REVIEW

Available from: www.jgld.ro/wp/archive/y2017/n1/a13
DOI: http://dx.doi.org/10.15403/jgld.2014.1121.261.haz

How to Diagnose and Treat a Cancer of Unknown Primary Site

Ciprian Tomuleasa[1,2,3*], Florin Zaharie[4,5*], Mihai-Stefan Muresan[4,6], Laura Pop[2], Zsolt Fekete[7,8], Delia Dima[3], Ioana Frinc[1,3], Adrian Trifa[3,9], Cristian Berce[10], Ancuta Jurj[2], Ioana Berindan-Neagoe[2], Mihnea Zdrenghea[1,3], Tudor-Eliade Ciuleanu[7,11]

Departments of 1) Hematology, 2) Research Center for Functional Genomics and Translational Medicine, 4) Surgery, 7) Radiotherapy/Oncology, 9) Genetics, and 10) Animal Facility; Iuliu Hatieganu University of Medicine and Pharmacy;
Departments of 3) Hematology, 6) Surgery, 8) Radiotherapy and 11) Chemotherapy, Ion Chiricuta Oncology Institute; 5) Department of Surgery, Octavian Fodor Regional Institute of Gastroenterology and Hepatology,
Cluj Napoca, Romania.

Address for correspondence:
Dr. **Ciprian Tomuleasa**
Departments of Hematology /Research Center for Functional Genomics and Translational Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Gheorghe Marinescu Str.23, 400014, Cluj Napoca, Romania.
ciprian.tomuleasa@umfcluj.ro

Received: 21.11.2016
Accepted: 07.01.2017

* contributed equally to this manuscript

ABSTRACT

Almost one in every three patients with advanced tumors have distant metastasis at the time of clinical diagnosis. In most cases, the primary tumor site is identified immediately, within a few days. But for some patients, the primary lesion cannot be found after the initial clinical assessment. These cases are called cancers of unknown primary origin (CUPs), a clinical diagnosis very difficult to manage by physicians due to the absence of a standard-of-care for the initial therapeutic regimen, as well as due to the impossibility to include these cases in randomized clinical trials. A cancer of unknown primary site is often associated with a poor prognosis as patients are usually treated with a non-selective empirical therapy. In the current paper, we summarize both the diagnostic challenges for patients with a cancer of unknown primary site as well as the current available therapeutic options, with emphasis on the management of this unique disease entity.

Key words: cancers of unknown primary site – differential diagnosis – clinical management.

Abbreviations: AFP: α-fetoprotein; AR: androgen receptor; Bcl 2: B-cell chronic lymphocytic leukemia/lymphoma 2; BM: basement membrane; HPV: human papilloma virus; CD: cluster of differentiation; CEA: carcinoembryonic antigen; CK: cytokeratin; CTC: circulating tumor cell; DTC: disseminated tumor cell; FISH: fluorescence in situ hybridization; GI: gastrointestinal; IHC: immunohistochemistry; MPO: myeloperoxidase; MSA: muscle-specific actin; OCT4: octamer-binding transcription factor 4; p504S: α-methylacyl-CoA racemase; PS: performance status; PSA: prostate-specific antigen; S100P: placental S100 protein; WT1: Wilms tumor 1.

INTRODUCTION

Almost one in every three patients with cancer has distant metastases at the time of clinical diagnosis. In most cases, the primary tumor and the metastases are identified concomitantly, but for some patients, the primary lesion cannot be found after the initial clinical assessment. In these cases, the diagnosis of cancer of unknown primary (CUP) is made, a clinical situation quite difficult to manage due to the absence of a standard-of-care for the initial therapeutic approach, as well as due to the impossibility to include these cases in randomized clinical trials [1-3]. Cancers of unknown primary (CUPs) represent up to 150,000 new cases diagnosed each year in the United States and the European Union, but the number may increase to 400,000 [4, 5]. In order to pinpoint the organ and tissue of origin, a complex assessment is carried out which includes patient history, physical examination, serum markers, histological examination including various tissue and cancer specific antibodies, as well as state-of-the-art imaging techniques. The physical examination includes palpation of breasts, and genitourinary and rectal examination. The first radiological tests are computer tomography (CT) and/or magnetic resonance imaging (MRI) of several body segments, with mammography when there is suspicion of a breast primary. If these are inconclusive, the next step is a combined positron emission tomography (PET) and CT (PET-CT). Immunohistochemical markers are the most important diagnostic tools in establishing tissue origin [6-12]. Usually peroxidase-labeled antibodies against various tumor-specific antigens determine the origin of a malignant cell, the most widely used being monoclonal antibodies against cytokeratin (CK) intermediate filaments, all of which have different

molecular weights or levels of expression for each type of cell. Communication between the pathology department and the clinic is crucial. Sometimes the expressed markers are non-specific [13-15].

A CUP is often associated with a poor prognosis as patients are usually treated with a non-selective empirical therapy. In the current review, we summarize both the diagnostic challenges for patients with a CUP as well as the current available therapeutic options.

