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# Detecting undetectable — epidemiology, etiology, and diagnosis of carcinoma of unknown primary — systematic review

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**Abstract:** Carcinoma of unknown primary (CUP) is a heterogeneous group of oncological diseases in which it is impossible to determine the primary tumor. The incidence is 3–5% of oncologic patients, but the survival time varies from 6 weeks to 5 months.
The diagnostics should begin with a clinical evaluation and basic laboratory tests. For CUP placed in head and neck the positron emission tomography — computed tomography is recommended; pancreatic or lung neoplasms are diagnosed with the computed tomography as well. Recently, the magnetic resonance, especially whole-body diffusion-weighted imaging has been introduced to the imaging panel. The lesion obtained during surgically removed metastases or biopsy material should be histopathological and molecularly examined to define the type of tumor. The basic immunoexpression panel should include cytokeratin-5/6, -7 and -20, EMA, synaptophysin, chromogranin, vimentin and GATA3 and molecular expression of *ERBB2*, *PIK3CA*, *NF1*, *NF2*, *BRAF*, *IDH1*, *PTEN*, *FGFR2*, *EGFR*, *MET* and *CDK6*. During the accurate diagnostics enable to classify malignancy of undefined primary origin as provisional CUP or finally confirmed CUP in which the primary place of tumor remains undetectable. The detailed diagnostics should be performed in highly specified centers to establish an accurate diagnosis and to initiate personalized treatment. Majority of patients are diagnosed with adenocarcinoma (70%), undifferentiated carcinoma (20%), squamous cell or transitional cell/uroepithelial carcinoma (5–10%), neuroendocrine tumor (5%) and with minor incidence other histological types, including melanoma.

**Keywords:** cancer, carcinoma of unknown primary, metastases, diagnostics.

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## Introduction

Carcinoma of unknown primary (CUP) described also as unknown primary tumor, carcinoma or adenocarcinoma of unknown primary, metastases of unknown origin, metastases from unknown primary tumors, or tumor of unidentified origin [1]. It is a group of neoplastic diseases in which the site where they originally developed cannot be found. The primary diagnostics is based on the exclusion of other detectable oncological problems. In patients with such diagnosis, metastatic changes, and lymph node involvement, may occur in any area of the body. The most common places where neoplastic outbreaks are detected are liver, lungs, bone, kidneys, skin, and lymph nodes. Commonly the systemic metastases affect three or more organs simultaneously. Lymph nodes are often the only point where abnormalities are visible [1–3].

CUPs constitute 3–5% of a significant group of malignant neoplasms [1, 2, 4–6]. However, the incidence differs among studies, depending on the area of interest, metastatic lesions location and guidelines selected in diagnostic process. According to all these sources, the estimated mortality in such group of patients is high. Average survival varies from 6 weeks to 5 months [5, 7–10].

The clinical symptoms are not specific and include weakness, painless enlargement of the lymph nodes, weight loss. The correct diagnostic course is based mainly on imaging methods, laboratory determination of tumor markers, histopathological or molecular markers. However, there is no universal pathway that will detect changes in all cases and the diagnostic course should be selected individually [1, 4, 11–13].

Most patients are diagnosed with adenocarcinoma (AC) — 70%, undifferentiated carcinoma (UC) — 20% squamous cell carcinoma (SCC) or transitional cell/uroepithelial carcinoma — 5–10%, a neuroendocrine tumor — 5%, and less often other histological types. CUP are the most frequently detected in head and neck (70%), where SCC is the most common (65–76%) [14].

CUP definition created by the US National Institute of Clinical Excellence (NICE) details three phases of clinical investigation. Patients with a malignancy of undefined primary origin (MUO) confirmed with a biopsy from a metastatic lesion or provisional carcinoma of unknown primary (pCUP) are referred for further cytological and histological examinations. If the determination of the neoplasm origin is impossible, the lesion is confirmed as carcinoma of unknown primary (cCUP) [11, 14]. The gradation of the stages depends on the histology, detection methods of the primary site and the specialist review. Diagnosis of MUO is based on limited number of tests, prior to comprehensive investigation. Histology and cytology enable to diagnose pCUP, being a metastatic-epithelial or neuro-endocrine tumor. Confirmed CUP is a stadium verified by specialist review and further specialized tests.

## Incidence of CUP

Incidence of CUP varies between country and ethnicity. 3–5% of all human cancers are caused by CUP [1, 2, 4–6]. According to Pavlidis *et al.* [4, 15] the overall age-standardized incidence per 100 000 people per year is 7–12 cases in the USA [16], 18–19 in Australia [17], 5–7 in the Netherlands [18], and 4–6 in Switzerland [10].

Between 1922 and 1981, in Yale-New Haven Hospital there were 1539 patients with CUP [7]. It accounted for 3% of all cases throughout the year of 1922 and remained constant in 1981. 82% (1268) of all cases were confirmed by microscopical examination, the rest only clinically. The average survival for both these groups was 5 months, 77% of patients died before the end of one year. The average age of patients was 62 years. Females and males were equally often diagnosed with CUP. The most common was adenocarcinoma (38.2%) of all histopathologically proven cases.

