Carcinoma of Unknown Primary: Focused Evaluation

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Abstract
The management of carcinoma of unknown primary (CUP) has evolved over the past decade, with the advent of sophisticated imaging and pathologic tests. Especially in the era of tailored therapeutics, this presents both an opportunity and a challenge. For the initial workup of a patient with CUP, a focused search for the primary cancer based on clinical presentation is recommended. Exhaustive use of unnecessary imaging, invasive studies, and pathologic tests adds to patient discomfort, is associated with significant cost, and often has a low yield for detection of a primary cancer. Physicians should be able to justify the need for ancillary tests and how results may impact the comprehensive management of patients. Over the next several years, physicians also need to focus their efforts on refining CUP subsets (e.g., isolated nodal, osseous, carcinomatosis presentations) and leveraging selective genomics and proteomics techniques to eventually deliver validated new therapeutic approaches to patients. This will require creative approaches to clinical studies, including establishment of international CUP cooperative groups and innovative designs. Just as physicians need to be selective in their diagnostic approach, they also need to be selective in their research efforts as they continue to impact quality of life and survival for patients with CUP. (JNCCN 2011;9:1406–1412)

Poor universal agreement exists on the definition of carcinoma of unknown primary (CUP). Some ambiguity comes from the literature (or lack thereof) regarding what constitutes CUP syndromes, particularly because the workup for primary tumor identification before labeling a tumor as CUP has not always been consistent. Opportunities for ambiguity have further increased with the emergence of robust immunohistochemistry and molecular profiling tools. The management of CUP has evolved over the past decade, with the advent of sophisticated imaging and pathologic tests. Especially in the era of tailored therapeutics, this presents both an opportunity and a challenge.

CUP cancers can be approached in two distinct ways, and this may impact patient management. On one hand, CUP management may be viewed as the epitome of personalized medicine as one applies the recent understanding of biological markers to this heterogeneous disease. On the other hand, some believe that CUP is functionally a homogeneous entity with disseminated metastatic disease at presentation and should be treated based on empiric experience with CUP as a whole, because individual variation does not have a significant impact on therapeutic approaches or survival. Today, the view of most practitioners probably falls somewhere between these two extremes. Some would like to believe that CUP lies closer to the former than the latter. Given the diagnostics and therapeutics currently available for CUP, and leveraging those available for known cancers, the therapeutic envelope is in fact slowly and steadily being pushed further for patients with CUP.

Although it is a heterogeneous disease, CUP has traditionally been treated largely as a single entity, and primarily with standard platinum-based combination
chemotherapies. Several combination treatments have been reported on in the past decade and these have helped physicians create a range of empiric therapies that can be used in patients with CUP, although not all are included in the NCCN Drugs & Biologics Compendium (NCCN Compendium).4-8

In the past several years, the primary focus of the field has moved away from evaluating additional broadly applicable empiric therapies, and moved toward focusing on innovative studies that may help select CUP subtypes.9 This refocus serendipitously meets the needs of the current health care economic environment, with its need for directed approaches that are both clinically effective and cost-effective, and has clear impact on patient quality of life and survival.

A concerted effort has been made in known cancers to evaluate driver oncogenes and druggable targets. So far no CUP-specific or unique biologic abnormalities that can be therapeutically exploited have been identified in patients with CUP. This lack of CUP-specific markers provides additional support for the belief that CUP is not a distinct entity, but rather unrelated groups of site-specific tumors that happen to share the property of having a diminutive primary that escapes detection. Consistent with this hypothesis, and with the increasing use of immunohistochemical studies and molecular profiling assays over the past several years, CUP subsets have been identified with profiles resembling those of metastatic disease with known primaries.10 Studies also suggest that some of these CUP subsets respond to chemotherapy regimens known to be effective against the corresponding anatomically defined cancers, further supporting a potential benefit from tailored treatment strategies.10

This article discusses the role of a focused approach to CUP diagnostics. This includes the role of various imaging, serum, and pathologic tests that are the moving pieces of the puzzle called CUP (Figure 1). Moving forward, this is a work in progress; a collective effort that selectively uses both tissue of origin (ToO)-based tests (immunohistochemistry and profiling) and target identification-based tests (prognostic and predictive biomarkers), which may ultimately provide the most benefits.

