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EVALUATION OF UNDIFFERENTIATED CARCINOMA FROM AN UNIDENTIFIED PRIMARY ORIGIN (REVIEW ARTICLES)

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Article Info.

Article history:

Received 01 October 2022

Revised 02 November 2022

Accepted 14 December 2022

Keywords:

unknown origin ,carcinoma , cancer

How to cite:

Zainab Al-Ali, Mostafa A. Alwash, Zena Mahdi Abd Ali.

Evaluation of undifferentiated carcinoma from an unidentified primary origin (review articles).

Aca. Intl. J. Med. Up.

2022;01(2):15-22

doi.org/10.56981/MU000124

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ABSTRACT

Cancers with an unidentified primary site at the start of treatment are classified as cancers with an unknown original primary site. Their prevalence among cancer patients is around 5%. One of challenges in medicine is an approach to those patients . The requirement for the early detection of malignancies or palliative care are still problem in medicine. A strategy for their treatment should be created to avoid prolong hospital stays and testing that won't improve therapy or the prognosis for those sufferers.

It's crucial to identify individuals whose diseases have good prognoses since they may benefit greatly from targeted therapy, including longer survival. A targeted search for the main tumor in those patients is advised. However, for those patients who will effectively employ targeted therapies, the combination of a robust immunohistochemistry panel with novel molecular knowledge may enable the development of a specific management strategy.

INTRODUCTION

A heterogeneous category of malignancies known as carcinoma of unknown primary original site (cupo) is distinguished by the presence of metastatic disease without a recognized source tumor at initial presentation. An estimated 2% to 5% of all cancer cases are CUPO, according to reports.¹ Despite the development of advance radiological techniques and targeted cancer therapies, the great effort management of CUPO still challenging. It should rely on the patient's response to treatment, the clinical

presentation, the pathological evaluation, and other factors.

The criteria for CUPO include a histopathological examination of specimens which the original site is unknown after obtaining a detail history, conducting a thorough clinical assessment, performing blood indexes account, hepatic assessment and urinary system function tests, imaging of thorax, topographical studies (CT) viewer of abdomen and pelvis and mammography.² Metastatic sarcoma, metastatic melanoma, and lymphoma. Because these diseases can be treated based on stage and histologic type, the majority of investigators also wish to exclude them.

Biological feature of carcinoma of unknown primary original site

It is possible for the primary tumor in CUPO to remain small and evade clinical detection, or it could vanish after triggering a metastasis. Additionally, the body's defenses may have ceased it or eliminated it. CUP might be a malignant tumor that affects the primary tumor's ability to metastasize or survive. CUP metastases may be genetically and phenotypically distinct, but this has not yet been established. Numerous research have examined the significance of chromosomal and molecular aberrations in CUPO, but to this far, no distinctive features of CUPO have been found in comparison to metastases from well-known original malignancies. There have been discovered complicated anomalies, including chromosomal 1 and 12 abnormalities.³ In 70% of CUPO cases with metastatic adenocarcinoma or anaplastic cancer, aneuploidy has been found. Ras (92%), Bcl-2 (40%), Her-2 (11%), and p53 (26%–53%) are only a few of the genes that have been shown to be overexpressed in CUP, however the existence of these abnormalities does not appear to affect either survival or treatment response.^{4 5 6} It has been hypothesized that in CUPO, the original tumor's angiogenic inefficiency induces a significant amount of programmed cell death and cell alteration, which results in a tumor that usually develops a metastatic phenotype; however, No clinical evidence exists to support the notion.⁷ Karavasilis and a coworker⁸ 81 CUPO tissue samples were assessed for thrombospondin-1, VEGF, and CD34 tissue expression levels. All cases had VEGF expression (83% of cases had high expression). It has also been evaluated how much angiogenesis there is in CUPO compared to metastases from known primary tumors, but no significant indications have emerged.⁹

Clinico-histopathologic Evaluation Of CUPO

In CUP situations, a thorough physical examination and family and personal medical history are crucial. Previous surgeries and lesions should be taken into consideration. It is also necessary to perform a detailed histopathologic evaluation of the biopsied tissue, which often includes immunohistochemical as well as H &E stains.¹⁰

Microscopical Assessment

In CUPO patients, a fine-needle aspiration biopsy is often sufficient; however, a core biopsy may be performed if possible. The first step in analyzing biopsy tissue should be light microscopy with H&E staining. On microscopical examination adenocarcinoma (60%), poorly differentiated adenocarcinoma (30%-35%), or undifferentiated carcinoma or neoplasm (30%-35%), are the most common types of CUPO cancers; squamous cell carcinoma (5%) and neuroendocrine tumors (2%) make up the remaining lesions. Adeno-squamous carcinoma, adenocarcinomas with neuroendocrine components, and sarcomatoid carcinoma are examples of mixed tumors that might infrequently show as CUPO.