Another data on the United States population from 1973 to 2008 delivered by SEER program gives slightly different results [5]. It consists of information about 106,641 patients, among them 78% (80,822) of all was confirmed histopathologically. The most common was AC — 36.1% (38,511), SSC — 8.5% (9058), not otherwise specified (NOS) 30.3% (32,357) and neuroendocrine 3.2% (3390). Incidence of CUP among all cancers was higher in females and Afro-Americans. The median survival was low, usually below 3 months. Over time there was less people diagnosed with CUP per 100,000 people, and less CUP per 100,000 cancers and in the end of these period — 2007 cancer of unknown primary site accounted for less than 2% of all cancers.

In Netherlands, the incidence of CUP in years 1984–1992 was 4% [8]. Among 1285 people, 1024 CUP were confirmed by histopathological examination, the most common type was AC — 479 (47%), poorly differentiated carcinoma (PDC) or poorly differentiated AC (PDA) — 453 (44%), while SSC was diagnosed in 76 (7%) patients. The rarest was undifferentiated malignant neoplasm — 16 (2%). The median survival time was equal to 11 weeks for histologically confirmed; 85% of patients died before the end of one year. For the patients with only clinical diagnosis, median survival time was 7 weeks. The average patient was 66 years old. Moreover, the condition was slightly more prevalent among males (53%).

Scottish Cancer Registry database consist of information about 50,941 patients from 1961–2010 period [9]. The number of diagnoses described as cancer of unknown primary site is equal to 3.9% of all cancer cases, and it was the third, after lung and colorectal cancer, cause of death due to cancer. For all this time females were more common diagnosed with CUP compared to males, also percentage of CUP were greater between females. However, both percentage of CUP and its amount is lower in the last decade of research 2001–2010 compared to previous ones. The CUP was greatly more common after the age of 40 years, also the survival time decreased with age, the median varies from 5.1–5.6 weeks, which is the worst result compared to other countries.

Percentage of people who were alive after 1 year fluctuates from 7.9 to 10.2%. Similar to previous research most common histological type was adenocarcinoma.

In the Swiss Cantons of Vaud and Neuchatel between 1984 and 1993, there were 699 cases of CUP which is equal to 1.7% of all cancers and 2.3% excluding non-melanomatous skin cancers [10]. Males were more often diagnosed. The median survival time was 11 weeks for histologically confirmed cases, which is 543, and 6 weeks for the non-histologically confirmed, 85% of patients died before 1 year. The median age for both groups was 71 and 79 years, respectively. In the histological studies most of the group was AC — 336 (62%), PDC or PDA — 122 (22%), SSC — 48 (9%) and undifferentiated malignant neoplasms — 37 (7%).

In all these studies the most common histological type of carcinoma of unknown primary site was adenocarcinoma [5, 8–10]. Depending on the research, females and males can be the majority, can be assumed that CUP is equally common in both sexes. Survival time varies from 6 weeks to 5 months (Table 1). Based on mentioned data it can be stated that averagely 13.45% (Fig. 1) of patients were alive after 1 year. Both, incidence of CUP per 100,000 people as well as CUP per 100,000 cancers was decreasing through time period.

**Table 1.** Survival among patients with CUP diagnosis.

| Research | | Survival |
|---|---|---|
| Yale-New Haven Hospital [7] | | 5 months[a] |
| USA-SEER [5] | | 3 months[b] |
| Netherlands [8] | Histologically confirmed | 11 weeks[b] |
| | Non-histologically confirmed | 7 weeks[b] |
| Scotland [9] | 1981–1990 | 5.3 weeks |
| | 1991–2000 | 5.1 weeks[b] |
| | 2001–2010 | 5.6 weeks[b] |
| Swiss Cantons [10] | Histologically confirmed | 11 weeks[b] |
| | Non-histologically confirmed | 6 weeks[b] |

[a] — mean [b] — median

## Etiology

Among different theories, there are leading two, which describe the formation of CUP. One states that CUP developed from a primary tumor which regressed and is undetectable. According to the other, the disease developed from stem cells that have undergone neoplastic transformation at a specific location.