THE BIOLOGY OF THE METASTATIC PROCESS

The metastatic process represents the dissemination, survival and multiplication of malignant cells originating from the primary tumor in distant anatomical sites. Cancers with unknown primary site per se are considered metastatic tumors at the moment of diagnosis. The route of a cancer cell's migration can be hematologic, lymphatic, intraperitoneal, intrapleural or through the cerebrospinal fluid. Metastasis is a cascade of events where malignant cells from the primary tumor first invade the basement membrane and the surrounding stromal tissue until they reach a blood or lymphatic vessel, where they are carried away with the circulation. In the bloodstream they become circulating tumor cells (CTCs) and the ones who survive and reach distant organs become disseminated tumor cells (DTCs) that have the potential to form secondary tumors [16-18].

In the local invasion phase, cells invade the surrounding tissue either as a group, a process termed "collective invasion", or as individual cells, a process named "single-cell invasion" [19-21]. In the latter case, in order to break the basement membrane (BM) cells may undergo epithelial to mesenchymal transition (EMT) [22]. This is a process driven by pleiotropic transcription factors (Twist, Snail, ZEB 1 and 2) which support epithelial cells to enter a mesenchymal state. After dissolving the BM, tumor cells reach the stroma where their malignant aggressiveness is influenced by a variety of tumor associated stroma cells that are characteristic for each state of the tumor progression. Tumor cells that invade the stroma encounter fibroblasts, endothelial cells, adiposities, macrophages and other cells of the immune system [23-25]. The intravasation takes place in the microvessels, by crossing the pericyte and endothelial cell barrier. Malignant tumors stimulate neoangiogenesis and form capillaries in which endothelial cells interact weakly and have low pericyte coverage of the walls. This process makes it easier for tumor cells to enter the circulation [26]. Tumor neoangiogenesis and a weak junction between endothelial cells are regulated by vascular endothelial growth factor (VEGF), cyclooxygenase-2 (COX-2), MMP1 and 2 [24], as seen in Fig. 1. Intravasation of mammary tumor cells was shown by Giampieri et al. to be determined by the transforming growth factor-β (TGF-β) that facilitates malignant cell penetration of the microvessel wall [27].

Once cancer cells reach circulation they have to resist the hemodynamic stress and the host's immune system. They do so by forming tumor cell clusters and co-opting platelets, basically coating themselves for protection [23]. The site where a CTC can stop is usually determined by the patterns of the blood circulation in the body. Once entrapped, CTCs either enter a latency stage that can last from months to decades or start colonizing the new location immediately. The mechanisms that trigger either of these stages are not yet well understood. From the microvessel in which they become entrapped tumor cells can extravasate by growing intraluminally, rupturing the walls of the microvessel and getting in direct contact with organ parenchyma, or individual cells can pass through the windows of endothelium and pericytes [28].

The environment where tumor cells become entrapped differs from that of the primary site. A theory through which DTCs adapt to the new environment hypothesizes that tumor cells create a premetastatic niche by modifying the environment to better suit their needs [29, 30]. Disseminated tumor cells can form micrometastases that do or do not have the potency of becoming macroscopic metastases. Actually, the majority of tumor cells either regress or remain dormant in the host tissue, which together with the destruction process in

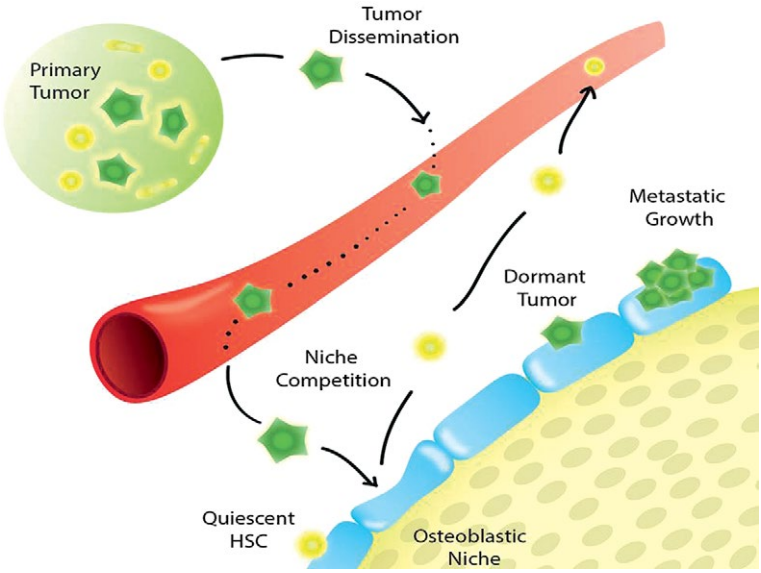

Fig. 1. Cellular mechanisms of metastasis in cancers of an unknown primary site

the blood stream makes the process of metastatic colonization inefficient.