Immunohistochemical Markers

The tumor lineage can be determined using immunohistochemical markers, which are often peroxidase-labeled antibodies towards certain tumor antigens.¹⁰ A battery of stains cannot take the place of effective interaction of the pathologist and the oncologist in making a proper management.

Cytokeratin (CK) intermediate filaments can be divided into 20 subtypes, each of which has a unique molecular weight and degree of expression in various malignancies and cell types. In order to categorize tumors according to where they originated, monoclonal antibodies to particular CK subtypes have been

utilized; the CK 7 and 20 stains are the most frequently applied in CUPO adenocarcinoma patients. While CK 20 is typically found in epithelium of the lower GIT, urothelium, and Merkel cells, CK 7 is typically expressed in malignant tumor of upper GIT, cholangiocarcinoma, pancreatic, lung, ovary, endometrial, and mammary malignant tumor.¹¹ The CK 20+/CK 7- phenotype reveals a primary colonic carcinoma; 75%-95% of colon cancers possess this staining pattern. Lung, breast, ovarian, and endometrial cancers are only a few of the cancer types that express CK 20/CK 7+. Pancreatic and cholangiocarcinoma cancers can either be CK 20/CK 7+ or CK 7+ with focal CK 20 positive. The utilization of thyroid transcription factor-1 (TTF-1) and surfactant apoprotein can help in identification of primary lung tumors from other CK 7+ types. 85% of lung malignancies are CK 7 positive. TTF-1 is stained positively in about 68% of lung adenocarcinomas and 25% of squamous cell lung malignancies.^{12 13} Occasionally it can be quite challenging to differentiate mesothelioma from adenocarcinoma.¹⁴ Indicators for the immunohistochemistry method that is widely used to identify mesothelioma involve calretinin, Wilms' tumor-1, and mesothelin. Calretinin, Wilms' tumor-1, estrogen receptor, and MOC-31 (or Ber-EP4) are some immunohistochemical markers that can be used in combination to assist in identifying peritoneal mesothelioma from serous papillary carcinomas when the morphologic characteristics are ambiguous.¹⁵ Hepatocytes that are both benign and malignant tend to express the hepatocyte paraffin 1 antibody, which can help with the immunohistochemistry identification of hepatocellular carcinoma.^{16 17} Breast carcinomas specifically produce the 15-kDa oncofetal protein known as gross cystic disease fluid protein 15 (GCDFP-15), which is a biomarker of apocrine differentiation. Expression is noted in 62%–72% of cases.^{18 19 20} The markers most frequently employed for diagnosis in instances thought to have a urothelial origin are uroplakin III, high-molecular weight cytokeratin, thrombomodulin, and CK.^{21. 22} The nuclear transcription factor caudal-related homeobox 2 (CDX-2), which is produced by the homeobox gene required for intestinal organogenesis, is detected in healthy epithelial cells of the colon and the majority of colorectal adenocarcinomas and is frequently applied as a diagnostic tool for gastrointestinal adenocarcinoma.²³ Table 1 shows different types of immunohistochemical markers used in carcinoma of unknown primary origin site.

Table 1. A differential diagnosis of CUPO is made using immunohistochemical staining.

Stain	Primary tumor
ER, PR, GCDFP-15, Her-2/neu	Breast cancer
TTF, CK 7, surfactant proteins	Lung cancer
Chromogranin, synaptophysin, neuron-specific enolase	Neuroendocrine tumor
β -Hcg, α -fetoprotein	Germ cell tumor
CK 7, CK 20, uroplakin III	Urothelial Malignancy
Calretinin	Mesothelioma
Hep Par-1	Hepatocellular carcinoma
CK 7, CK 20, CDX-2, CEA	Colorectal cancer

