Cancer of Unknown Primary Sites: What Radiologists Need to Know and What Oncologists Want to Know

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OBJECTIVE. In this article, we review the role of imaging in cancer of unknown primary site (CUP) diagnosis and management and the utility of immunohistochemistry, serum tumor markers, and molecular profiling in the optimized care of CUP patients.

CONCLUSION. With advances in imaging, pathology, and molecular medicine, the diagnosis and management of CUP have evolved into more personalized and site-specific therapies. A multidisciplinary integrated approach among oncologists, pathologists, and radiologists is extremely important.

Keywords: carcinoma of unknown primary sites, diagnosis, imaging, immunohistochemistry, occult primary

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Cancer of unknown primary sites, (CUP) or occult primary tumors, is not rare, accounting for approximately 2% of all malignancies diagnosed in the United States in 2011 [1]—the seventh to eighth most frequently occurring cancer in the world [2, 3]. The estimate of new cases of CUP in the United States was 31,000 per year in 2012 [4], decreased from 45,230 per year in 1995 [5]. Modern technologies, including MDCT, MRI, PET, and immunohistochemistry have evolved over the past decade, enabling the identification of the primary site of disease in more patients, thereby facilitating site-specific therapy. A multidisciplinary integrated approach among oncologists, radiologists, and pathologists is extremely important and has been emphasized in recent guidelines of management of CUP [1, 2]. In this article, we review the overall diagnostic approach and the role of imaging, immunohistochemistry, serum tumor markers, and molecular profiling.

Terminology and Definition

The terminology used for CUP is variable according to pertinent studies and guidelines. CUP or occult primary tumors, the commonly used terminologies in the literature, potentially include all types of malignancies, including carcinomas, sarcomas, and lymphomas [2]. The term "carcinomas of unknown primary sites" is reserved for epithelial malignancies, excluding nonepithelial types of cancers, such as sarcomas, lymphomas, and melanomas, because these other tumor types are usually easily identified on immunohistochemistry and are amenable to specific therapy [6]. However, carcinoma of unknown primary sites has been used interchangeably with CUP or occult primary tumors.

The definition of CUP or occult primary tumors is histologically confirmed metastatic tumor for which the site of origin is not identified through a standardized diagnostic workup [7]. The patients with presumed CUP are confirmed after detailed standardized evaluation fails to identify the primary site. Currently, there is no consensus on how much diagnostic workup is sufficient before confirming CUP and the standard varies across institutions and countries.

Overview of Diagnostic Approach to Cancer of Unknown Primary Site

CUP sites have a wide variety of clinical presentations due to metastatic disease, such as palpable masses, pain, or dyspnea, as well as their abnormal findings on initial imaging tests, such as multiple lung nodules on a chest radiograph, a destructive lesion on a bone radiograph, and multiple liver masses on an abdominal ultrasound image [8]. According to large postmortem cohort studies, the most common primary sites include lung (27%), pancreas (24%), liver or bile duct (8%), kidney or adrenal glands (8%), colorectum (7%), genital system (7%), and stomach (6%) [9]. However, small subsets of patients have more favorable clinical features or a treatable type of tumor that is more responsive to chemotherapy or locoregional therapy.

The diagnostic approach should identify such patients to achieve improved response and survival benefits.

The evaluation of presumed CUP should be stepwise and focused. Even though each diagnostic algorithm differs according to the clinicopathologic presentation, the overall diagnostic approach to presumed CUP can be summarized as follows: initial evaluation and biopsy, additional selective workup based on clinicopathologic presentation to identify specific subsets, and focused precise immunohistochemistry or gene profiling to direct the choice of treatment, such as site-specific therapy or personalized targeted therapy [1, 10–12] (Fig. 1).

The goals of initial standard workup and biopsy are as follows: confirming histologically that the lesions are indeed metastatic, identifying the cell lineage (and likely primary sites) of the cancer, and guiding further selective tests to identify the favorable or treatable subsets of patients [13]. The minimal required standard workup includes a thorough physical examination, including head and neck, rectal, pelvic, and breast examinations; basic blood and biochemical surveys; fecal occult blood testing; CT of the chest, abdomen, and pelvis; and histology of the biopsy sample. Identifying tumors with specific cell lineages, such as lymphoma, sarcoma, melanoma, or germ cell tumor, is important because these tumors are amenable to distinct therapies [13]. Fortunately, these tumors are usually easily identified on H and E staining and a limited immunohistochemistry panel.

The purpose of the second step, additional selective tests to identify specific subsets of patients, is to provide site-specific therapy to the patients who have favorable clinical features or treatable types of tumors [14]. At this stage, given the clinical, radiologic, and pathologic information from the initial evaluation, it is important to select and guide further tests to avoid exhaustive imaging and invasive tests because nontargeted studies rarely detect the primary site and confusion can result from false-positive results. Close communication among oncologists, pathologists, and radiologists is of paramount importance. Particular clinicopathologic findings should guide the choice of imaging studies, and the findings from imaging studies may suggest additional pathologic tests.