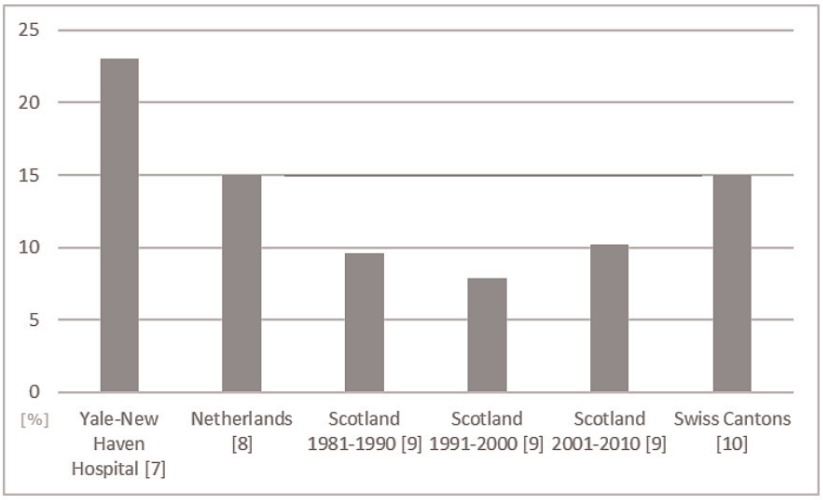


**Fig. 1**. Percentage of CUP patients who survived 1 year.

## *The metastatic theory*

In accordance with the literature the metastatic theory states that CUP tumors are in fact metastases arising from very early primary cancer, which is in dormant state or in regression. The first theory is supported by the following arguments. Available data state that 80% of primary tumors remain unidentified in antemortem diagnosis and 30–70% in autopsy. It is suggested that this proportion highlights the regression as a probable cause of primary tumor being unidentified, which is the first hallmark of CUP. Additionally, even if found, primary tumor of pancreas or lung appears as a small and asymptomatic nodule, which is incoherent with the typical picture of lung or pancreatic carcinomas. This phenomenon renders the dormancy as a consecutive hallmark of CUP [19]. Another thesis states that the reason for metastatic phenotype of primary carcinoma is its angiogenic incompetence, which drives cells to marked apoptosis and cells' death. It subsequently forces the tumor cells to metastasize. However, there were not any clinical trials performed to prove this theory [20].

Another concept supporting the metastatic theory, is based on characteristics of nucleic acid of cancerous cells of CUP checked by oligonucleotide arrays technology. It was stated that CUP has tissue-specific gene-expression profile, which means that there are common gene profiles of CUP and the genes characteristic for epithelial tissue. Therefore, CUP should be considered as typical metastases originating from specific carcinomas. The theory is supported by the study of 100 primary tumors containing 12,533 genes, grouped in 11 tumor types, which demonstrated that in 75 to 87% of cases carcinomas expressed the gene profiles corresponding to the tissue and site of origin [19].

CUP is considered as metastatic carcinoma itself, from the beginning of diagnosis. The process of emerging CUP as a metastasis of primary tumor relates to formation of premetastatic niche, which is a modification of local environment for better accommodation of cancerous cells. The formation process of the metastatic niche is still unclear, but it seems to be elucidated by mechanisms mentioned in the article. First concerns the testicular cancer, where the testis scarring was associated with metastatic germ cell cancer. Another one explains the presence of metastatic tumors in the retroperitoneum, inguinal canal, or mediastinum, caused by migration of embryonic rest cells (germ cells). The other thesis suggests that if the genetic alternations affected all germ-line cells, CUP could occur in the same way as primary immunodeficiency disorders seen in monozygotic twins. The last one stating that CUP arises from stem cells with multiple differentiation capacity, but this view is going to be discussed further in subsequent paragraph [21]. Therefore, the formation of metastatic niche is essential for CUP emergence, but the molecular mechanism still puzzles scientists.

## *The stem cell theory*

The stem cell theory is built on the migratory and proliferative potential of stem cells, called cancer stem cells (CSCs). The theory states that the stem cells, which are burdened with molecular alterations or premalignant, originate from their natural tissue, migrate away and give rise in a random location. It is important to realize that stem cells, which create a new tumor, do not have to origin from the existing cancer, but are molecularly damaged and are able to migrate and give rise for tumor in distant locations. According to the authors, this mechanism is a key to understand the formation of cancer of unknown primary. Often, never do emerge a cancer in the primary site of stem cells, which explains high percent of primaries being not found even on postmortem examination. Aforesaid theory presenting stem-cells origin of CUP is not considered commonly, hence is deflated by some arguments presented in the literature:

- Adult stem cells do not divide often, while for cancer to emerge, the cells should have a higher proliferative ratio.
- Stem cells pose rather small population within a tissue, therefore the probability of aleatory carcinogenic event in this kind of cells is low in comparison to other larger cells populations within a tissue.
- The role of stem cells in tissue is to self-renew. Carcinogenesis depends on the acquired deficiency in cell phenotype, which is advantageous for tumor formation. This phenotype is more likely to occur when the cell differentiates, than when self-renews [14].

On the other hand, all above arguments are refuted by the following, which seems to confirm stem cell theory:

- Authors highlight that life starts before birth and the stem cells divide actively during in-utero development. Stem cells had just acquired many heritable DNA alterations during embryonic development, which manifest during adulthood as possible carcinogenesis. The alternations may also have positive impact on proliferative rates of stem cells during adulthood.
- Despite of stem cells being a small population within a tissue, they are still at the stages of embryonic development, which increases the chance of possible carcinogenic damage of the genetic material during mitotic divisions.
- Stem cells have three cellular features, which are necessary for carcinogenesis. They include migration ability, high proliferation rate and self-renewal capacity. All these properties are observed right in stem cell population [22].

The data from 2015 reveal, that the rate of normal stem cell proliferation in a tissue is strongly correlated with the development of cancer in that origin [3]. However, it is crucial to remember, that stem cells can migrate from the place of their origin to the new tissue, and they can become cancer stem cells (CSC) either before or after this migration. It may be an answer for unpredictable location of CUP and lack of its primary.