### A Focused Approach to Radiology and Invasive Studies

#### Diagnostics on Initial Presentation and Follow-Up

CT scan of the chest, abdomen, and pelvis is routinely performed to search for the primary tumor, evaluate the extent of spread, and select the most favorable biopsy site. A mammogram is indicated in women who present with metastatic adenocarcinoma and clinical presentation suggestive of breast cancer (interestingly, autopsy studies suggest that a breast profile may be a rather uncommon CUP presentation).11 In selected women with isolated axillary adenopathy, nonsquamous pathology, and a negative mammography and ultrasound, bilateral breast MRI is a sensitive modality to evaluate for occult primary breast carcinoma.12-14

Conventional workup for neck adenopathy presenting as squamous cell carcinoma CUP includes either CT or MRI and triple endoscopy with biopsy of the suspicious sites. Because a superficial biopsy of the tonsil can miss a small primary tumor within the deep crypts, bilateral tonsillectomies are recommended for patients presenting with this CUP entity.15

The role of 18F-fluorodeoxyglucose positron emission tomography (18F-FDG PET) scan in the diagnostic workup of patients with disseminated CUP remains unproven. Data suggest that PET-CT is a recommended diagnostic study in patients with squamous cell cancer and cervical adenopathy.16,17 In prospective and retrospective studies, a primary head and neck squamous tumor is identified in approximately 25% to 30% of these patients. In this group of patients, FDG-PET is useful because it may help guide the biopsy of the putative primary (PET to be

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**Figure 1** The CUP puzzle.

Abbreviations: CUP, carcinoma of unknown primary; IHC, immunohistochemistry.
performed before panendoscopy); determine the extent of disease; facilitate the appropriate treatment, including limiting radiation fields (thereby minimizing xerostomia); and assist with surveillance.\textsuperscript{18}

Outside of the neck adenopathy presentation, PET studies suggest a primary tumor detection rate of approximately 20\% to 30\%; unfortunately these studies are mostly small, retrospective, heterogenous, and difficult to interpret.\textsuperscript{19} Moller et al.\textsuperscript{20} recently reviewed 18F-FDG PET/CT as a diagnostic tool in patients with extracervical CUP. After a comprehensive literature search, they identified 4 publications (152 patients) that had evaluated 18F-FDG PET/CT in patients with CUP with extracervical metastases. All 4 studies were retrospective and heterogeneous in their inclusion criteria, study design, and diagnostic workup before 18F-FDG PET/CT. 18F-FDG PET/CT detected the primary tumor in 39.5\% of patients with extracervical CUP. The lung was the most commonly detected primary tumor site (~50\%). Pooled estimates of sensitivity, specificity, and accuracy of 18F-FDG PET/CT in detecting the primary tumor site were 87\%, 88\%, and 87.5\%, respectively. The investigators concluded that 18F-FDG PET/CT may have a role in identifying the primary tumor in extracervical CUP; however, prospective studies with more uniform inclusion criteria are warranted.

In the era of cost-containment, the decision to pursue a PET scan should be justified, and rarely are a PET-CT and an intravenous contrast scan needed repeatedly in the same patient. Although some practical situations exist in which a PET-CT scan may be considered in the diagnostic and follow-up period, prospective studies to evaluate these indications remain a challenge. Three selective scenarios that the author has encountered in her clinic include 1) in the evaluation of disease extent before locoregional therapy (including surgery) in patients presenting with solitary metastatic disease; 2) in the follow-up of patients with osseous CUP who are undergoing active therapy (in whom MRI is not indicated); and 3) as an alternative in patients with a severe iodine dye allergy. In these situations, PET may be cost-effective and may suffice as the predominant imaging study to evaluate the extent of disease and therapy response.

**Invasive Studies**

Exhaustive use of upper endoscopy and colonoscopy in all patients with CUP increases patient discomfort, introduces significant costs, and has a low yield for detection of a primary cancer. Beyond symptoms or clear radiographic indications, the role of these modalities is limited to a select group of patients (if a primary tumor is detected on endoscopy [performed because of concerning symptoms/signs], it should not be considered CUP but rather referral bias and suboptimal evaluation before CUP designation).