The goal of the third step, focused immunohistochemistry or molecular-genetic profiling for the choice of treatment, is to provide individualized therapy for selected patients to

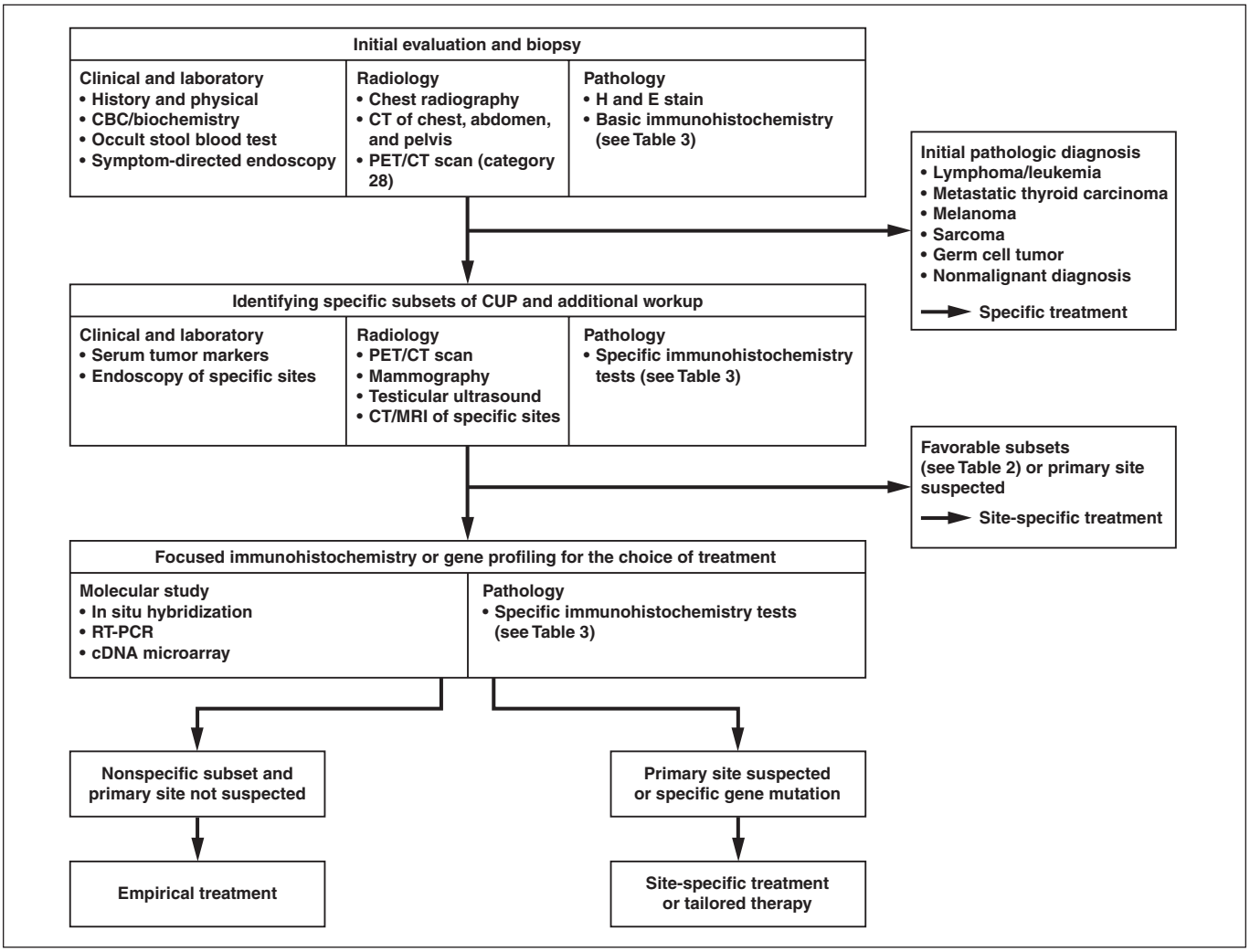


Fig. 1—Flowchart shows integrative approach of patients with cancer of unknown primary site (CUP). RT-PCR = reverse transcriptase polymerase chain reaction, cDNA = complementary DNA.

achieve better response to chemotherapy and longer survival gain [10]. In the 2013 National Comprehensive Cancer Network (NCCN) guidelines (version 1), molecular profiling is not recommended as part of the routine evaluation because more data from prospective clinical trials are necessary to confirm whether molecular profiling can improve the prognosis of patients with CUP [1]. However, in many institutions, selective immunohistochemistry stains and molecular profiling are used to predict primary sites, enabling site-specific therapy or identification of specific genetic mutations amenable to newer molecular targeted therapies [11].

Imaging Modalities in Cancer of Unknown Primary Site Diagnosis

In the initial standard evaluation, contrast-enhanced CT of the chest, abdomen, and pelvis is essential to search for the primary tumor, evaluate the extent of disease and possible pattern of spread, and select amenable biopsy sites [12, 15]. Scanning of additional regions, such as the head and neck, should be used in selected cases, such as cervical, axillary, or supraclavicular node metastases.

The routine use of mammography in all women with presumed CUP is controversial. Even though detecting the breast cancer has potential benefit in patients with CUP site and mammography is a quick and noninvasive test, mammography has a relatively low detection rate of breast cancer [8]. In the 2013 NCCN guidelines, mammography or breast ultrasound is recommended in women presenting with axillary or supraclavicular node adenocarcinoma metastases or mediastinal, lung, peritoneal, retroperitoneal, liver, bone, or brain metastases. If mammography is nondiagnostic and if there is any suggestive histopathologic evidence for breast cancer, breast MRI or ultrasound is indicated [1].