### *Molecular changes that may underlie the development of CUP*

Besides the metastatic theory and stem cell theory scientists examined molecular and chromosomal abnormalities as propitious factors for CUP formation. Studies give the bases to consider the process of CUP formation as a result of some pathological changes: angiogenesis activation (50–89% of cases), activation of protein kinase B (AKT) or mitogen-activated protein kinase (MAPK) (20–35%), epithelial-mesenchymal transition markers or hypoxia-related proteins (16–25%), over-expression of oncogene (10–30%). One possible source of mentioned changes is chromosomal instability, which may cause drag resistance, aggressive course and unfavorable prognosis [23]. These abnormalities concern chromosome 1 (1p) — it was proved in 12 of 13 CUP in the examination — which commonly consistent with other advanced solid tumors. Then, additional copy of chromosome 12 short arm i (12p), which is characteristic for germ-cell tumors and favorably makes the tumor responsive to cisplatin-based chemotherapy. Another abnormality is an aneuploidy detected in 70% cases of CUP with undifferentiated carcinoma or metastatic adenocarcinoma. According to the studies CUP presented overexpression of following onco-genes: Ras (92%), Bcl-2 (40%), Her-2 (11%), c-Myc [5], p53 (26–53%). This high titer of Ras/Her2 may be beneficial for patients, while searching for the therapies which target these molecules. EGFR protein and proto-oncogene c-Kit were found not influencing pathogenesis of CUP and therefore having no prognostic implication [19].

It is important to notice that angiogenesis in CUP is relatively higher than in metastases from known primaries. It corresponds with the fact, that unfavorable

(means with worse prognosis) CUP malignancies are marked by higher micro-vessel density than CUP form favorable group (with more optimistic prognosis). The angiogenesis correlates positively with tissue expression of vascular endothelial growth factor (VEGF) — strong expression in 83%, and negatively with thrombospondin-1 (TSP-1) — strong expression in 20%. However, neither VEGF nor TSP-1 had any association with clinicopathological parameters [19]. CUP have also common expression of matrix metalloproteinase (MMPs), which enable metastasis by degradation of extracellular matrix, and tissue inhibitor of metalloproteinases type 1 (TIMP-1), which regulates MMPs activity. Both factors are involved not only in tissue remodeling, but also in tumor angiogenesis, modifying tumor cell progression [24]. Unfortunately for the advance in medical research, described features of CUP do not differ from those seen in normal advanced tumors with known primaries [19].

Summing up the molecular and genetic features described above, even though they are present in CUP, do not pose factors that are unique and thereby would be helpful in the investigation of primary carcinoma, determining the actual mechanism of CUP formation or setting appropriate treatment. The studies also highlight some risk factors, which may be significant for CUP formation. They include smoking cigarettes, high quartile of waist circumference, rather weak association of CUP and alcohol consumption or level of education [23].

## Diagnosis

The complex diagnostic process should take place in highly specialized oncological centers, having multidisciplinary staff from various medical fields including oncologists, radiologists, pathologists, genetics, immunologists and others (Fig. 2). The more experienced the team members are and the more they understand the natural history of different types of cancer, the diagnostic process is faster, less expensive, and demands using fewer techniques [11].

To achieve that goal a combination of histopathological investigations, immunohistochemistry, electron microscopy, molecular diagnosis and imaging technology, including mostly computed tomography (CT), magnetic resonance imaging (MRI), mammography and FDG-positron emission tomography (PET) is crucial. Establishing a profile of the CUP precede contact with the clinical oncologist.

### *The initial stage of diagnostics*

The first step in the diagnosis of the neoplastic process is taking the patient's history and physical examination. The patient should be asked about all his symptoms (nature, duration, location), medical history (past diseases, chronic diseases, chemical and infection exposure, pregnancy outcome, previous medical procedures), addictions,