Tumor Markers and Cytogenetics Analysis

A PSA test should be performed on men who have adenocarcinoma and bone metastases. In order to assess for extragonadal germ cell cancers, β -HCG and α -FP levels are typically evaluated in patients with undifferentiated or poorly differentiated carcinoma (particularly when a midline tumor is present). The majority of tumor markers, such as carcinoembryonic antigen, CA-125, CA 19-9, and CA 15-3, are not specific, making it difficult to pinpoint the location of the main tumor.^{24 25} Similar to immunohistochemical testing, we believe that cytogenetic analysis is no longer useful in light of the widespread usage of assays that can distinguish between epithelial malignancies and lymphomas. During a Motzer and associates study,²⁶ a cytogenetic study was performed on 40 patients who had CUPO

& poorly differentiated cancer. Genetic testing was used to diagnose 17 patients (42%) of whom 12 (30%) had cytogenetic abnormalities typical of germ cell cancers (eg, isochromosome 12p, increased 12p copy number, or deletion of the long arm of chromosome 12). Pantou and associates²⁷ 20 CUPO spaceman were examined & cytogenetics helped identify the original tumor in 5 patients (4 with lymphoma and 1 with Ewing sarcoma). The other samples contained a variety of intricate cytogenetic patterns. It can be challenging to interpret the findings of former investigations when suggestion the of lymphoma is elevated and the immunohistochemistry findings are ambiguous, a situation occasionally seen with poorly differentiated neoplasms, our institution occasionally requests cytogenetic and B cell and T cell gene abnormalities to confirm the diagnosis of lymphoma.

Radiological evaluation in CUPO

In CUPO cases, CT of the abdomen and pelvis is frequently done for diagnosis of the underlying tumor, assess the severity of the disease, and choose the best location for the biopsy. Retrospective research on the use of abdomen CT in 46 CUPO cases with metastatic adenocarcinoma or undifferentiated carcinoma was done in the 1980s by McMillan and colleagues²⁸, in 21 cases, the original tumor location was eventually found. In 16 of these individuals, a CT of the abdomen revealed it, and in 65% of those individual, it revealed further, frequently undetected metastatic disease. Ultrasound study and contrast investigations of the urinary and gastrointestinal systems were inferior to CT. Latent main tumors were discovered in 179 of 879 CUPO patients in a research by Abbruzzese and colleagues²⁹ (20%), however in the era of advanced imaging, this number should have been much lower. Panendoscopy and CT or MRI scans are part of the standard work up for cervical CUPO (usually neck adenopathy) that presents with squamous cell carcinoma. For all cases representing with squamous cell cervical CUPO, an ipsilateral (or, preferable, bilateral) tonsillectomy has been advised since a superficial tonsil biopsy may actually miss a tiny original tumor.^{30 31}

Positron Emission Tomography Scan

Given that most of the records data are retrospective, the place of positron emission tomography (PET) in the investigative process for cases with disseminated (noncervical) CUPO is still up for dispute. The majority of the PET scans in CUPO are performed on individuals with squamous cell carcinoma and cervical lymphadenopathy, a disease group in which the benefit of PET has been amply demonstrated about 21% to 30% of patients with cervical CUPO have primary tumors, but these results come from few research.^{32 33 34} The preponderance of medical specialists concur that 18F-fluorodeoxyglucose (FDG)-PET is beneficial for this diseased group because it may aid in guiding the biopsy, assessing the severity of the disease, facilitating the appropriate treatment (including radiation beams), and assisting in disease surveillance.

302 cases with CUPO cervical metastases were included in 16 FDG-PET studies assessed by Rusthoven and colleagues³⁵. These studies were published between 1994 and 2003. Panendoscopy or CT/MRI were included in the standard work-up. Both diagnostic procedures (panendoscopy and CT/MRI) were carried out prior to the diagnosis in 10 of the 16 studies. FDG-PET had an overall sensitivity, specificity, and accuracy rate of 88.3%, 74.9%, and 78.8% in identifying unknown primary malignancies. About 25% of tumors were discovered by FDG-PET that were missed by traditional testing, and 27% of patients had previously undetected regional or distant metastases. Additionally, in two small retrospective research, 20% of CUPO patients who did not have cervical cancer had their primary tumor discovered by PET.^{36,37} In some institutions, patients with cervical CUPO, those with solitary metastatic cancer management depends on the violence of the illness), those with iodine allergies, and those with no proof of disease who are receiving postsurgical adjuvant treatment frequently have PET-CT (because PET results could affect outcome and therapy strategy).