**Serum Tumor Markers and Cytogenetics**

Men presenting with adenocarcinoma and bone metastases should have their serum prostate-specific antigen (PSA) level evaluated for presence of prostate cancer. \(\beta\)-Human chorionic gonadotropin and \(\alpha\)-fetoprotein levels are usually measured in men diagnosed with a midline undifferentiated or poorly differentiated carcinoma to evaluate for extragonadal germ cell tumor. Beside these two clear indications, most tumor markers, including carcinoembryonic antigen (CEA), CA 125, CA 19-9, and CA 15-3, are nonspecific and not helpful in establishing the site of the primary tumor. Similarly, cytogenetic analysis is archaic for the workup of CUP. Pantou et al.\textsuperscript{21} reported that most CUP samples have complex cytogenetic patterns, and interpreting other older studies evaluating the role of isochromosome i(12p) or gene rearrangement studies for lymphoma\textsuperscript{22} is difficult because, in the era of sophisticated immunohistochemistry, cytogenetic analysis is rarely if ever indicated.

**Focused Approach to Pathologic Workup**

Pathologic evaluation of CUP tissue is often the missing piece of the CUP puzzle. Sometimes it is a good “fit,” and other times discordancy between the pathology and other parts makes it incredibly perplexing. Several limitations must be taken into account as various steps involved with the pathologic approach to CUP are discussed.

Ideally, a core biopsy is preferred, given the need for immunohistochemistry in most cases.\textsuperscript{23} On light microscopy, most CUPS are identified as adenocarcinoma (60\%); poorly differentiated adenocarcinoma, carcinoma, or undifferentiated neoplasm (30\%); squamous cell carcinoma (5\%); or neuroendocrine cancer (4\%).\textsuperscript{23} Occasionally, CUP present as mixed tumors, such as adenosquamous carcinoma, adenocarcinoma with neuroendocrine features, and sarcomatoid carcinoma. An ideal selective approach to pathology would include a tiered
diagnostic algorithm (Figure 2). The initial (first) tier in this algorithm includes hematoxylin-eosin staining and immunohistochemistry using peroxidase-labeled antibodies against specific tumor antigens that help suggest the tumor lineage. The use of immunohistochemistry in CUPs is based on the premise that concordance exists in the expression profiles of primary and metastatic cancers. Once the tumor lineage is established (e.g., carcinoma, sarcoma, lymphoma, melanoma), the second-tier tests help suggest the cancer’s profile (i.e., predict the possible primary site). Except PSA, most immunohistochemistry assays have a modest specificity and sensitivity. They perform the best when used in groups, which gives rise to patterns that are strongly indicative of certain profiles and can assist with treatment planning. For example, the TTF-1/CK7+ and CK20+/CDX-2+/CK7- phenotypes have been reported to be very suggestive of lung and lower gastrointestinal cancer profiles, respectively, although they have not been prospectively validated in the absence of a primary cancer. 24–27 The third tier consists of additional biomarkers, especially those with a therapeutic intent (i.e., druggable), and this requires integration of imaging and clinical presentation (use of KRAS, HER2, ALK mutations). 28–30 Somewhere between tier 2 and 3 is an emerging subset of studies, including molecular profiling, as discussed later.

Several groups have studied the role of immunohistochemistry patterns and decision trees based on immunohistochemistry specificity, sensitivity, and predictive values. These data are helpful and must be adopted in clinical practice to optimize tailored therapy and minimize unnecessary costs. DeYoung et al.,31 in their comprehensive review of immunohistochemistry markers, suggest that the sequence of interpretation of immunostains should be governed by their relative statistical values, moving from most specific to least, or from the highest positive predictive value to the lowest. Dennis et al.,32 published an excellent study profiling 27 candidate markers using immunohistochemistry in 352 primary adenocarcinomas. Ten markers were selected (PSA, TTF1, CDX2, CK20, CK7, GCDFP-15, ER, CA 125, mesothelin, and lysozyme). The markers were used in a diagnostic table and decision tree, which enabled correct prediction of the primary site (pancreas, lung, colon, breast, stomach, ovary, prostate, and other carcinomas) in 88% of the initial samples. A separate validation cohort of 120 primary and metastatic adenocarcinomas showed the same accuracy (88%). Park et al.,33 evaluated expression profiles of tumor markers in adenocarcinomas from 7 primary sites: colorectum, stomach, pancreas, bile duct, breast, lung, and ovary. Marker algorithms and a decision tree with immunohistochemistry, including TTF1, GCDFP-15, CDX2, CK7, CK20, and DPC4, reported an accurate prediction in 75% of cases (metastatic cancers with known clinical truth).