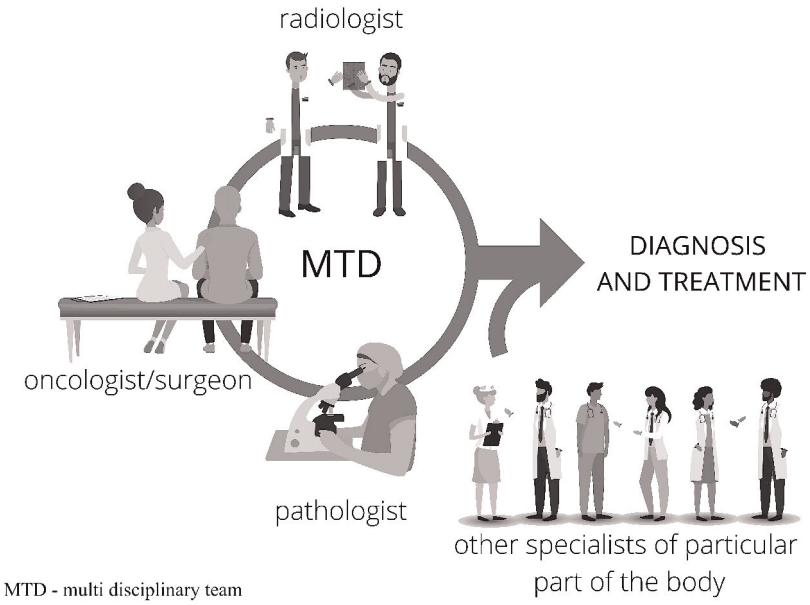


**Fig. 2**. Role of multidisciplinary team (MTD) in diagnostics and treatment of carcinoma of unknown primary origin.

diet, as well as the occurrence of cancer in the family. It is very important to collect a thorough interview direct with the patient in a proper environment, because of possibility to find key information about the origin of the cancer, e.g. primary site situated in the nasopharynx should be considered when alcohol and tobacco using appears in a patient's history. Confirmed CUP (cCUP) usually have a unique natural history that consists of early dissemination and a short duration (<3 months) of disease signs. Clinical symptoms for CUP can be non-specific and vague (e.g. weight loss and pain) or might be absent at the time of diagnosis despite the presence of palpable tumor mass [1, 4]. The physical examination should also be very thorough e.g. a painless enlarged cervical lymph node is the most common presentation of the head and neck CUP [25]. In addition to the basic activities, it should also include palpation of breasts, genitourinary and rectal examination [18]. Then, basic blood tests, biochemical tests, urinalysis, fecal occult blood testing should be performed [26, 21].

In CUP the primary neoplastic focus cannot be found, but based on the location of the metastasis or lymph node involved, the histopathological tumor subtype and the routes of cancer cell's migration (such as: hematologic, lymphatic, intraperitoneal, intrapleural or through the cerebrospinal fluid), search for the starting point of the tumor can be narrowed and treatment that may be effectively introduced. A study of 347 patients with confirmed CUP showed that in 95% of patients with this disease, the

presence of metastasis in sentinel lymph node is consistent with the typical pattern of spreading of the other cancers. This is called the "sentinel node theory" [12].

The age of the patient should be analyzed, as the isolated metastases to the organs of the abdominal cavity most often occur in patients aged 80–90 years, in the thoracic organs — in the age of 90–95, and in the brain and bones — under the age of 50 [11, 21, 25].

## *Imaging diagnostics*

Usually, patients come to the doctor only with basic imaging tests such as chest X-ray, abdominal ultrasound or CT of the small part of the body that is insufficient for the final diagnosis or with exam too old to state current status of the disease. It also happens that these tests are performed incorrectly e.g. absence of arterial and urographic phases that enable accurate diagnosis of neoplasms originating in the transitional epithelium of the urinary tract or absence of paranasal sinuses or superior mediastinal region on MRI and CT scans important in case of cervical lymph nodes enablement [11]. When the abnormality on chest X-ray is found, the CT of the chest, abdomen and pelvis should be performed. CT scan determines the character, extent of the disease and the optimal site for biopsy is selected. There is evidence that CT enables detection of the primary lesion in 1/3 of CUP patients [1]. This method makes it possible to set the diagnosis of most pancreatic and lung cancer patients and one third patients with colorectal lesion, especially when the virtual colonoscopy is performed. In such localization the accuracy of these scans is better than of the FDG-PET.

Women with an enlarged axillary, mediastinal or cervical lymph node are referred for a mammography (MMG). However, it should be remembered that this method has a sensitivity of about 20% and if the result is negative and there is a strong suspicion of primary breast tumor, additional MRI, tomosynthesis and/or spectral mammography is indicated [4, 11].

If the above tests are inconclusive, the next step is PET and PET-CT, that allow the detection of 30% more changes than a conventional contrast CT scans. PET-CT is an effective method, particularly in detecting primary neoplastic focuses whose metastases are located within the head and neck. This method helps to correctly diagnose the origin of a CUP better than MRI (22–44% vs. 20–27%) especially when it is SSC (53–77% of the cases) placed in cervical lymph nodes [1, 11, 27]. In 27–30% of cases PET-CT allows to visualize changes, that were not observed in any other imaging methods. The sensitivity and specificity is estimated to be 43–88% and 33–97% in all CUP localizations but in the region of head and neck they reach nearly 88.3, 75.9 and 78.8% [11]. Sensitivity is even higher than 3-Tesla MR (94.4% vs. 88.2%) in the opposite to specificity, which is around 71.4–76.2% when using DWI (DWI-diffusion-weighted imaging) [11, 28]. The accuracy of both of these methods in the diag-

nosis of metastatic disease achieved a similar result — about 79%. For advanced head and neck cancers or even suspicion of neoplastic process whole-body (FDG-PET-CT) imaging should be recommended despite basic work-up in other neoplasms. It accelerates the diagnostic process, helps doing an accurate biopsy in endoscopy and reduces costs [11, 29–31].

When dealing with metastatic lesion or neuroendocrine tumor it is a proper path to resign from CT or MRI and use PET-CT instead using 68Ga-DOTA-NOC receptor [4]. Monitoring of the therapy might be the long-term process that makes cumulative dose quite significant, so a powerful alternative to PET-CT is PET-MR due to lower dose of ionizing radiation [30]. It is shown that both hybrid imaging techniques (FDG-PET-CT and FDG-PET-MR) with very similar effectiveness detect the primary cancer and metastasis with comparably high visible lesions. Differences were observed in cervical CUP sites assed better with PET-MRI in the opposite of pulmonary lesions when PET-CT gave better results [32]. The primary lesions detected by PET-CT positive predictive value and negative predictive value rates 91.35 and 40%, respectively [33]. Currently, beside of FDG and 68Ga-DOTA other more specific isotopes are used in case of suspicion of less commonly type of tumor; e.g. 11C-Acetate (kidney, prostate), 11C-Methionine (brain, prostate, head and neck squamous cell carcinoma), 131I, 123I (thyroid gland), analogues of somatostatin (neuroendocrine tumors) [34]. It has to me mentioned that PET-CT should be performed before the biopsy because, the biopsy is procedure that may cause an iatrogenic inflammatory process that would result in a false positive on this imaging test.