The use of ^{18}F-FDG PET or PET/CT has been increasing in the management of patients with CUP. Several studies have found that PET/CT detects more primary sites (24–40%) than CT or MRI (20–27%) [16–17]. However, the majority of these were retrospective studies with a small number of patients. Even though PET/CT is an attractive diagnostic tool in oncology, its utility in CUP has not been validated by a large-scale prospective clinical study [1]. In the 2013 NCCN guidelines, the routine use of PET/CT for initial evaluation is not recommended and PET/CT may be warranted in some situations.

There are several circumstances in which the use of FDG PET/CT is justified. PET/CT is recommended in patients with squamous cell cancer who present with malignant cervical adenopathy [10]. A primary head and neck squamous tumor is identified in approximately 50% of these patients [17, 18]. In this group, PET/CT is useful because it may help guide the biopsy of the suspected primary site; determine the extent of disease, including the radiation field; and enable the appropriate treatment. In cases of extracervical CUP, PET/CT is preferred when CUP manifests as localized disease or a single-site metastasis to find the primary site as well as to determine disease extent before locoregional treatment, such as surgery or radiation. Indeed, PET/CT has been reported to change the patient management plan in 34.7% of patients with CUP [19–21]. However, its utility is limited in patients with widespread metastases because it is difficult to distinguish the primary site from metastatic foci, and PET/CT may result in false-positive lesions.

Role of the Radiologist in Cancer of Unknown Primary Site Diagnosis

Radiologists must be part of multidisciplinary teams with oncologists and pathologists for optimized care of patients with CUP [7, 13, 22, 23]. Radiologists must have a clear understanding of the clinical questions at hand, the goals and role of imaging at each diagnostic step, and imaging limitations (Table 1). Not only is it essential for radiologists to be familiar with imaging findings of primary tumors and patterns of spread, it is also necessary to be knowledgeable in the differential diagnosis of the patterns, selection of lesions amenable to biopsy, selection and optimization of imaging protocols in both the diagnosis and follow-up of disease, and concordance or discordance of imaging and histopathologic findings.

In the ideal scenario, the primary site of disease or specific tumor subset is suspected on imaging and then confirmed by immunohistochemistry or other tests (Fig. 2). Knowledge of specific imaging findings of primary tumors as well as metastatic patterns of primary cancers is obviously helpful. However, the primary site is found in less than 30% of patients with presumed CUP during the workup, and only a minority of patients, about 20%, can be categorized as favorable or treatable on the basis of clinical features, radiologic findings, metastatic patterns, and immunohistochemistry [1].

TABLE 1: Summary of What Oncologists Want to Know and Radiologists Need to Know

What Oncologists Want to Know	What Radiologists Need to Know
What is the primary tumor site?	Imaging findings of primary tumors and metastatic tumors Metastatic pattern of primary cancers Limitations of imaging diagnosis
What is the appropriate site and method for biopsy?	Risk (complication) and benefit (diagnostic yield for specific sites and methods)
What is the subset into which my patient can be classified?	Thorough knowledge of each subset Further diagnostic steps for each subset
Is further imaging necessary, and if so, what is the appropriate next diagnostic test?	Recommendations for focused tests based on initial evaluation and clinicopathologic context Optimization of imaging protocol Recommendations for sufficient imaging test to avoid exhaustive workup
Are the results of imaging, immunohistochemistry, and laboratory tests concordant?	Adequate feedback based on typical and atypical metastatic-progression pattern of suspected primary cancers Importance of communication with oncologists and pathologists
Is the current targeted therapeutic regimen effective?	Appropriate use of changes in tumor vascularity, density, and other functional parameters in addition to size changes in response assessment to targeted therapy

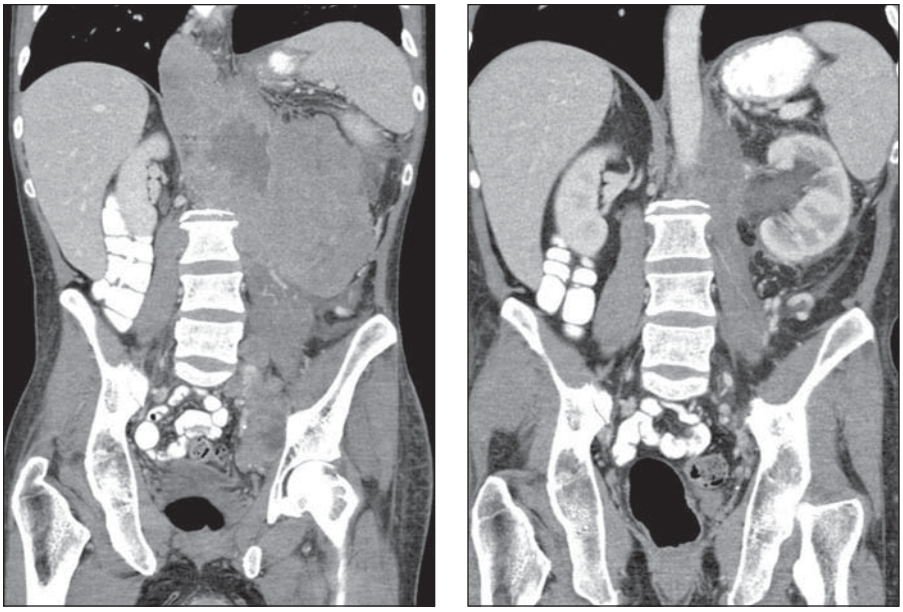


Fig. 2—38-year-old man with favorable subset who presented with midline poorly differentiated carcinoma.
A, Coronal image from initial contrast-enhanced CT shows large heterogeneous retroperitoneal mass with internal necrotic component. Scrotal ultrasound did not reveal any abnormality. Pathology revealed poorly differentiated carcinoma. Serum β-HCG and α-fetoprotein were elevated. On basis of clinicopathologic and imaging findings, patient was treated for extragonadal germ cell tumor.
B, Coronal contrast-enhanced CT after three cycles of platinum-based chemotherapy shows mass has significantly decreased in size.