As previously stated, in the clinical management of most CUPs, the origin of the primary tumor cannot be identified, even in an autopsy setting [31, 32]. For such situations, several hypotheses have been stipulated in order to try to explain and understand the molecular basis that facilitates the development of the metastatic niche [33-36]. One such hypothesis might emerge from the pathological findings described for testicular cancer, where testis scarring can be found associated to metastatic germ cell cancers; potential explanations concern regression or involution of the primary tumor (*burned-out primary* theory). Furthermore, one other theory describes an *in utero* failure of migration of fully-differentiated embryonic rest cells (extragonadal germ cells), thus explaining the phenomenon of finding such tumors in the retroperitoneum, mediastinum or in the inguinal canal (i.e undescended testis) [33]. One other theory suggests that if a genetic lesion impacts all cells of a the germ-line, CUPs can occur, as seen in monozygotic twins with primary immunodeficiency disorders (X-linked hyperimmunoglobulin M syndrome) [37]. One last theory which has attempted to explain the biology of CUPs is the adult stem cell theory [34-36]. This theory states that some CUPs might arise from adult stem cells with a multiple lineage differentiation capacity. A wide reserve of precursor stem cells is still located in the connective tissue after birth. In order to support this theory, some studies [34] have proved that hematopoietic stem cells have the capacity to differentiate into liver, muscle, skin or even gastrointestinal cells, which can suffer neoplastic transformation and become primary tumors.

CLINICAL EVALUATION

A cancer of primary unknown site is a clinical diagnosis. It usually presents as metastatic disease and its origin is found only in a limited number of cases. A CUP has several very important characteristics, which differentiate it from any other cancer. These cancers have a short history of symptoms, have a very early metastatic disease in the absence of an easily identifiable tumor mass. The clinical natural evolution of a cancer of unknown primary site is very aggressive and often unpredictable, as depicted in Fig. 2. Patients present to the hospital with three or even more organs being involved early in the course of the disease, and metastases having sometimes a different pattern when compared with metastatic cancer of a known origin (Fig. 3). In more than 75% of cases, the primary tumor is found post-mortem at autopsy [38]. In such cases, the clinical evaluation should be thorough and systematic as to predict the T0 and guide the site-specific treatment.

The minimal diagnostic work-up always starts with a complete medical history of the patient and accurate physical examination, followed by basic blood and biochemistry tests, urinalysis, fecal occult blood testing and by radiography of the suspected site of origin [39]. If the primary site is still not identified and if the patient has no contraindications, a baseline intravenous contrast CT scan should be performed for the chest, abdomen and pelvis [40] (Fig. 4). In men, the prostate-specific antigen (PSA) test might be contributory. Women must undergo mammography and optionally a vaginal

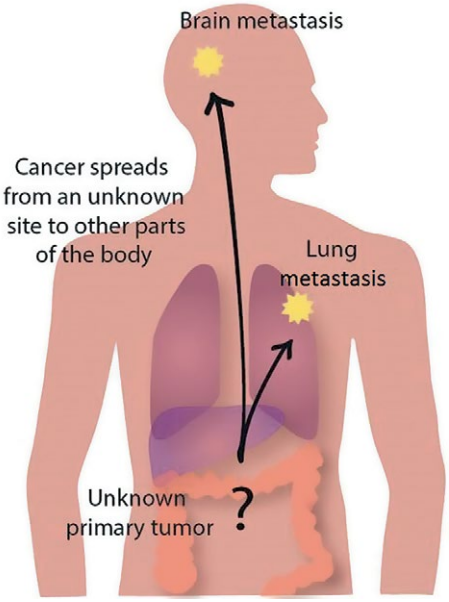

Fig. 2. Distant metastasis pattern for cancers of an unknown primary site.

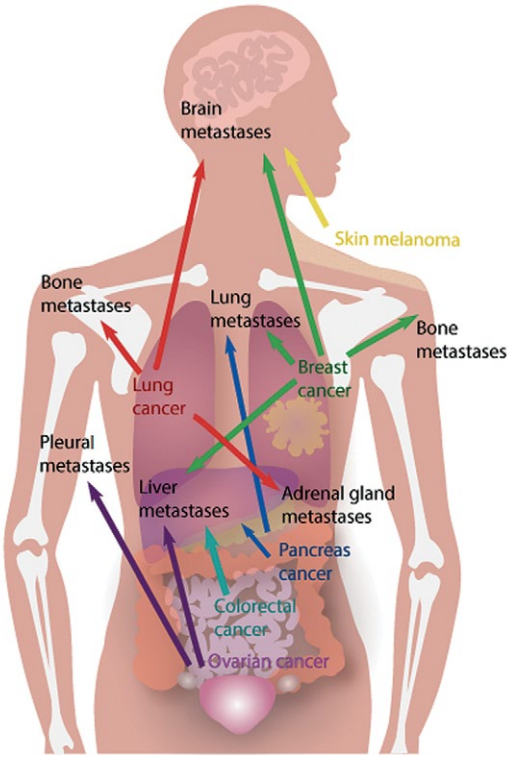

Fig. 3. Multiple sites of metastasis for cancers of an unknown primary site.

ultrasonography. For women with axillary lymphadenopathy and negative mammography, MRI of the breasts might provide higher sensitivity for diagnosing a breast primary. Mastectomy after a negative breast MRI is very unlikely to be useful [41]. For patients with suggestive symptoms, organ specific endoscopies and imaging are performed.

