP lymph nodes and breast & lung was identified as the primary origin site.

Many review articles show that the most common histopathological entities are adenocarcinoma (66.6%), followed by squamous cell carcinoma (13.15%), and undifferentiated neoplasm (10.52%) carcinoma with neuroendocrine differentiation (7.8%). Routine H & E stained sections are often adequate and appropriate for the definitive diagnosis of many pathological lesions. However, in such cases like CUP where light microscopic examination of tissue sections is inconclusive, IHC can efficiently back up histopathology [10]. IHC is a laboratory technique used to detect specific antigens in tissues or cells based on antigen-antibody recognition at the light microscopy level [11].

Tissue procurement is the first step in the workup for tumors of unknown primary origin followed by proper staining techniques along with finalizing the appropriate immune marker panel and interpretation of stain by the pathologist [6, 11]. All the steps must be supervised by a well-trained and highly skilled pathologist with good knowledge of interpretation of IHC markers to minimize the errors and get straight to the diagnosis [6].

Morphology is the foundation upon which the interpretation of IHC studies rests. Site of involvement and line of differentiation (epithelial, mesenchymal, germ cell, lymphoid) are very important to identify and find out the CUP cases [11]. The pattern of expression of the antigen, whether nuclear, cytoplasmic, membranous or extracellular, is very essential along with the nature of the antigen to interpret the results of CUP cases. Using positive and negative controls in staining methods are also very important to standardize the IHC technique [6].

In the current study, different panels of antibodies are used depending upon their histopathological features. Epithelial markers like pan CK, other cytokeratins, CK7, CK20 CK 5/6, EMA, mesenchymal markers like vimentin, SMA were used in first panel. To exclude neuroendocrine differentiation synaptophysin and chromogranin are also available. CD 45 for lymphoid origin was used. Accordingly, a second panel is performed to include organ-specific markers like CDX2, TTF-1, PAX-8 for colonic, thyroid, lung or ovary primary, respectively. Other markers used in the current CUP study cases include ER, PR Her2Neu, CD34, CD99, S100, p16 and many more.

Keratins are family of intermediate filaments found in epithelial cells of all types hence they are specific markers for an epithelial lineage. Once the initial differentiation is made, more specific cytokeratins such as CK7 and CK20 can be used to better characterize an epithelial tumor [12]. The patterns are not entirely specific; cytokeratin expression in epithelial cells indicates possible primary sites in the CUP cases. The CK7 positive & CK20 negative profile is the commonest in CUP cases but not useful to identify specific primary site of origin. Hence further histopathological features and additional markers are required to find out the primary in CUP cases [6, 13, 14].

Many valuable immunological markers in CUP cases workup are ER, Her2neu, GATA 3, mammoglobin A, for breast origin. Estrogen receptor (ER) is better expressed in primary breast carcinomas than secondaries [15]. ER's positive expression is found in endometrium, thyroid and ovarian carcinomas, henceforth ER has limitations in CUP

cases. Currently GATA3 is indeed a sensitive marker for breast carcinomas [1], and its expression can vary in different subtypes of breast cancer. In recent studies, it has been reported to have varying positivity rates in different types of breast carcinoma: Ductal, Lobular, Triple-negative breast carcinoma and Metaplastic breast carcinoma, positive rate reported at 91%, 100%, 43% and 54% respectively [1, 16]. These percentages indicate the likelihood of GATA3 expression in these specific subtypes of breast cancer. It's an important marker used in diagnosing and characterizing breast cancer cases. The use of GATA3 can be helpful in distinguishing between transitional cell carcinoma (TCC) and high-grade prostatic adenocarcinoma. It serves as a valuable immunohistochemical marker in this context, aiding in the differentiation and accurate diagnosis of these two distinct types of cancer [6].

The Wilms tumor antibody (WT1), known as a nuclear transcription factor, plays a key role in normal urogenital development. In the context of CUP diagnosis, WT1 is primarily utilized for identifying ovarian serous carcinomas, primary peritoneal adenocarcinomas, and fallopian tube serous carcinomas [6].