The remaining majority of patients with CUP often present with widespread metastases to the nodes, liver, lung, brain, bones, or peritoneum (Fig. 3). In those cases, radiologists should suggest the most appropriate biopsy site and recommend further focused imaging or other appropriate tests.

In all patients with CUP, tissue diagnosis is compulsory because adequate tissue specimens provide information on the histologic appearance of the tumor and allow immunohistochemistry [23]. Radiologists are commonly asked to determine the lesion most amenable for biopsy, a selection that is often based on the most superficial target and least invasive method. Radiologists can perform imaging-guided percutaneous biopsies to obviate more invasive procedures in many cases, which are generally accurate, safe, and time-saving [24] (Fig. 4). Solid portions of masses with contrast enhancement on CT or FDG-uptake on PET are regarded as better diagnostic targets. Surgical or endoscopic biopsy may also be suggested on the basis of diagnostic imaging findings, providing our colleagues with information regarding potential best targets and routes of approach. Indeed, the ability of a CT or MRI to detect a primary tumor in the head and neck ranges from 9.3% to 23%, rising to 60% when subsequent endoscopic biopsies are directed at suspicious imaging findings [25].

Another important role of radiologists is to prevent exhaustive, unnecessary, and costly diagnostic workups. Radiologists are consultants to nonradiologist colleagues in selecting the most appropriate imaging test to answer the clinical question posed. Conversations with ordering physicians lead to optimized and efficient imaging tests. Imaging protocol optimization is also important. For example, in CT, arterial phase scanning of the chest and upper abdominal organs (liver, pancreas, and kidneys) and portal venous phase scanning of the abdomen and pelvis provide dual-phase abdominal imaging, which is helpful in detection of hypervascular liver metastases as well as neuroendocrine tumors and renal cell carcinoma [26]. Multiplanar image reconstruction can also enhance the detection of biliary or bowel tumors [27]. If MRI is warranted as a next diagnostic test, the addition of diffusion-weighted MRI to the standard MRI protocol can increase the detection rate of primary sites [28].

It is also of paramount importance to give feedback to oncologists regarding the concordance of imaging, clinical, and immunohistochemical findings (Fig. 4). Sometimes interpretation of immunohistochemistry findings does not fit the clinical and radiologic mani-

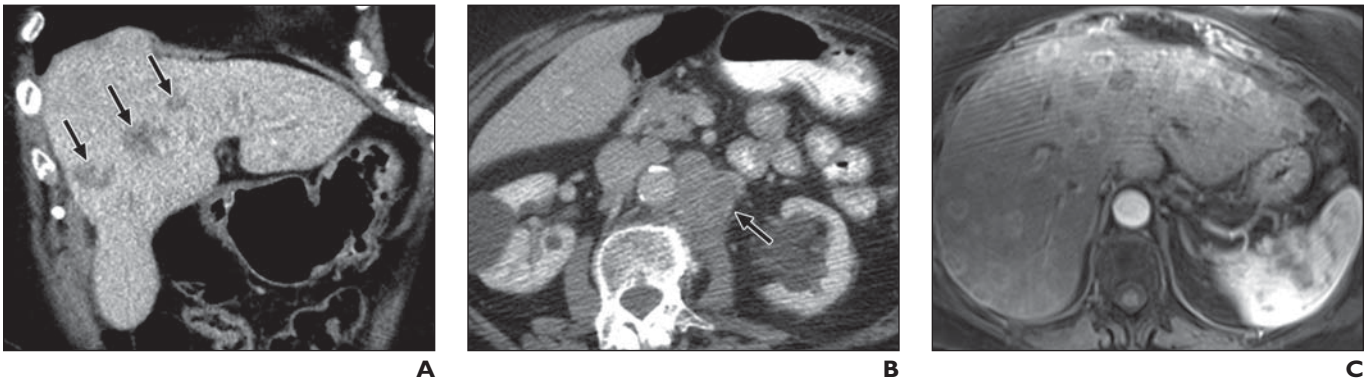


Fig. 3—78-year-old woman with loss of appetite and abdominal pain.
A, Coronal contrast-enhanced CT image shows multiple low-attenuation liver lesions (*arrows*).
B, Axial CT image shows enlarged retroperitoneal lymph nodes (*arrow*). CA125, CA 19–9, and β-HCG were elevated. Pathology from liver lesions revealed metastatic adenocarcinoma with immunohistochemistry positive for CK7, CK20, p53, and SMAD4 (intact) and negative for TTF-1, CDX-2, estrogen receptor, progesterone receptor, hepatocyte, PAX8, napsin, glypican-3, and β-HCG.
C, Contrast-enhanced MR image obtained after 3 weeks because of worsening abdominal pain shows extensive new hepatic metastases. Patient died 2 months later.

festations because of pitfalls, such as inadequate tumor tissue, tissue antigen changes during processing, inappropriate methodology (such as excessive antigen retrieval), misinterpretation, and interobserver variability [10].