Up to this day, the usefulness of a PET-CT is not clearly defined and physicians have sometimes different opinions regarding the use of this technology for the identification of the primary site of a malignancy. Still available data shows that

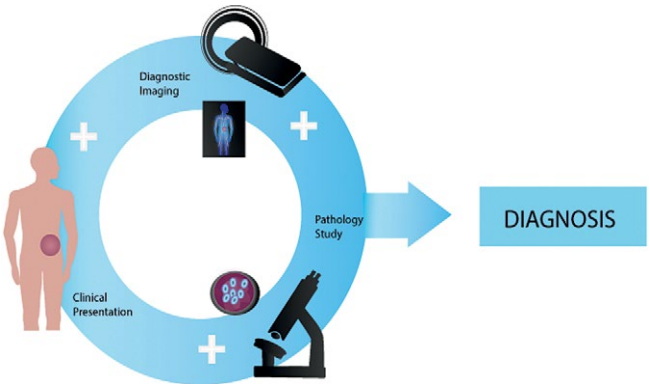

Fig. 4. Diagnosis of cancers of an unknown primary site

a PET-CT identifies correctly the origin of a CUP better than MRI (22-44 % vs. 20-27%) [42, 43], but the cost effectiveness of using it has yet to be proven, preventing it from being considered a standard-of-care. A PET-CT is considered to be of utmost importance in the evaluation of certain anatomical localizations such as the head and neck and the investigation of cervical lymphadenopathy [44]. The diagnosis of the primary is not the only important aspect of using a PET-CT for cervical squamous cell carcinoma, but also for evaluation for loco-regional therapy with curative intent [45]. In case of metastatic neuroendocrine tumors ^{68}Ga-DOTA-NOC receptor PET-CT has been proven to be superior both to classic CT and MRI, as well as to OctreoScan [50].

Serum markers are complementary to the clinical examination and together with immunohistochemistry (IHC) staining and molecular biology play an important role in guiding the clinical towards a proper diagnosis. Serum carcinoembryonic antigen (CEA) is not a very specific protein marker, as it is elevated mostly in cancers of epithelial origin; it has been proven useful in the follow-up of such malignancies. Prostate specific antigen (PSA) may lead to a clear diagnosis of an occult prostate cancer whereas women with an elevated CA-125 might have ovarian cancer. Elevated levels of β-human chorionic gonadotropin (β-HCG) and α-fetoprotein (AFP) in a man with a mediastinal mass or with malignant masses in the retroperitoneum may be suggestive for an extragonadal germ-cell tumor [71].

PATHOLOGY AND GENETIC DIAGNOSTIC TESTS

The diagnosis of CUPs is mainly done by immunohistopathology, but also the medical history of the patient is very important. CUPs are mainly diagnosed from the biopsy, which is firstly stained with hematoxylin–eosin in order to determine the type of cancer. Most of these cancers are adenocarcinomas (60%) or poorly differentiated or undifferentiated carcinomas (30-35%). There could be also other types of cancers such as squamous cell carcinomas (5%) or neuroendocrine cancers (2%) [52] (Fig. 5). After establishing the type of cancers it is important to identify the tumor lineage, which is done by an IHC test. The diagnostic algorithm of the pathology department starts with a joint IHC staining for cytokeratins (CK) 7 and 20, for a rough differential diagnosis. CK7 and CK20 are the most common IHC stainings, with CK20 being positive for gastrointestinal (GI) and urothelial carcinomas and CK7 positive for lung, endometrial, breast, ovarian or thyroid carcinomas [53-55]. Of special interest is staining for CK5/6 in lung carcinomas, where the adenocarcinoma is negative for CK5/6 and the squamous cell carcinomas is positive for the very same staining. Afterwards, highly specific stainings and assays are performed (Table I) [56].

Even though there are many biomarkers that can be used for the identification of the primary site of CUPs by IHC, this task is still very difficult, mainly due to the fact that this technology is an interpretative and subjective technique. But also because the CUPs specimens are small and you cannot use a large scale of biomarkers to test and there are tumors that present the same biomarkers. To overcome this problem the molecular profiling of CUPs tumors was developed and proved to be feasible for

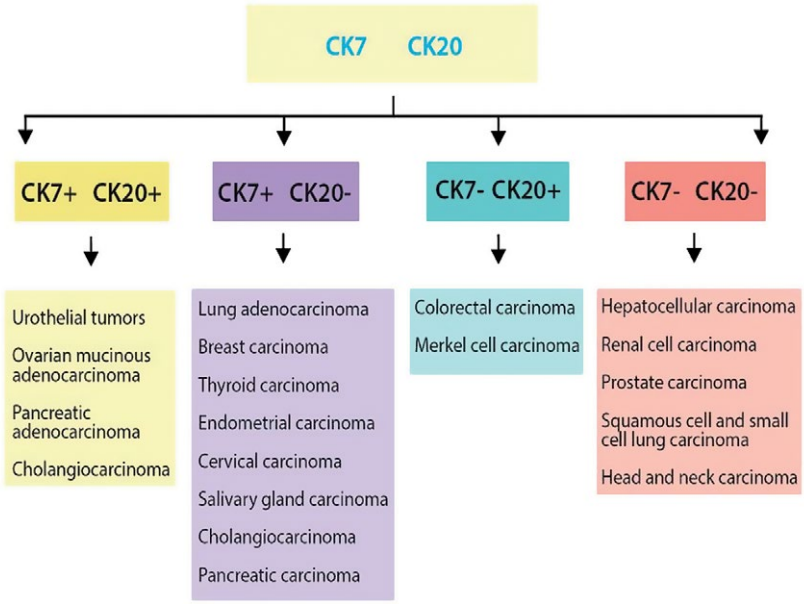

Fig. 5. Pathology staining algorithm for cancers of an unknown primary site.