Immunohistochemistry is not without its limitations; several factors affect tissue antigenicity (antigen retrieval, specimen processing, and fixation); interpretation of immunohistochemistry in tumor (nuclear, cytoplasmic, membrane) versus normal tissue; inter- and intraobserver variability; and tissue heterogeneity and inadequacy (given small biopsy sizes). Communication between the clinician and pathologist is essential and cannot be replaced by an exhaustive battery of stains.

**Integration of Immunohistochemistry With Profiling and its Impact on Management**

**Rationale for Profiling**

The accurate diagnosis of the primary site in CUP would impact patients’ prognosis and management, allowing site-specific therapy. Molecular profiling...
of tumors offers the chance of a systematic human cancer classification, and is a promising technique to improve the site of origin diagnosis in patients with CUP. Molecular profiling methods using various platforms, including DNA microarray and reverse transcriptase polymerase chain reaction (RT-PCR), have been used to evaluate the ToO in metastatic samples. The data on known metastases have been validated using independent blinded sets of tumor samples, in which the reference diagnosis is known, with an accuracy of 80% to 90%.34–39

These genomic technologies that provide large-scale gene expression profiles based on mRNA or microRNA are currently in commercial application. Given the feasibility of using mRNA/microRNA from small biopsy samples available as formalin-fixed paraffin-embedded (FFPE) tissue (using a quantitative RT-PCR platform), this is a practical approach for clinic use.

**CUP Studies and Molecular Profiling**

The University of Texas MD Anderson Cancer Center, in collaboration with The Sarah Cannon Cancer Center, reported the first large CUP series evaluating the feasibility of a 10-gene RT-PCR assay to identify the ToO in patients with CUP.40 Diagnostic FFPE specimens from 120 patients with CUP were studied, and ToO assignments rendered by the assay were correlated with clinical and pathologic features and response to therapy. The assay was successfully performed in 104 patients (87%), and a ToO was assigned in 63 patients (61%). In the remaining 41 patients (39%), the molecular profiles were not specific for the 6 tumor types detectable with this assay. The ToO most commonly identified were lung, pancreas, and colon; most of these patients had clinical and pathologic features consistent with these diagnoses.

Monzon et al.41 described a multicenter validation of a predefined 1550-gene expression profile to identify the ToO. Four institutions processed 547 frozen specimens (metastatic, poorly differentiated, undifferentiated primary cancers) representing 15 ToOs using oligonucleotide microarrays. The study found an overall sensitivity of 88% and overall specificity of 99%. Performance within the subgroup of metastatic cancers was found to be slightly lower than that of the poorly differentiated and undifferentiated primary tumor subgroup. This study did not include patients with CUPs.

Two groups have evaluated the role of microRNA in ToO profiling. The first study by Ferracin et al.42 evaluated microRNA profiling in 101 FFPE primary cancers and metastasis samples using a microarray platform. Forty samples representing 10 cancer types were used to define a cancer-type–specific microRNA signature, which was used to predict primary sites of metastatic cancers. A 47-microRNA signature was identified and used to estimate ToO probabilities for each sample. Overall, accuracy reached 100% for primary cancers and 78% for metastases. This signature was applied to an independent published dataset of 170 samples, and prediction was found within the first 2 options (differential) in 86% of the metastasis cases (the first prediction was correct in 68% of cases). This signature was also applied to predict 16 CUP samples, and maintained the accuracy. The second study by Varadhachary et al.43 exclusively evaluated FFPE metastatic tissue from 104 patients with CUP, 87 of whom had sufficient tumors for testing. The assay quantitated 48 microRNAs and assigned 1 of 25 tumor diagnoses using a biologically motivated binary decision tree and K-nearest neighbors (KNN). The assay predictions were compared with clinicopathologic features and, when suitable, therapeutic response. The assay result was consistent or compatible with the clinicopathologic features in 84% of cases processed successfully. The authors concluded that microRNA profiling may be particularly helpful in patients for whom the immunohistochemistry profile of the metastasis is nondiagnostic or leaves a large differential diagnosis.