Identification of Favorable or Treatable Subsets

One of the most important roles of radiologists is the identification and diagnosis of patients with treatable malignancies or potentially favorable outcomes, guiding additional appropriate workup in this group. Subsets of patients with favorable clinicopathologic features and recommended evaluation in these scenarios are summarized in Table 2. For example, radiologists can confirm the subsets of isolated lymph node metastases in the axilla, cervical, or inguinal areas. In these cases, PET/CT is helpful to exclude distant metastases and enable locoregional therapy. In some unique subsets, such as peritoneal serous papillary carcinomatosis in women or poorly differentiated carcinoma in the midline in young men, radiologists can offer directed differential possibilities [8].

Adenocarcinoma identified in isolated axillary nodes without an apparent primary tumor is a unique favorable subset. Occult breast cancer should be suspected, and further evaluation should be oriented toward finding breast cancer. If mammography or breast ultrasound cannot find a breast mass, dynamic contrast-enhanced breast MRI should be considered [14, 29]. Additional immunohistochemistry can be considered for detection of estrogen-progesterone receptors, gross cystic disease fluid protein-15 (*GCDFP15*), or overexpression of *ERBB2* (formerly *HER2*) [7]. Women presenting with metastatic disease spread typical of breast cancer (axillary nodes, hepatic, bone, and pleural metastases) without an apparent primary breast mass should be examined for a breast primary, and additional immunohistochemistry with the markers listed is recommended [14].

Peritoneal carcinomatosis of unknown primary sites can be caused by metastases from ovarian, gastrointestinal, and breast cancer, among other primary sites. Biopsy from peritoneal deposits of tumor can reveal relatively specific histologic features of serous carcinoma (e.g., papillary architecture, psammoma bodies), which can represent either spread from the ovary–fallopian tube or a peritoneal primary [14]. Clinical features, spread patterns, and pathologic findings of primary peritoneal disease are equivalent to those of ovarian–fallopian tube carcinoma. The radiologic impression on initial CT is crucial to the further management of these patients [22]. Further diagnostic examinations include identifying an elevated level of serum CA125 and immunohistochemistry of the tissue biopsy for detecting expression of estrogen receptors and Wilms tumor-1 (WT1), and paired-box gene-8 (PAX8) immunostaining [7], although, generally, the histologic appearances of this particular tumor type are often sufficiently distinctive and immunohistochemistry is not always required.

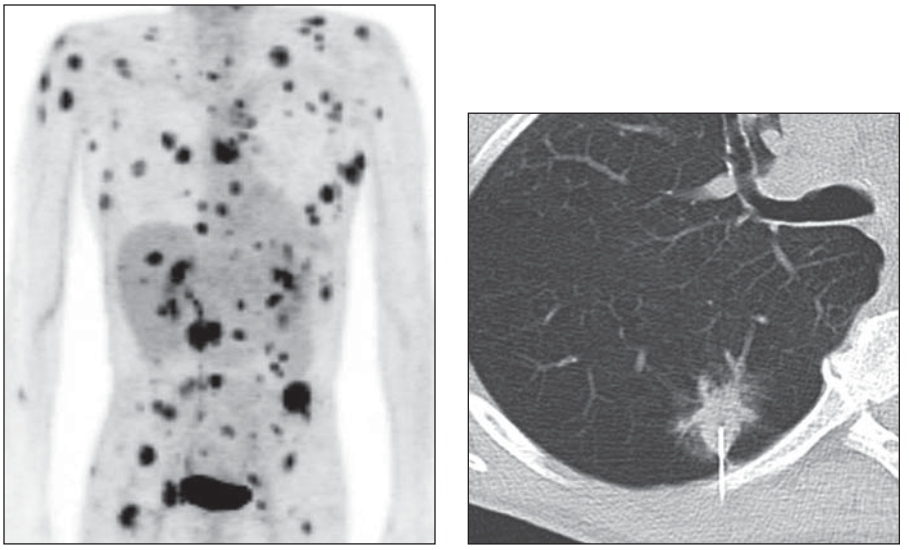


Fig. 4—61-year-old man who presented with skin nodules.
A, Coronal maximum-intensity-projection ^{18}F-FDG PET/CT image shows widespread FDG-avid lesions involving skin, lung, liver, and lymph nodes. Biopsy of skin nodule revealed metastatic adenocarcinoma. Immunohistochemistry showed positive CK7 and SMAD4 and negative CDX2, TTF1, and napsin A. This immunohistochemistry profile is not specific for any primary site.
B, Multiple lung nodules were present. Axial chest CT image shows biopsy of dominant nodule in right lung. Immunohistochemistry of lung mass was positive for CK7, TTF1, and napsin A and negative for CK20, CDX2, and prostate-specific antigen. Finding of CK7-positive/CK20-negative/TTF1-positive is highly specific for adenocarcinoma of lung. Immunohistochemistry profile of skin nodule (TTF-negative) was different from those of lung mass (TTF-positive). This case illustrates that metastatic foci can sometimes have different patterns of protein expression from primary sites, which may lead to misdiagnosis.