There are five markers (PAX8, pVHL, RCC, CD10, and KIM-1) in the renal system that are valuable for confirming the diagnosis of clear cell renal cell carcinoma (RCC). Among these markers, PAX8 stands out as the most sensitive and moderately specific marker for detecting thyroid follicular cell tumors, RCCs, ovarian & endometrial adenocarcinomas [3]. It is consistently identified in renal epithelial neoplasms, with conventional clear cell RCC displaying a sensitivity ranging from 88% to 98% [17].

Over 90% of prostatic acinar adenocarcinomas (ADCs) typically do not express CK7 and CK20, except for prostatic ductal adenocarcinomas [17]. Markers for prostatic cancer are highly specific and sensitive for predicting the site of origin. 2 important markers in this context are prostatic specific antigen (PSA) and the more recently described NKX3.1. These markers play a crucial role in the diagnosis and monitoring of prostate cancer. Several markers, including p40, CK5/6, p63, CK903, SOX2, and desmocollin, can indicate squamous differentiation. Squamous cell carcinomas, whether originating in the lung, cervix, or tumors displaying a squamous immunophenotype like thymus tumors, consistently exhibit strong positive staining for both p63 and p40 [18].

CDX2 is a transcription factor primarily found in intestinal epithelium. It serves as a highly sensitive immunomarker for gastrointestinal adenocarcinomas (ADCs) [17]. However, it can also be expressed in tumors of pancreas, bile ducts, bladder, uterine cervix, endometrium, and ovary. Typically over 90% of colorectal ADCs and small bowel ADCs exhibit positivity for CDX2. Nevertheless, a significant reduction or loss of expression of CK20 and CDX2 is frequently observed in medullary carcinoma of the large intestine. In contrast, mucinous ADCs of the lung tend to be positive for CDX2 and CK20, while negative for TTF1 and napsin A [18].

In the germ cell lineage, diagnosing primary testicular tumors has been aided by IHC. This involves assessing transcription factors such as octamer-binding transcription factor 4 (OCT4), Sal-like protein 4 (SALL4), SRY (sex-determining region Y)-box 2 (SOX2), and SOX17. These markers have been confirmed to be highly sensitive and specific, making them valuable tools for differentiating between various types of germ cell tumors [18]. This study highlights certain limitations related to the availability of few markers.

IHC is indeed an efficient and cost-effective method for determining the site of origin in cases CUP [6]. The analysis of clinical, histopathological, and immunohistochemical data plays a pivotal role in diagnosing metastatic CUP origin. Initial histopathological interpretation provides crucial insights into the tumor's location, and this information is complemented and further refined by the results obtained through immunohistochemical tests [1].

In conclusion, the study found that CUP cases comprised approximately 0.99 (~1) % of all cases referred to the Histopathology Department at M. P. Shah Medical College in Jamnagar. The most frequent presenting sites were lymph nodes, followed by the liver and lung. The histological classification of these cases was primarily adenocarcinoma, squamous cell carcinoma, undifferentiated neoplasm, and carcinoma with neuroendocrine features. Thorough IHC workup, it was determined that cervical lymph nodes were the most common location for metastatic CUP. Pathologists and lab managers are recommended to utilize IHC within the appropriate histological context by employing panels of markers rather than individual markers. Additionally, staying updated with the introduction of new markers is deemed a significant aspect of patient diagnosis and management. This approach ensures a more comprehensive and accurate assessment of tissues and contributes to improved patient care.

Declarations

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Conflict of interest: Nil

References

1. Kandalaft PL, Gown AM. Practical applications in immunohistochemistry: Carcinomas of unknown primary site. Archives of Pathology & Laboratory Medicine. 2015;140(6):508–523.
2. Varadhachary G, Abbruzzese JL. Carcinoma of unknown primary. Abeloff's Clinical Oncology; c2020. p. 1694–702.
3. Selves J, Long-Mira E, Mathieu M-C, Rochaix P, Ilié M. Immunohistochemistry for diagnosis of metastatic carcinomas of unknown primary site. Cancers. 2018;10(4):108.
4. Shivaji VS, Wilson JC, Schmidt NL, Kolokythas O, Lalwani N. Carcinoma of unknown primary with hepatic metastases: A need of judicious and contemplative diagnostic algorithm. Abdominal Radiology. 2020;46(1):257-267. https://doi.org/10.1007/s00261-020-02630-3
5. Stella GM, Senetta R, Cassenti A, Ronco M, Cassoni P. Cancers of unknown primary origin: current perspectives and future therapeutic strategies. Journal of Translational Medicine. 2012, 10(1). https://doi.org/10.1186/14795876-10-12
6. O.R Omar E, Haussein N, Ahmed A. The role of immunohistochemistry in the workup of malignant neoplasms of unknown primary origin at Khartoum Oncology Hospital. Asian Pacific Journal of Cancer Care. 2021;6(4):441–447. doi:10.31557/apjcc.2021.6.4.441-447
7. Schofield J, Oien K. Standards and datasets for