One might anticipate a rise in the usage of PET-CT scans in the CUPO context in the near future, particularly with the adding of IV contrast to the procedure. To further understand how cost-effective PET is, larger, well-designed research are needed.

Magnetic Resonance Image

For evaluating solitary axillary lymph node metastases and supposed hiding primary mammary cancer, MRI is an established technique (after negative mammography and U/S findings). Olson and colleagues³⁸ looked at 40 women who had axillary node metastatic illness but no initial mammographic tumor. Using a specialized breast coil, an MRI was used to detect a primary tumor in 28 women (70%). A modified radical mastectomy was performed on 5 out of 12 women who had negative breast MRI results; on 4 of them, no tumor was discovered in the mastectomy specimen. According to these results, magnetic image can successfully diagnose breast cancer in up to 75% of women who have axillary lymphadenopathy.

ROLE OF Molecular Study (DNA MICROARRAY & RT- PCR) IN Evaluation Of CUPO

Reverse transcription polymerase chain reaction (RT-PCR) methods and the usage of DNA microarrays are expected to be ultimately beneficial in this matter. It can be challenging to create therapeutic choices for CUPO patients, particularly those needing focused therapy.

The gene profiles of known tumors must be used as a training set for gene expression studies in order to accurately represent the tumor types that are believed to be prevalent in the research population. To create predictive algorithms from the gene expression profiles, neural network software has been applied. The software is often trained using a collection of gene profiles from well-known tumors, ideally from metastatic locations. It is then possible to utilize the algorithm to determine where the test tumor originated. Massive gene expression datasets for well-known cancer types that have just become available may be beneficial for CUPO. Researchers have discovered conserved expression patterns in malignant tissue and, as a result, been able to predict the genesis tissue by using expression data from healthy, differentiated tissues.^{39 40 41 42}

Su and colleagues⁴³ explained how to build a 1st molecular categorization scheme for the 11 diseases that comprise 70% of all cancer-related mortality using wide-ranging RNA profile and conducted learning techniques. The subsets of predictor genes comprised genes whose expression was particular to the tissue of origin and genes whose expression was increased in cancer. 100 primary carcinomas from 10 common tumor categories were employed by the authors. Using 110 of the 9,198 genes that were only marginally expressed in these growths, a predictive algorithm was created. The algorithm was subsequently evaluated on an additional 75 blinded sections, including 12 metastatic sections, and in more than 90% of those instances, it correctly identified the primary tumor. The classification of 11 out of the 12 metastatic test cases was accurate.

To sequence and verify a cross-platform support vector machine (SVM) model to identify the main tumor, Tothill and colleagues⁴⁴ used data from quantitative PCR (low-density array to allow the usage of both fresh-frozen and formalin-fixed paraffin-embedded tissue) and a microarray. They used SVM classifiers to analyze 13 patients with CUPO, and in 11 cases, information from the patients' clinical histories corroborated the predictions.

Predictions must be backed by clinical and pathologic evidence because the primary tumor site in CUPO is unclear, making it difficult to validate the primary tumor site. The effectiveness of molecular studies in CUPO is now being evaluated through prospective indirect validation trials.

CONCLUSION

A thorough investigation for the primary neoplasm is indicated in CUPO cases. It is essential to identify patients with predictive value favorable disease because they may experience significant therapeutic benefit from targeted therapy and live longer. However, because resistance to the available cytotoxic therapy frequently emerges, the prediction value is dismal for the majority of CUPO patients. We predict higher overall response rates with new tailored treatments for chosen CUPO cases because response rates among identified tumor forms have gradually increased over the previous ten years. We intend to develop a customized therapy regimen for CUPO patients using an extensive immunohistochemistry panel (guided approach) and the use of developing molecular data. It is unclear whether CUPO differs

from metastases of recognized primary tumors in terms of its molecular genotype-phenotype. Finding certain CUPO-related molecular and biochemical targets may aid in the selection of effective targeted treatments for the disease's various patients.

Abbreviation: CUPO=carcinoma of unknown primary original site., GIT= gastrointestinal tract ,SVM= support vector machine. CT computerized tomography, DNA = deoxyribonucleic acid ,RNA ribonucleic acid ,RT_PCR= Reverse transcription polymerase chain reaction. U/S = ultrasound . PET = positron emission tomography. MRI= magnetic resonance image .

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