Young men with poorly differentiated malignant neoplasms involving predominantly mediastinal or retroperitoneal areas should be suspected of having an extragonadal germ cell tumor or metastasis from a testicular primary (Fig. 2). Extragonadal germ cell tumor affects mostly young men under 50 years old and is clinically characterized by midline distribution, mediastinal-retroperitoneal masses, or lung metastases [30]. Histologic examination in this subset often shows embryonal carcinoma, but other components of a malignant germ cell tumor (e.g., seminoma, yolk sac tumor, teratoma, choriocarcinoma) may also be identified. The radiologic impression on CT of the chest, abdomen, and pelvis is important to raise a clinical suspicion. The differential diagnosis also might include lymphoma, retroperitoneal sarcoma, retroperitoneal fibrosis, and sarcoidosis. Additional immunohistochemistry tests, including β-HCG, α-fetoprotein (AFP), placentalike alkaline phosphatase (PLAP), and octamer-binding transcription factor-4 (OCT4), can aid in the diagnosis [13]. In these patients, an elevated serum level of β-HCG or AFP further supports the diagnosis of extragonadal germ cell tumor.

Role of Serum Tumor Markers

Even though serum epithelial tumor markers in patients with CUP are generally known to be overexpressed in a nonspecific way [7], a panel of tumor markers is often used in the initial evaluation of patients with CUP because they are readily available and sometimes help to narrow the differential diagnosis lists and can be greatly helpful in particular cases. For example, identifying elevated levels of serum β-HCG and AFP in a young man with poorly differentiated carcinoma of midline distribution, serum CA125 in women with primary peritoneal serous adenocarcinomatosis, CA15–3 in women with an isolated axillary node adenocarcinoma metastasis, and prostate-specific antigen (PSA) in men with

TABLE 2: Favorable Subsets in Cancer of Unknown Primary Sites: Further Diagnostic Steps

Subsets	Equivalent Tumor	Recommended Evaluation
Isolated axillary nodal metastasis with adenocarcinoma in women	Breast cancer	Mammography and/or breast MRI/ultrasound, immunohistochemistry stains (estrogen receptor, progesterone receptor, GCDFP15, mammaglobin, *ERBB2*
Isolated cervical nodal metastasis with squamous cell carcinoma	Head and neck cancer	Neck CT and/or MRI, consider PET/CT, panendoscopy, molecular studies (EBV, HPV16)
Squamous cell carcinoma involving inguinal node	Genital and anorectal cancer	Gynecologic examination, anoscopy, cystoscopy, pelvic ultrasound or MRI
Peritoneal carcinomatosis of a serous papillary histology in women	Ovarian cancer	Serum CA125, immunohistochemistry stains (WT1, PAX8)
Poorly differentiated carcinoma in the midline in young men	Extragonadal germ cell tumor	Serum β-HCG and AFP, immunohistochemistry stains (β-HCG, AFP, PLAP, OCT4)
Blastic bone metastases and serum PSA elevation in men	Prostate cancer	Serum PSA, immunohistochemistry stains (PSA)
Metastatic neuroendocrine tumor with unknown primary tumor	Neuroendocrine tumor	Immunohistochemistry stains (chromogranin, synaptophysin), octreotide scanning

Note—GCDFP15 = gross cystic disease fluid protein-15, EBV = Epstein-Barr virus, HPV = human papilloma virus, WT1 = Wilms tumor-1, PAX8 = paired-box gene-8, AFP = α-fetoprotein, PLAP = placentalike alkaline phosphatase, OCT4 = octamer-binding transcription factor-4, PSA = prostate-specific antigen.

blastic bone metastases can give important clues to identify primary tumors [7] (Table 2) and thus guide therapies.

Epithelial tumor markers, including carcinoembryonic antigen (CEA), CA19–9, and CA125, are frequently obtained in patients with widespread metastatic disease. CEA is not a specific marker and is present in the majority of epithelial tumors but shows high sensitivity because a high serum CEA level is almost always indicative of epithelial tumors [31]. Instead, CEA is very helpful for monitoring treatment response [32]. The combination of CA19–9 and CA125 is sometimes helpful for predicting primary sites, such as gynecologic and pancreatobiliary cancers, even though both markers can be elevated in advanced metastatic disease. If CA19–9 is highly increased and CA125 is negative (or mildly increased), the results further support the diagnosis of pancreatic cancer rather than gynecologic cancer [33]. In cases of advanced gynecologic cancers, such as ovarian cancer and endometrial cancer, serum levels of both CA19–9 and CA125 are generally significantly increased [34]. These markers are also useful for monitoring treatment response.

TABLE 3: Stepwise Approach to Immunohistochemistry

Steps	Diagnosis	Useful Immunohistochemistry Markers
1: Cell lineage	Carcinoma	Pancytokeratin, such as AE1/AE3
	Lymphoma	LCA
	Melanoma	S100; HMB45
	Sarcoma	Pancytokeratin-negative and LCA-negative
2: Subtype of carcinoma	Squamous cell carcinoma	CK5/CK6, p63
	Germ cell tumor	PLAP, OCT4, AFP, β-HCG
	Neuroendocrine tumor	Chromogranin, synaptophysin
	Hepatocellular carcinoma	HEPPAR1, canalicular pCEA/CD10/CD13
	RCC	RCC, CD10, PAX8
	Thyroid carcinoma	TTF1, thyroglobulin
	Adenocarcinoma	CK7 or CK20
3: Primary site of adenocarcinoma	Colorectum	CDX2 (with CK7-negative, CK20-positive)
	Lung	TTF1, napsin A (with CK7-positive, CK20-negative)
	Pancreatobiliary	CDX2; loss of SMAD4 (with CK7-positive, CK20-positive)
	Breast	GCDFP15, mammaglobin, estrogen receptor (with CA125-negative)
	Ovary	Estrogen receptor, CA-125, WT1, PAX8
	Prostate	PSA, PrAP