Greco et al.44 presented an interesting retrospective study that compared the molecular profile results with the latent primary cancer diagnosed over the course of a patient’s life/treatment. Of 501 patients, 38 (7.6%) with CUP (data from 2000–2008) had their latent primary-site tumor subsequently identified during life. Among these patients, 28 (74%) had adequate initial tissue biopsies available for molecular profiling with an RT-PCR assay. The assay was performed on FFPE biopsy specimens in a blinded fashion, and the assay results were compared with clinicopathologic data and the actual latent primary sites. Among the 28 patients, 20 (71.4%) RT-PCR assays were successfully completed. Of these assay predictions, 15 (75%) were correct, corresponding to the actual latent primary sites identified after the initial diagnosis of CUP.
Several themes have emerged from these molecular profiling studies: 1) the indirect validation methods using immunohistochemistry, pattern of spread, and treatment response is feasible; what is currently not clear is how discordant results from immunohistochemistry and molecular profiling should be interpreted; 2) insufficient tumor quantity for assay is not an uncommon circumstance, especially if exhaustive immunohistochemistry assays have been performed (~15%–20% in clinical practice); and 3) although useful in some subtypes, the current usefulness of the profiling approach is limited by the paucity of effective drugs for several CUP profiles. Physicians who treat CUP are excited about the emerging significance of molecular profiling, and continued experience with commercial ToO assays helps them recognize when and where these tests will be most helpful. As novel therapies are developed for additional known cancers, this will significantly impact the appropriate CUP subtypes. In practice, the niche for profiling assays are the cases with a poor immunohistochemistry profile and for which two completely different therapies are indicated (based on pathology differential), with an impact on quality of life and possibly survival.

The authors can envision using an integrated algorithm that helps identify a patient’s CUP profile using directed immunohistochemistry and molecular profiling in selected cases. The most compelling case may be made for “osseous” predominant CUP presentation, in which the differential suggests cholangiocarcinoma and lung, breast, renal, poorly differentiated melanoma, thyroid, and other cancers. This group of patients rarely shows a specific immunohistochemistry profile, and therapies differ (cytotoxic vs. biologics). Regrettably, this group is also the one for which tissue procurement is a complicated task and inadequate samples are not uncommon.

**Making Progress**

Increasing evidence (latent primaries, immunohistochemistry patterns, and molecular profiles) shows that CUPs are probably unrelated groups of site-specific tumors that happen to share the property of having a diminutive primary that escapes detection. Unfortunately, efforts to study CUP using collaborative research and novel approaches have lagged behind those for other solid tumor types. The traditional prospective randomized trial design to adequately answer the novel treatment, immunohistochemistry, biology, or profiling question is likely not feasible, because an adequately powered trial would require more than 500 patients and still run the risk of providing ambiguous results because of the very heterogeneous presentations of CUPs. Furthermore, a well-defined regulatory path to study new CUP therapies does not exist. And finally, over the past several years, a shift from anatomically defined favorable subsets to pathologically defined favorable subsets (basically, pathology trumps radiology) has clearly occurred. All of these factors push physicians toward a focus on leveraging genomic and proteomic techniques to refine CUP subsets. In the past decade, some advances have been made in the management of patients with CUP, with the promise of more to come. Delivering validated new therapeutic approaches to patients will require creative approaches to clinical studies, including establishment of international CUP cooperative groups and innovative designs. Just as physicians need to be selective in their diagnostic approach, they also need to be selective in their research efforts as therapeutic options continue to expand in this sizeable patient population.

**References**