reporting cancers Dataset for histopathological reporting of cancer of unknown primary (CUP) and malignancy of unknown primary origin (MUO) July 2018. https://www.rcpath.org/uploads/assets/555302b1-8b11-4d8a-a24431d0693d3287/ G167-Dataset-for-histopathological-reporting-of-cancerof-unknown-primary-CUP-and-malignancy-of-unknownprimary-origin-MUO.pdf.
8. Handorf CR, Kulkarni A, Grenert JP, Weiss LM, Rogers WM, Kim OS, Monzon FA, Halks-Miller M, Anderson GG, Walker MG, Pillai R, Henner WD,. A multicenter study directly comparing the diagnostic Accuracy of Gene Expression profiling and Immunohistochemistry for Primary Site Identification in Metastatic Tumors. American Journal of Surgical Pathology. 2013;37(7):1067-1075. https://doi.org/10.1097/pas.0b013e1828309c4
9. Zaun G, Schuler M, Herrmann K, Tannapfel A. CUP Syndrome. Deutsches Aerzteblatt Online. 2018 03 09;. https://doi.org/10.3238/arztebl.2018.0157
10. Buchwalow IB, Böcker W. Diagnostic immunohistochemistry. Immunohistochemistry: Basics and Methods; c2009. p. 109–27. Doi:10.1007/978-3-642-04609-4_13
11. Dabbs DJ. Diagnostic Immunohistochemistry: Theranostic and genomic applications. Philadelphia, PA: Elsevier; c2023.
12. Painter JT, Clayton NP, Herbert RA. Useful immunohistochemical markers of tumor differentiation. Toxicologic Pathology. 2009;38(1):131–41. Doi:10.1177/0192623309356449
13. Tuffaha M, Guski H, Kristiansen G. Recommendations for the Utility of Immunohistochemistry in Tumor Diagnosis. Immunohistochemistry in Tumor Diagnostics [Internet]. Springer International Publishing; c2017. p. 257-8. https://doi.org/10.1007/978-3-319-53577-7_32 11.
14. Oien K, Dennis J. Diagnostic work-up of carcinoma of unknown primary: from immunohistochemistry to molecular profiling. Annals of Oncology. 201209;23:x271-x277. https://doi.org/10.1093/annonc/mds357
15. Tagliaferri B, Quaquarini E, Palumbo R, Balletti E, Presti D, Malovini A, *et al*. Role of androgen receptor expression in early stage ER+/PgR−/ HER2– breast cancer. Therapeutic Advances in Medical Oncology; c2020. 01;12:175883592095835.https://doi.org/10.1177/1758835920958355
16. Ni Y, Tsang JYS, Shao M, Chan S, Cheung S, Tong J, *et al*. GATA-3 is superior to GCDFP-15 and mammaglobin to identify primary and metastatic breast cancer. Breast Cancer Research and Treatment. 2018;169(1):25-32. https://doi.org/10.1007/s10549-0174645-2
17. Lin F, Liu H. Unknown Primary/Undifferentiated Neoplasm. Handbook of Practical Immunohistochemistry. Springer New York. 2015. p. 119-63. https://doi.org/10.1007/978-14939-1578-1_11
18. Lin F, Liu H. Immunohistochemistry in undifferentiated neoplasm/tumor of uncertain origin. Archives of Pathology & Laboratory Medicine. 2014;138(12):1583–610. doi:10.5858/arpa.2014-0061-ra

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