Note—LCA = leukocyte common antigen, HMB45 = human melanoma black-45, PLAP = placentalike alkaline phosphatase, OCT4 = octamer-binding transcription factor-4, AFP = α-fetoprotein, HEPPAR1 = hepatocyte paraffin-1, RCC = renal cell carcinoma, pCEA = polyclonal carcinoembryonic antigen, PAX8 = paired-box gene-8, TTF1 = thyroid transcript factor-1, CDX2 = caudal type homeobox transcription factor-2, GCDFP15 = gross cystic disease fluid protein-15, WT1 = Wilms tumor-1, PSA = prostate-specific antigen, PrAP = prostatic acid phosphatase.

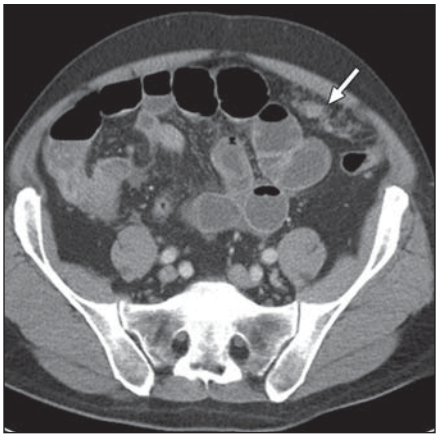
Role of Immunohistochemistry

In the diagnostic approach of patients with CUP, clinical and radiologic features can give a big picture or work as a road map to further steps, and pathology with immunohistochemistry can give the answer at each step and provide the final diagnosis. A panel of immunohistochemistry stains should be interpreted together with the morphologic characteristics and clinical presentation.

The stepwise approach of immunohistochemistry is summarized in Table 3. The immunohistochemistry goes through a systematic approach as follows: The first step (basic immunohistochemistry panel) defines the cancer cell lineage, whether carcinoma, melanoma, lymphoma, or sarcoma. The initial panel of immunohistochemistry antibodies would typically include a marker for lymphoma (leukocyte common antigen [*LCA*]), a marker for melanoma (*S100*), and a marker for carcinoma (broad-spectrum cytokeratins, such as *AE1/AE3*). Of note, although vimentin is often used as a marker for sarcoma, this antigen is not specific for mesenchymal lineages and may occasionally be detected in carcinomas and melanomas. There is no general marker of sarcoma that is useful in an initial broad panel; the lack of staining for cytokeratins and *LCA* might suggest sarcoma.

The second step identifies the subtype of carcinomas such as squamous cell carcinoma, adenocarcinoma, solid carcinomas (liver or renal cancers), or neuroendocrine tumors and germ cell tumors that may resemble carcinomas [11]. Squamous cell carcinomas, adenocarcinomas, and neuroendocrine tumors usually show distinctive features on standard H and E staining, but poorly differentiated tumors (in each of these groups) may be challenging, and immunohistochemistry markers are helpful to arrive at a definitive diagnosis (e.g., *CK5/6* and *p63* for squamous cell carcinoma, chromogranin and synaptophysin for neuroendocrine tumors). For germ cell tumors, PLAP is a highly sensitive (although not entirely specific) marker; more recently developed embryonic stem cell transcription factor markers, such as OCT-4, are much more specific for germ cell tumors. Monoclonal antibodies to specific cytokeratin subtypes have been used in an attempt to classify carcinomas according to the site of origin. The two most common cytokeratin stains used for CUP are CK7 and CK20, and the combination of CK7 and CK20 immunohistochemistry profiling has been helpful to identify primary tumor sites, although these markers alone are not specific [23]. For example, a CK7-negative/CK20-positive phenotype is often associated with carcinomas of colorectal origin, whereas a CK7-positive/CK20-negative phenotype is seen in a wide variety of carcinomas, including carcinomas of the lung, breast, thyroid, pancreas, and female genital tract.

The third step predicts the primary site of adenocarcinoma from the immunohistochemistry staining pattern, which enables site-specific treatment. Adenocarcinomas frequently show an immunohistochemistry staining pattern highly suggestive of a single primary site on the basis of a combination of markers, such as CK7, CK20, CDX2, TTF1, PAX8, and breast or ovarian markers (estrogen receptor, progesterone receptor, mammaglobin, and GCDFP15). There are several particularly important stains, such as GCDFP15 and mammaglobin for breast cancer, TTF1 for lung cancer (with CK7-positive, CK20-negative) (Fig. 4) CDX2 for colorectal cancer (with CK7-negative, CK20-positive) (Fig. 5), and WT1 and PAX8 for ovarian cancer [11].