Table I. Important biomarkers used for the differentiation of the tumor lineage in CUPs.

Primary site	Biomarkers
Adrenal cortical neoplasm	Mart-1, inhibin-α, calretinin, SF-1
Alveolar soft part sarcoma	TFE3
Angiomyolipoma	HMB-45, SMA
Atypical lipomatous tumor	MDM2, CD45
Breast carcinoma	GATA3, ERM GCDFP-15, TFF1, MGB
Chordoma	Cytokeratin, S100
Choriocarcinoma	β-HCG, CD10
Desmoplastic small round cell tumor	Cytokeratin, CD99, desmin, WT1
Embryonal Carcinoma	SALL4, LIN28, OCT4, NANOG, CD30, SOX2
Endocervical adenocarcinoma	PAX8, p16, CEA, HPV ISH, loss of PAX2
Endomerial carcinoma	PAX8/PAX2, ER, vimentin
Endometrial stromal sarcoma	CD10, ER
Epithelioid sarcoma	CD34, loss of INI1
Ewing sarcoma/PNET	CD99, Fli-1, NKX2-2
Follicular dendritic cell tumor	CD21, CD35
Gastrointestinal stromal tumor	CD117, DOG1
Lower gastrointestinal tract tumor	CDH17, SATB2, CDX2, CK20
Upper gastrointestinal tract	CDH17, CDX2, CK20
Hepatocellular carcinoma	ARG1, glypicn-3, HepPar-1, AFP
Histiocytosis X	CD1a, S100
Hyaline trabecular adenoma of the thyroid	MIB-1
Intrahepatic cholangiocarcinoma	pVHL, CAIX
Low-grade fibromyxoid sarcoma	MUC4
Lung adenocarcinoma	TTF1, napsin A
Mast cell tumor	CD117, tryptase
Melanoma	S100, mart-1, HMB-45, MiTF, SOX10, PNL2
Merkel cell carcinoma	CD20, MCPyV
Mesothelial origin	Calretinin, WT1, D2-40, CK5/6, mesothelin
Myeloid sarcoma	CD43, CD34, MPO
Myoepithelial carcinoma	Cytokeratin and myoepithelial markers, may loss INI1
Myxoid and round cell liposarcoma	NY-ESO-1
Neuroendocrine origin	Chromogranin, synaptophysin, CD56
Ovarian clear cell carcinoma	pVHL, HNF-1, KIM-1, PAX8
Ovarian serous carcinoma	PAX 8, ER, WT1
Pancreatic, acinal cell carcinoma	Glypican-3, antitrypsin
Pancreatic ductal adenocarcinoma	MUN5AC, CK17, mapsin, S100P, IMP3
Pancreatic neuroendocrine tumor	PR, PAX8, PDX1, CDH17, islet-1
Pancreatic solid pseudopapillary tumor	Nuclear β-catenin, loss of E-cadherin, PR, CD10, vimentin
Papillary RCC	P504S, RCCma, pVHL, CD10, PAX8, KIM-1
Prostate adenocarcinoma	PSA, PSAP, ERG, NKX3.1
RCC clear cell	PAX8/PAX2, RCCma, pVHL, CD10, KIM-1
Rhabdomyosarcoma	Myogenin, desmin, MyoD1
Salivary duct carcinoma	GATA3, AR, GCDFP-15, Her-2/neu
Seminoma	SALL4, LIN28, OCT4, CD117, D2-40
Sex cord stromal tumors	SF-1, inhibin-1, calretinin, FOXL2
Smooth muscle tumor	SMA, MSA, desmin, calponin
Solitary fibrous tumor	CD34, BCL2, CD99
Squamous cell carcinoma	P40, CK5/6, p63, SOX2, desmocollin-3
Synovial sarcoma	TLE1, cytokeratin
Thymic origin	PAX8, p63, CD5
Thyroid follicular cell origin	TTF1, PAX8, thyroglobulin
Thyroid medullary carcinoma	Calcitonin, TTF1, CEA
Translocational RCC	TFE3
Urothelial carcinoma	GATA3, UPII/UPIII, S100P, CK5/6, CK903, p63, CK20
Vascular tumor	ERG, CD31, CD34, Fli-1
Yolk sac tumor	SALL4, LIN28, glypican-3, AFP

diagnosis of CUPs. The molecular diagnosis of CUPs is based on the evaluation of the messenger RNA (mRNA), microRNAs (miRNAs), DNA or epigenetics. Commercially available tests are based on the evaluation of the mRNA and miRNA expression. Such tests include the Pathwork Tissue of Origin (TOO) [57], biTheranostics Cancer type ID (CTID) [58] or the miRview mets2 [59]. These assays can be used for formalin-fixed paraffin embedded (FFPE) tissue or cytology specimens, and they require a small amount of tissue in comparison to IHC. When comparing the results of these three tests with the IHC results TOO showed a sensitivity of 87-94%, CTID 72-95% sensitivity and miRview 82-90% sensitivity in identifying the primary site of the CUPs. Even though the molecular tests show good sensitivity there are also some disadvantages. In 10% of the cases the diagnosis is not possible due to the poor quality of RNA and in some cases the test presents some difficulties. Such, TOO is not ideal for sarcoma and CTID is not really feasible for pancreatic, colorectal and gastroesophageal cancers. Another important disadvantage of the molecular test is the cost which can reach 3000-4000 US dollars, whereas IHC can cost up to 100-200 US dollars [8].