It seems that a new method that can bring very good results in the diagnosis of CUP is the WB-DWI (Whole body DWI) technique that shows comparable sensitivity (50 vs. 54%) and specificity (93 vs. 95%) as FDG-PET-CT but is safer, since patients are not exposed to ionizing radiation. Moreover, it has costless, better available, and final diagnosis is made in a shorter time [11–14].

Recently, the role of DCE-MRI (dynamic, contrast-enhanced magnetic resonance imaging) has also been discussed as a method that shows similar sensitivity and specificity to the DWI-MR and FDG-PET-CT methods [35]. It should be remembered that for each patient the method that will allow for the final determination of the exact diagnosis may be different and the tests should be selected individually for each case [12, 13].

### *Histopathological and immunohistochemical diagnostics*

A crucial role for diagnosis is a histologic evaluation, which gives the possibility to categorize tumors, depending on the degree of differentiation and mutated cells [4, 21, 26, 28].

The most frequently used method for patients with poorly differentiated tumors (30%) is fine-needle aspiration (FNA) — which provides cytological material or core

needle biopsy (CNB) — which provides a solid piece of tissue. The most common sampling sites are lymph nodes, liver, bones, lungs, serous cavities, and central nervous system. [14] The sample might particularly provide not enough material to be evaluated properly [1, 11]. Taking into consideration the metastatic neck lymph nodes, the diagnostic sensitivity ranges from 83 to 97% with a specificity of 91–100% when performed by an experienced histopathologist using conventional staining methods [3]. During routine microscopical evaluation type of cells, the structure they make up and their relation to the surrounding tissues are determined. An additional staining allow visualization of other components as mucus, glycogen, fats, reticulin and collagen fibers. This allows to identify the type of neoplasm, while the determination of the primary lesion location is in most cases not possible [14].

Finally, immunohistochemistry (IHC) tests verify the histopathological findings (type, subtype) and establish tissue of origin (TOO) by its markers. [1, 11, 21, 26]. The procedure has limited the value of light microscopic examination (rarely successful in identifying the primaries) same as routine staining with hematoxylin and eosin or even additional staining [1]. Current European and American guidelines also emphasize the importance of meticulous histopathological examination, including the assessment of tissue and cell morphology, and the use of IHC to determine tumor malignancy and exclude highly curable non-cancerous cancers [26].

Two main markers: cytokeratin-7 (CK7) and -20 (CK20) are tested as first and they are needed to profile possible primary site. Based on the 2015–2020 study, after profiling cytokeratins 7 and 20 in a group of 307 patients with CUP, at this stage of diagnosis, the primary site could be defined or confirmed in 73% of patients [35]. CK7 is positive e.g. for lung, endometrial, breast, ovarian or thyroid carcinomas and CK20 for gastrointestinal (GI) and urothelial ones (Fig. 3). Negative to AC and positive for SSC is CK5/6, used when there is lung cancer suspicion.

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 because this technology is an interpretative and subjective technique. Another problem is that there are tumors that present the same biomarkers or that CUPs specimens might be small, and the large scale of biomarkers cannot be used. It should also be taken into consideration that in most of those patients occurs nonspecific elevation of multiple markers (Table 2). To manage this problem, the molecular profiling of CUPs was developed. Based on the result of the histopathological examination and the data from the patient's interview, the range of markers that will be most effective to measure in a specific case can be estimated [14].

The key role of the study of immunohistochemical markers is emphasized in the case of liver tumors. It has been proven that 86% of liver tumors are metastatic lesions. Imaging, as well as pathomorphological analysis, can establish the neoplastic meta-

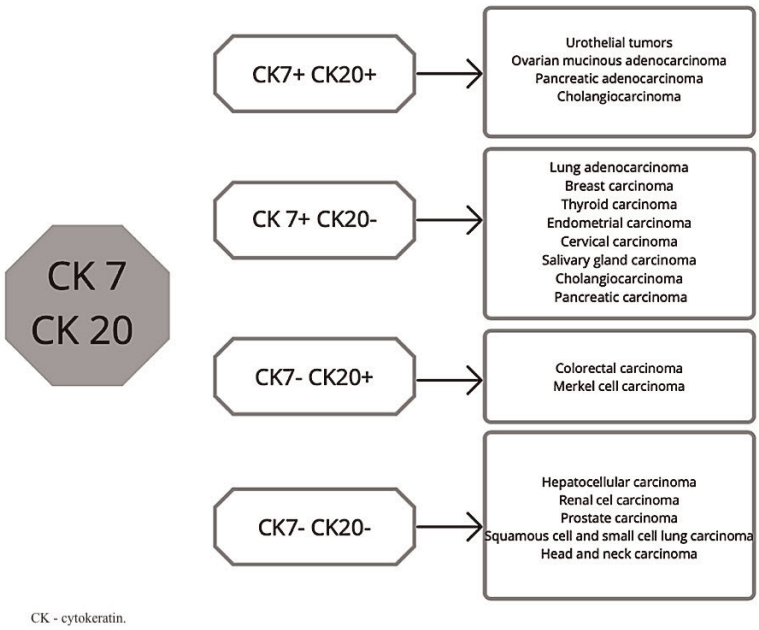


**Fig. 3**. The role of Cytokeratin 7 and Cytokeratin 20 in CUP diagnosis.

static nature of the tumor, while the precise determination of the type and subtype and therefore the possible location of the primary tumor is most likely with the immunohistochemical examination [36].