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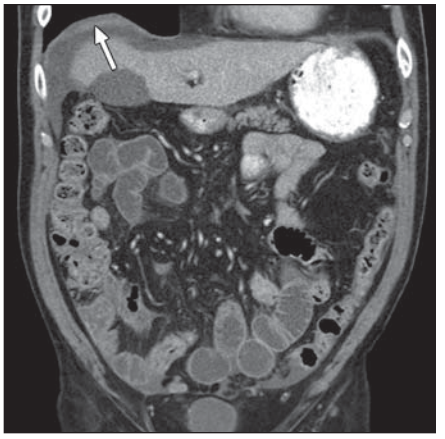

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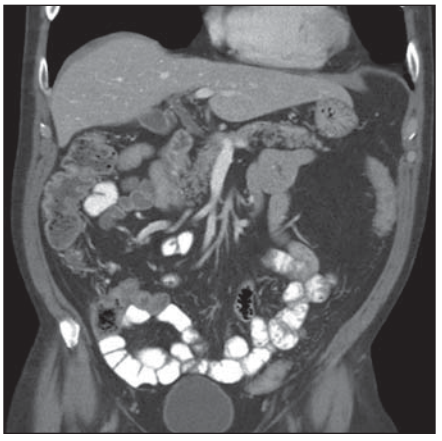

C

Fig. 5—72-year-old man with abdominal pain. **A** and **B,** Axial (**A**) and coronal (**B**) images from initial contrast-enhanced CT show diffuse peritoneal thickening and nodularity (*arrow*) as well as ascites, suggestive of peritoneal carcinomatosis. Multiple dilated small bowel loops are present, likely secondary to serosal disease. Colonoscopy was negative, and multiple blind colonic biopsies revealed normal colonic mucosa. Diagnostic paracentesis and fine-needle aspiration of peritoneal nodules were performed. Immunohistochemistry was CK20-positive, CK7-negative, and CDX2-positive, which is highly suggestive of colorectal cancer. Patient responded well to combination of colon cancer chemotherapy and targeted agent (FOLFIRI [folinic acid, 5-fluorouracil, and irinotecan] and bevacizumab).
C, Coronal image from contrast-enhanced CT after 13 cycles of treatment shows no evidence of disease.

Role of Molecular Profiling

Molecular profiling methods have been developing rapidly in various platforms, such as reverse transcriptase polymerase chain reaction, cDNA microarray, and microRNA profiling. The goal of these different molecular profiling methods is the same: to predict the primary site of CUP thereby allowing site-specific therapy. There are several commercial kits for identification of tissue of origin, with accuracy rates of 33–93% [7]. According to a recent study, use of the 10-gene CUP assay could identify a tissue of origin in 61% of patients with CUP [35]. Because increasingly specific immunohistochemistry markers continue to be developed and such gene assays are usually based on algorithms developed using relatively well-differentiated examples of tumor types, it is debatable whether these gene assays provide useful information for poorly differentiated tumors beyond a well-designed immunohistochemistry panel. Another important application of molecular testing is to identify specific gene mutations such as EGFR, K-ras, *ERB2*, and

ALK alterations, which are targets of new molecular agents [7]. In the near future, it seems likely that molecular profiling will become an important modality for patients with CUP, particularly to identify specific gene mutations for targeted chemotherapeutic agents.

Treatment of Cancer of Unknown Primary Site Based on Workup Findings

The management of patients who are confirmed to have favorable subsets or treatable types of CUP after the stepwise diagnostic approach should follow specific guidelines that are based on site-specific therapy or treatment guidelines of metastatic cancer with a known primary tumor. Responses and survival are similar to those of patients with relevant known primary tumors. Patients in unfavorable subsets are treated with empirical chemotherapy based on combination regimens of platinum or taxane, but responses and survival are generally poor [7].

As individualized treatment has emerged as an important concept, several studies have been performed or are ongoing to validate the site-specific therapy on the basis of immunohistochemistry results or molecular profiling. In 2008, CUP patients with an immunohistochemistry profile of colon cancer (CK20-positive/CK7-negative/CDX2-positive) were treated with colon cancer–specific chemotherapy regimens rather than empirical therapy. These patients showed responses and survival similar to those with known advanced colorectal cancer [35], which are better than the outcome of empirical chemotherapy to CUP (Fig. 5).

Regarding molecular targeted therapy for treatment of CUP, there have been only two studies using bevacizumab and erlotinib (alone or combined with paclitaxel and carboplatin) [36, 37]. However, the patients in these studies were not selected by the profiles of target expression, and further investigation of targeted therapy based on expressed targets in patients with CUP is warranted.

Treatment Response Assessment to Therapy

Early treatment response assessment to therapy is also an important role of imaging. Tailored targeted therapy to patients with CUP has been increasing because of recent advances in molecular profiling and new drug development. Many targeted therapeutic agents are cytostatic rather than cytotoxic. Hence, tumor size changes may not constitute appropriate criteria for the early treatment assessment [38]. Therefore, response assessment in patients treated with targeted agents should include appraisal for reduced vascularity or tumor density on conventional CT or MRI as well as parameter changes in functional imaging, such as perfusion CT or MRI, diffusion-weighted MRI, or FDG PET.

Summary

The diagnosis and management of patients with CUP has evolved and will continue to change. Currently, individualized therapy, especially the use of targeted therapeutic agents with or without conventional chemotherapy, has dramatically improved the overall survival of patients with advanced colorectal, lung, pancreatic, breast, ovary, renal, and hepatocellular carcinomas over the past decade. These improvements in care compel us to identify sites of primary disease when possible, a task much more important and sophisticated now than a decade ago. Radiologists, in collaboration with oncologists and pathologists, should adopt a multidisciplinary and integrative stepwise diagnostic approach, considering imaging and clinicopathologic data, for optimized focused care of patients with CUP.

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