Another important test for identification of the primary origin of a squamous cell carcinoma of unknown primary was the evaluation of human papilloma virus (HPV) infection in the metastatic neck lesion. It was observed that the HPV infection status was a strong predictor of a primary cancer of the oropharynx [60]. Over the years, several other molecular tests have been developed and comparison studies have showed that these tests are better used as complementary test to the IHC panels and that they are better employed in cases where the IHC

Table II. All the molecular diagnosis tests commercially available.

Assay	Platform	Tissue	Number of genes	Accuracy
Veridex	RT-PCR mRNA	FFPE	10	76 %
Pathwork diagnostics	cDNA microarray	Frozen/FFPE	1500	89 %
Rosetta genomics MiRview mets-2	Microarray miRNA	FFPE	64 miRNAs	92 %
BioTheranostics CancerType ID	RT-PCR mRNA	FFPE	92	86 %
CupPrint	Microarray miRNA	FFPE	495 miRNAs	85 %

FFPE: formalin-fixed paraffin embedded

panel testing is inconclusive. The commercially available panels up to date for the diagnosis of CUPs are presented in Table II. All these tests are based on the gene and miRNA expression evaluation by RT-PCR or microarray [61].

Due to the fact that CUPs diagnosis is very difficult, mainly because of the unknown primary site, which is very important in the identification of the optimal treatment options, both IHC and molecular methods of diagnosis should be employed and used in a complementary manner. The IHC panel testing has improved during the years but still there are difficult cases where this technique is inconclusive. This is where molecular testing has demonstrated promising results and has been able to help clinicians decipher the primary site of CUPs and prescribe the optimal therapeutical option. Two randomized studies that evaluated the efficacy of molecular profiling-based treatment in metastatic cancers are currently recruiting patients (NCT01827384 and NCT02152254). Still, until now, scientists cannot decide whether a CUP with a molecular signature of a specific primary behaves similarly to a typical metastatic cancer [62].

The differential diagnosis of the origin of a carcinoma usually includes the lung, breast, kidney, ovary, uterus, upper GI tract, pancreatobiliary tract, urinary tract, thyroid, prostate, liver, and adrenal gland. Numerous biomarkers have been studied and suggested as useful for that purpose and include CK7, CK20, estrogen receptor (ER), mammaglobin (MGB), thyroid transcription factor 1 (TTF1), uroplakin, napsin A, caudal type homeobox 2 (CDX2), Wilms tumor 1 (WT1), OCT4 (octamer-binding transcription factor 4), and paired box gene (PAX) 8.

Thyroid transcription factor-1 (TTF-1), surfactant apoprotein (PE-10), CK7 and CK20 are useful in the differential diagnosis between primary and metastatic lung adenocarcinoma. TTF-1, PE-10 and either CK7 or CK20 cannot properly tell the difference reliably between a primary pulmonary carcinoma and a metastatic malignancy of the lung in FNA biopsy specimens because of their low sensitivity and specificity. An IHC panel based on CK7/CK20, TTF-1, and PE-10 antibodies may be useful in the differential between a primary and a metastatic adenocarcinoma of the lung. An adenocarcinoma is probably a primary lung tumor when it is CK7 positive/CK20 negative and either TTF-1 positive or PE-10 positive [63].

Thyroglobulin is of special interest as it helps the differential diagnosis for entrapping of thyroid follicles by medullary carcinoma, as well as for mixed medullary-follicular tumors out of which we emphasize the rare paraganglioma-like variant, where cells have an inconspicuous cytoplasm, significant nuclear atypia with occasional bizarre or binucleated cells, coarse and granular nuclear chromatin with occasional grooves and intranuclear inclusions. These cancers are positive for the Fontana-Masson staining for melanin, for calcitonin and for S100 [64, 65].

The differential diagnosis of metastatic breast carcinoma to the skin from primary sweat gland carcinomas is of special importance as 25% of patients with breast cancer develop cutaneous metastases. The IHC panel includes mammaglobin, gross cystic disease fluid protein (GCDFP) 15, p63, basal cytokeratins (CK5, CK14, and CK17), androgen receptor, and PAX5. The basal phenotype of skin metastases from breast carcinoma (CMBC) cases have the potential to metastasize to the skin, apart from other known metastatic sites, such as the brain and bones. Furthermore, the percentage of mammaglobin expression in CMBC appeared similar to its previously reported expression in primary breast cancer. This finding may indicate preservation of this marker from primary breast cancer to the metastatic one [66]. Urothelial malignancies with a squamous morphology raise the differential diagnosis between a pure primary squamous cell carcinoma, an urothelial carcinoma with squamous differentiation and a secondary involvement by squamous cell carcinoma. An IHC panel of three urothelial-associated antibodies (uroplakin III, S100P, and GATA3) and two squamous-associated antibodies (CK14 and desmoglein-3) [67] identifies squamous and urothelial differentiation in most instances suggesting potential diagnostic utility.