## *Molecular diagnostics*

One of the most advanced diagnostic methods is based on molecular structure using the evaluation of the messenger RNA (mRNA), microRNAs (miRNAs), DNA or epigenetic. There are some commercial molecular test available such as Pathwork Tissue of Origin, biTheranostics Cancer type ID or miRview mets2 [37–39]. This method differs from the others in its advantages and disadvantages (Table 3).

Although molecular methods give the most accurate results they should not be performed one at time but as a complementary manner [21]. The detection of human papilloma virus (HPV) DNA is also valuable in diagnostics. Its presence in neoplastic metastasis indicates squamous cell carcinoma in 78% of cases. The methods used to detect virus genetic material are: in-situ hybridization (ISH) or polymerase chain reaction (PCR), detecting HPV DNA or by HPV E6/E7 RNA expression detected by quantitative reverse transcriptase-PCR (qRT-PCR). The marker that can also be used interchangeably with HPV DNA is p16, a human tumor-suppressor protein.

EBV (Epstein-Barr virus) and HPV detecting in the metastatic lymph node is very important for further diagnostics and treatment and to predict the patient's outcome.

**Table 2.** Examples of biomarkers that are detected in different types of cancer [14].

| Primary site | Biomarkers |
|---|---|
| Adrenal cortical neoplasm | Mart-1, inhibin-α, calretinin, SF-1 |
| Angiomyolipoma | HMB-45, SMA |
| Breast carcinoma | GATA3, TFF1, MGB, ERM GCDFP-15, PgR, myoglobin |
| Chordoma | Cytokeratin, S100 |
| Choriocarcinoma | β-HCG,CD10 |
| Embryonal carcinoma | SALL4, LIN28, OCT4, NANOG, CD30, SOX2, CD117, PLAP |
| Endocervical adenocarcinoma | PAX8, p16, CEA, HPV ISH, loss of PAX2 |
| Endometrial stromal sarcoma | CD10, ER, PgR |
| Epithelioid sarcoma | CD34, loss of INI1 |
| Ewing sarcoma | CD99, Fli-1, NKX2-2 |
| Gastrointestinal stromal tumor | CD117, DOG1 |
| Lower gastrointestinal tract tumor | CDH17, SATB2, CDX2, CK20 |
| Upper gastrointestinal tract tumor | CDH17, CDX2, CK20 |
| Hepatocellular carcinoma | ARG1, glypicn-3, HepPar-1, AFP |
| Hyaline trabecular adenoma of thyroid | MIB-1 |
| Intrahepatic cholangiocarcinoma | pVHL, CAIX |
| Lung adenocarcinoma | TTF1, napsin A |
| Melanoma | S100, mart-1, HMB-45, MiTF, SOX10, PNL |
| Mesothelial origin | Calretinin, WT1, D2-40, CK5/6, mesothelin |
| Myeloid sarcoma | CD43, CD34, MPO |
| Myoepithelial carcinoma | Cytokeratin and myoepithelial markers, loss of INI1 |
| Neuroendocrine | Chromogranin, synaptophysin, CD56 |
| Ovarian clear cell carcinoma | pVHL, HNF-1, KIM-1, PAX8, ER, WT1, vimentin, TFF1 |
| Pancreatic, acinar cell tumor | Glypican-3, antitrypsin |
| Pancreatic neuroendocrine tumor | PR, PAX8, PDX1, CDH17, islet-1 |
| Prostate adenocarcinoma | PSA, PSAP, ERG, NKX3.1 |
| Rhabdomyosarcoma | myogenin, desmin, MyoD1 |
| Salivary duct carcinoma | GATA3, AR, GCDFP-15, Her-2/neu |
| Seminoma | SALL4, LIN28, OCT4, CD117, D2-40 |
| Solitary fibrous tumor | CD34, CD99, BCL2 |
| Smooth muscle tumor | SMA, MSA, desmin, calponin |
| Squamous cell carcinoma | P40, CK5/6, p63, SOX2, desmocollin-3, CK34BE12, p16 (+/–) |
| Synovial sarcoma | TLE1, cytokeratin |
| Thymic origin | PAX8, p63, CD5 |
| Thyroid follicular cell origin | TTF1, PAX8, thyreoglobulin |
| Urothelial carcinoma | GATA3, UPII/UPIII, S100,CK5/6, CK34BE12, p63,CK20, p40 |
| Vascular tumor | ERG, CD31, CD34, Fli-1 |

**Table 3.** Advantages and disadvantages of molecular diagnostic methods.

| Advantages | Disadvantages |
|---|---|
| small amount of tissue needed | 10% — still impossible to diagnose |
| high sensitivity (TOO — 87–94%, CTID 72–95%, miRview — 82–90%) | TOO is not ideal for sarcoma |
| | CTID is not really feasible for pancreatic, colorectal and gastroesophageal cancers |
| | high costs (20–30x higher than IHC) |

These assays should be implemented in the clinical routine for every CUP case. EBV turns out to be associated with poorly or undifferentiated nasopharyngeal carcinoma (NPC) or its nonkeratinizing types [40].

