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Review

# Unknown primary tumors

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ABSTRACT

An unknown primary tumor (UPT) is defined by the presence of a metastatic cancer without a known primary site of origin despite a standardized diagnostic workup. Clinically, UPTs show rapid progression and early dissemination, with signs and symptoms related to the metastatic site. The molecular bases of their biology remain largely unknown, with no evidence as to whether they represent a distinct biological entity.
Immunohistochemistry remain the best diagnostic tool in term of cost-effectiveness, but the time-consuming "algorithmic process" it relies on has led to the application of new molecular techniques for the identification of the primary site of UPTs. For example, several microarray or miRNA classifications of UPTs have been used, with an accuracy in the prediction of the primary site as high as 90%. It should be noted that validating a prediction of tissue origin is challenging in these patients, since most of them will never have a primary site identified. Moreover, prospective studies to determine whether selection of treatment options based on such profiling methods actually improves patient outcome are still missing. In the last few years functional imaging (i.e. FDG-PET/CT) has gained a main role in the detection of the site of origin of UPTs and is currently recommended by the European Association of Nuclear Medicine. However, despite recent refinements in the diagnostic workup, the site of origin of UPT often remains elusive. As a consequence, treatment of patients with UPT is still empirical and inadequate.



**Contents**



*Abbreviations:* αFP, alpha-fetoprotein; βHCG, human chorionic gonadotropin; CA, cytosine-adenosine; CEA, carcinoembryonic antigen; CK, cytokeratin; EGFR, epidermal growth factor receptor; ECM, extracellular matrix; EANM, European Association of Nuclear Medicine; FFPE, formalin-fixed, paraffin-embedded; FDG, 18F-fluorodeoxyglucose; FU, fluorouracil; HBV, hepatitis B; HCV, hepatitis C; IHC, immunohistochemistry; miRNA, microRNA; MMP, matrix metalloproteinase; mRNA, messenger RNA; MVD, microvessel density; NET, neuroendocrine tumors; NOS, not otherwise specified; NSCLC, non-small cell lung cancer; OS, overall survival; PET, positron emission tomography; PLAP, placental alkaline phosphatase; PSA, prostate specific antigen; pts, patients; RR, response rate; RT-PCR, reverse transcription polymerase chain reaction; SPET, photon emission tomography; SVM, microarray support vector machine; CT, computed tomography; TIMPs, tissue inhibitor of metalloproteinases; TSP1, thrombospondin-1; TTF-1, thyroid transcription factor-1; UPT, unknown primary tumor; VEGF, vascular endothelial growth factor

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## 1. Introduction

Unknown primary tumor (UPT) is defined by the presence of a metastatic cancer without a known primary site of origin, despite a standardized diagnostic workup. UPT comprises a range of cancer types and subtypes. Lymphoma, leukemia, melanoma, germ cell tumor and sarcoma are usually excluded. Thus, UPT translates to carcinoma of unknown primary, of which 90% are adenocarcinomas or poorly differentiated carcinomas, 5% are squamous carcinomas and 5% are neuroendocrine carcinomas [1]. UPT demonstrates common characteristics, such as aggressiveness, early dissemination and silent primary tumor. The clinical course is often dominated by symptoms and signs related to metastases. These tumors are usually associated with unknown biology [2]. The primary tumor may either have a slow growth, or become involuted and undetectable. It has been hypothesized that cancer may remain dormant until subclones with angiogenic phenotypes arise and lead to metastases [3].

UPT represents a real diagnostic problem and a therapeutic challenge. Several groups have proposed different diagnostic and therapeutic protocols for this complex disease [4]. It can be hypothesized that detection of the primary tumor may optimize treatment planning, which, in turn, may improve patient outcome. Recently, new molecular tests have been applied to the identification of molecular signatures of a tissue of origin. The impact of the genomic classification on patient outcomes is currently under study.

In this article, we review the molecular features characterizing UPT, and highlight recent advances in the diagnosis and treatment of this disease, which will hopefully improve the prognosis of patients.

## 2. Epidemiology

It is generally reported that UPTs account for 2.3–4.2% of all neoplasms [1]. They represent the seventh to eighth most frequently occurring cancer in the world and the fourth commonest cause of cancer death in both males and females [5]. The annual age-adjusted incidence per 100,000 population in USA is 7–12 cases, in Australia 18–19 cases and in the Netherlands 5.3–6.7 cases [6]. The median age for occurrence is around 60 years, with a marginally higher frequency in males. However, since other diagnoses are often reported for patients with UPTs, this heterogeneous group of tumors is misrepresented in tumor registries and its exact incidence is unknown. Identification of the primary tumor occurs before death in less than 20–30% of cases, even when extensive investigations take place. The primary tumor is identified at autopsy in 50–75% of cases, usually as a small deposit in the lung or pancreas (both comprising approximately 25% of cases) [7]. The overall prognosis of these tumors is very poor, with a median survival of 4–12 months despite treatment, with <50% of patients alive at 1 year and <10% at 5 years after diagnosis [8].

## 3. The pathologist's perspective

Most of UPTs in adults are histologically classified as adenocarcinomas NOS (40–60%) or undifferentiated carcinomas (30%) and the remainder as poorly differentiated tumors, squamous cell carcinoma and small cell carcinoma [1,9].

Although the extremely poor prognosis of these tumors often discouraged a strong effort in defining their origin [10], early attempts to employ serological methods underscored the potential of this approach in challenging areas of conventional histopathology [11]. Since today some advanced histotype-specific therapies can significantly improve survival of a patient with UPTs, it is mandatory to outline histogenetic features and biological profile of these tumors.

Although globally accepted immunohistochemical guidelines on this issue are not available, some acceptable general principles are largely present in medical literature. They are usually based on “algorithmic processes” that, starting with some screening tests, continue along a rational scheme progressively excluding or confirming possible diagnostic hypotheses on the basis of positivity or negativity for known markers, until a final diagnosis is reached [12]. This method represents a good cultural approach, but it has to be followed with prudence and criticisms in order to avoid dangerous mistakes.

A critical point is to rule out an anaplastic lymphoma, a mistake with ethical and legal implication. A CD45 preliminary testing is considered mandatory by most pathologists, taking into consideration that a cytokeratin (CK) positivity is a possible, though extremely rare, finding in some haematological malignancies [13]. Another preliminary screening test is represented by a restricted immunohistochemical “melanoma panel” (S-100, HMB-45). Because of spontaneous regression, malignant melanoma can manifest with metastatic disease in the absence of a primary lesion. Moreover, it possesses a great microscopic mimicry, being able to simulate malignant tumors of any kind of histogenesis. A panCK is needed to verify a possible epithelial origin. A vimentin test, by itself, is not demonstrative of any specific origin since epithelial tumors can exhibit an associate positivity for this marker that, rarely, could be expressed also in the absence of CK, as in adrenocortical carcinoma. Conversely, mesenchymal tumors, such as epithelioid sarcoma or angiosarcoma, may be CK-positive.

In the presence of panCK-positive tumors, a combined test for CK7 and CK20 is notoriously a useful intermediate step before to select immunoprofiles suggestive of colorectal carcinoma (CK7−/CK20+), transitional carcinoma, pancreatic carcinoma (CK+/CK20+), hepatocarcinoma, renal cell carcinoma, prostate adenocarcinoma (CK7−/CK20−), non-small cell lung cancer, breast and endometrial carcinoma (CK7+/CK20−) [14].

In any case, in the presence of a poorly differentiated panCK-positive tumor or an adenocarcinomatous NOS tumor, it is mandatory to exclude neoplasms that can be efficaciously treated. Currently,