THERAPEUTIC MANAGEMENT

Most patients diagnosed with a CUP (75-80%) have a dismal prognosis and will die within six months. Scientists from the Ioannina University Hospital analyzed the treatment of over 700 patients with CUP [68], as well as cases with liver metastases of a CUP [69] and have reported a response rate of only 20% and a median survival of just 6 months for patients treated with platinum-based, taxane-based or other combination regimens. Targeted therapy may play an important role, as proven by the studies of the Minnie Pearl Cancer Research Network [70, 71]. A treatment with carboplatin and paclitaxel in the first line with or without maintenance therapy with erlotinib and bevacizumab achieved a response rate of 53% and an overall survival of 13 months.

The use of bevacizumab plus erlotinib in second line was not as effective, with the median survival being 7 months and only 10% of patients responding [72-76].

The 20% of CUPs with a better response to therapy and better prognosis include men with poorly differentiated carcinoma with midline nodal distribution, women with papillary adenocarcinoma of the peritoneal cavity, squamous cell carcinoma that involves the head and neck lymph nodes, women with adenocarcinoma that involves only axillary lymph nodes, men with blastic bone metastases and a high PSA, neuroendocrine carcinomas of unknown primary site, isolated inguinal lymph nodes and patients with a single small, resectable metastasis that can be treated by surgical excision (and/or radiotherapy).

Serous papillary peritoneal carcinomatosis is treated using the same protocols as stage III and IV ovarian adenocarcinoma, with surgical cytoreduction and chemotherapy with platinum plus paclitaxel; 30-40% of the patients achieve a complete response after the primary treatment, 70% a partial response and the median survival is 3 years [77].

In cases with poorly differentiated neuroendocrine carcinoma several regimens have been applied without proof of any combination being superior, with a complete response rate of 21% and with a median survival of 15 months: platinum-based, taxane-based, 5FU or capecitabine combinations, dacarbazine, streptozotocin, temozolomide and irinotecan. Molecular targeted treatments consist of everolimus and sunitinib. Thirteen percent of the patients have a long-term survival [78]. In the case of well-differentiated neuroendocrine carcinoma the survival at 5 years can be more than 50%. A poorly differentiated carcinoma with midline distribution has the same treatment protocol as a germ-cell tumor and receives chemotherapy with platinum. The median survival is 12 months, 20% of the patients achieve complete response and the overall response rate is around 45% [79].

Women with a metastatic adenocarcinoma in an axillary lymph node are treated with axillary clearance and mastectomy or breast irradiation to obtain a proper loco-regional control, followed by adjuvant systemic chemotherapy with or without trastuzumab and hormone therapy, if there is estrogen receptor expression [80]. In the absence of surgical resection or radiotherapy of the breast tissue around 40% of patients develop a clinically evident breast primary.

In the case of a squamous cell carcinoma in lymph nodes of the head and neck, the clinical management is the same as in the case of locally advanced squamous cell carcinoma of the head and neck. It starts in the department of surgical oncology with radical neck dissection, followed by external beam radiotherapy of the pharynx and uni- or bilateral neck lymph nodes, associated with chemotherapy [81, 82].

In CUPs of the GI tract, especially CK20+, CK7, CDX2+ adenocarcinoma with a colon cancer profile, therapy and response rates are similar to patients with colon adenocarcinoma. The median survival is 24 months [83].

Cases with blastic bone metastases associated with a high PSA level are treated as metastatic prostate cancer, androgen-deprivation therapy or upfront docetaxel chemotherapy. Isolated metastatic lymph nodes with squamous cell carcinoma are treated with local surgical dissection, with or without radiation oncology therapy [84]. These patients have a very good response rate and prognosis.

As stated before, CUPs are mainly divided between the good prognosis group and the poor risk group. Petrakis et al. [86] separated CUPs using a robust multivariate and CART analysis into patients with low, intermediate and high risk. The score is called I-SCOOP (Ioannina Score for CUP Outpatient Oncologic Prognostication) and takes into consideration the clinical and pathological subgroup, performance status (PS) and leukocyte count. The clinical and pathological subgroup represents the physician‘s best guess on the origin of the cancer (based on IHC and clinical examination), PS is a time-honored parameter and the leukocyte count is associated with the inflammatory reaction associated with the cancer. Other prognostic markers are the number and sites of metastases, the presence of liver involvement, baseline serum levels of albumin, lactate dehydrogenase and alkaline phosphatase.

Since CUPs have an estimated incidence of 3-5% of all cancers and by definition are already metastatic when discovered, even in the absence of the origin of primary tumor, the role of surgery in such circumstance is strictly related to obtaining a pathology specimen, reduce the burden of symptoms or sometimes offer local control [85]. Tailored-suited therapy should be offered to different subsets of patients as for equivalent patients with a known primary tumor but with metastatic disease, thus trying to achieve long-term disease control. In many situations, the clinical onset of CUPs resides in the appearance of an isolated lymphadenopathy. Independent to the location of the lymph node metastasis (cervical, supraclavicular, axillary or inguinal), a pathology specimen should be obtained in order to facilitate future direct targeted-therapy. The preferred approach should consist of an excisional biopsy (for non-bulky nodes), or incisional biopsy/core-needle biopsy (for bulky nodes) [87].