To avoid potentially unnecessary diagnostic research for the primary lesion, there are studies about Comprehensive Genomic Profiling (CGP), which identify pathogenic changes in genes. It was used in a randomized trial, named CUPISCO, that allows to assign patient to personalized treatment [41]. Based on the retrospective analysis of samples collected from neoplastic focuses classified as CUP, it was found that in 96 out of 303 (31.7%) of these patients, characteristic genetic alterations could be found. The main genomic changes concerned the genes *ERBB2* (7.3%), *PIK3CA* (6.3%), *NF1* (5.6%), *NF2* (4.6%), *BRAF* (4.3%), *IDH1* (3.3%) %), *PTEN*, *FGFR2*, *EGFR* (3.6% each), *MET* (4.3%), *CDK6* (3.0%). That identification seems to allow using effective site-specific treatment or immunotherapy options with clinical benefit that correlated with longer progression-free (PFS) and overall survival (OS) rates [21, 41].

## *Surgical diagnostic methods*

Recently, there has been a lot of research on surgical methods for the diagnosis of head and neck CUP. One of them is transoral robotic surgery (TORS), which is a microinvasive technique that allows 3D visualization (mainly using the da Vinci Xi® robot) of spaces within the head and neck and provides access to spaces that are difficult to reach with traditional surgery. It is a way of allowing radical excision of the tumor, and through this obtaining tissue material that may be sufficient for histopathological and immunohistochemical analysis [42].

Other methods are transoral laser microsurgery (TLM) and transoral endoscopic electrocautery (TOEC). A systematic review of 777 patients with CUP of the head and neck determined that the rates of identifying the primary site were respectively: 60% in TORS, 80% in TLM, and 40% during TOEC [43].

## Conclusion

CUP is an epidemiological issue demonstrating high mortality rate in patients and challenging multidisciplinary medical teams [11]. Hence multifactorial and holistic diagnostics including imaging, laboratory, molecular and pathological tests proves valuable to set a personalized treatment.

## Contribution statement

J.O., I.W., O.P., W.Z., F.B. conceived the concept of the review. J.O., I.W., O.P., W.Z. performed the research and analyzed the information. J.O., I.W., O.P., W.Z. wrote the paper. J.O., O.P., F.B. revised and edited the manuscript for final submission.

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## Conflict of interest

None declared.

## Abbreviations

AFP — alpha-fetoprotein
AR — androgen receptor
ARG1 — arginase 1
BCL2 — B-cell lymphoma 2
β-HCG — human chorionic gonadotropin
CAIX — carbonic anhydrase IX
CEA — carcinoembryonic antigen
CD — cluster of differentiation
CDH17 — cadherin-17
CDX2 — caudal type homeobox 2
CK — cytokeratin
CK34BE12 — cytokeratin 34 beta E12
D2-40 — podoplanin
DOG1 — discovered on gastrointestinal stromal tumors protein 1
DPC4 — deleted in pancreatic carcinoma 4
ER — estrogen receptor
ERG — erythroblast related gene
ERM — epiretinal membrane
Fli-1 — friend leukemia integration 1
GATA3 — GATA binding protein 3
GCDFP-15 — gross cystic disease fluid protein 15
HepPar-1 — hepatocyte paraffin 1

Her-2/neu — receptor tyrosine-protein kinase erbB-2
HMB-45 — human melanoma black
HNF-1 — hepatocyte nuclear factor 1
HPV — human papillomavirus
INI1 — integrase interactor 1
KIM-1 — kidney Injury Molecule-1
LIN28 — Lin-28 homolog A
MGB — medulloblastoma
MIB-1 — mindbomb E3 ubiquitin protein ligase 1
MiTF — melanocyte inducing transcription factor
MPO — myeloperoxidase
MSA — muscle-specific actin
MyoD1 — myogenic differentiation 1
NANOG — homeobox protein NANOG
NKX2-2 — Homeobox protein Nkx-2.2
NKX3.1 — NK3 Homeobox 1
OCT4 — octamer-binding transcription factor 4
p — protein
PAX — paired box
PDX1 — pancreatic and duodenal homeobox
PgR — progesterone receptor
PLAP — placental alkaline phosphatase
PNL — percutaneous nephrolithotripsy
PR — progesterone receptor
PSA — prostate-specific antigen
PSAP — prostate-specific acid phosphatase
pVHL — Von Hippel–Lindau protein
SALL4 — sal-like protein 4
SATB2 — special AT-rich sequence-binding protein 2
SF-1 — steroidogenic factor 1
SMA — smooth muscle actin
SOX — SRY-box transcription factor
TFF1 — trefoil factor1
TLE1 — transducer-like enhancer of split 1
TTF1 — thyroid transcription factor 1
UPII/UPIII — uroplakin protein II/III
WT1 — Wilms tumor 1

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