Some CUPs present as peritoneal carcinomatosis of a serous papillary histological type in female patients, having a potential tumour equivalent to ovarian cancer. For such patients, optimal surgical debulking, ideally with a R0 resection should be attempted, by means of aiding platinum-taxane-based chemotherapy. Inconsistent data regarding the benefit of the use of HIPEC for these patients has not reached a consensus. On the other hand, if isolated axillary nodal metastases are found, surgical conduct should pursue to axillary lymph node dissection and radical modified mastectomy or whole breast irradiation with or without adjuvant chemotherapy and hormone therapy. In more than 50% of the cases, subclinical breast cancer is found on MRI scans. Squamous cell carcinoma metastases found in cervical lymph nodes, without involvement of supraclavicular nodes, should be managed with bilateral modified radical neck dissection, associated or not to external-beam radiation therapy with platinum based chemoinduction. Skin tumoral metastatic deposits should be surgically resected, leading the treatment options subsequently towards systemic therapy.

In our own experience as in other series of patients, Krukenberg's tumours have been found to be the most frequent, bearing not only radiologic signs of malignancy, but also serologic marker levels, highly suspicious for ovarian carcinoma (unpublished data). Surgical management consisted

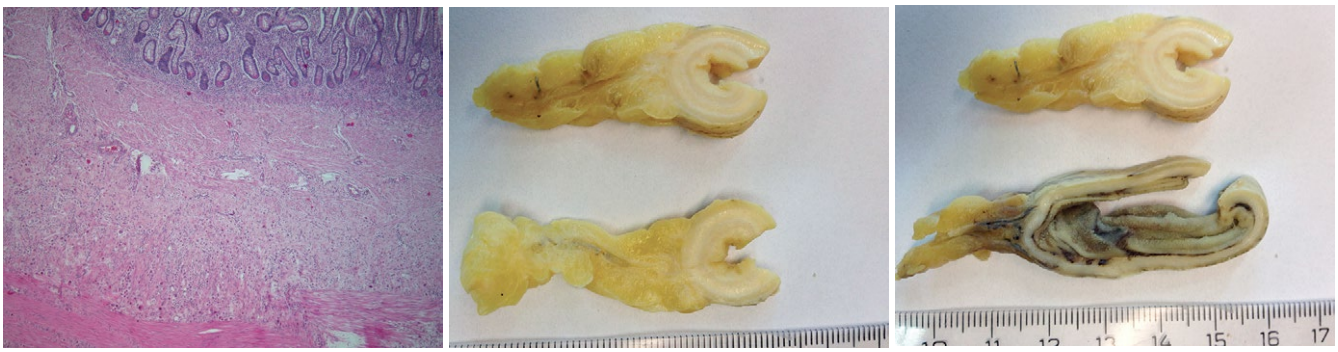

Fig. 6. Gastrointestinal CUP: a. H&E staining (x10) for a cancer of unknown primary site that has metastasized to the wall of the small intestine); b and c: surgical specimens of a cancer of unknown primary site located in the wall of the small intestine.

of optimal cytoreductive surgery, after a frozen section pathology examination. At the definitive pathology report, the ovarian origin was withdrawn, being more in favor of a GI primary source (Fig. 6 a-c). The macroscopic primary tumor was not found when the upper and lower GI tract was examined by endoscopy. For selected cases, where a gastric origin was suspected, platinum-taxane systemic chemotherapy was administered followed by subsequent radical gastrectomy. If colorectal origin seemed more probable, FOLFOX-based systemic chemotherapy, without further surgery was the preferred treatment. We also encountered two isolated cases of GI obstruction caused by intraluminal metastases of lobular invasive breast carcinoma without palpable breast lesions, where the resection of the obstructed bowel segment was performed, followed by bilateral mastectomy and targeted adjuvant chemotherapy, a procedure used by other authors [84, 85].

CONCLUSION

The present limitations in diagnosing and treating an unknown primary cancer remain a major challenge in comparison with other malignancies. Randomized clinical trials in which one would compare overall survival and progression-free survival with empirical chemotherapy versus a personalized therapy might help define the standard of care. Diagnostic assays have improved significantly in the last decade with the introduction of new IHC stains and when IHC fails to make an adequate differential diagnosis, molecular tests may aid diagnosis. Still, translational research and molecular diagnostic need further testing. Extending survival or attempts to achieve a cure is possible today only in a subgroup of patients.

Conflicts of interest: No conflict to declare.

Authors' contribution: C. T., M.-S. M., F. Z., L. P. and Z. F. wrote the manuscript. D. D., I. F., A. T., C. B. and A. J. gathered the literature, synthethised the data in tables, provided excellent insight into the differential diagnosis of cancer of unknown primary site and were responsible for providing the images that support the text. I. B.–N., M. Z. and T. C. corrected the manuscript. All authors approved the final version of the paper.

Acknowledgements: The present paper was supported from a national research grant awarded to Ciprian Tomuleasa (PNII-RU-TE-2014-4-1783), as well as by internal grants of the Iuliu Hatieganu University awarded to Florin Zaharie, Mihai Muresan and Ioana Frinc. The authors gratefully acknowledge the support of the excellent students involved in the current project (Grigore Gafencu, Sergiu Pasca, Vlad Moisoiu, Lorand Magdo, Patric Teodorescu and Sonia Selicean), as well the support of Professors Alexandru Irimie, Patriciu Achimas-Cadariu and Cosmin Lisencu.

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