Brain Metastasis from CUP

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Brain metastases (BMs) are the most common intracranial tumours in adults and occur up to 3–10 times more frequently than primary brain tumours. BMs may be the cause of the neurological presenting symptoms in patients with otherwise previously undiagnosed cancer. In up to 15% of patients with BMs, the primary tumour cannot be identified. These cases are known as BM of cancer of unknown primary (CUP) (BM-CUP). CUP has an early and aggressive metastatic spread, poor response to chemotherapy, and poor prognosis. The pathogenesis of CUP seems to be characterized by a specific underlying pro-metastatic signature. This entry is a review of modern diagnostic and therapeutic approaches to brain metastases from unknown primary tumor.

Keywords: brain metastases, unknown primary tumor, genetics

1. Introduction

Brain metastases (BMs) are the most common intracranial tumours in adults and occur up to 3–10 times more frequently than primary brain tumours [1]. Population-based data reported that about 8–20% of patients with cancer are affected by symptomatic BMs, although an incidence up to 40% has been observed in autoptic series. BMs may be the cause of neurological presenting symptoms in patients with otherwise previously undiagnosed cancer. More commonly, the origin of BMs is found in the lung (20–56% of patients), breast (5–20%), and skin (melanoma) (7–16%). However, in up to 15% of patients with BMs, the primary tumour cannot be identified. These cases are known as BM of cancer of unknown primary (CUP) (BM-CUP) [1][2]. CUP is the seventh/eighth most common malignancy, accounts for 2–5% of all malignancies, and is the fourth most common cause of cancer-related deaths. CUP manifests, by definition, by metastases. It is more commonly characterised by early and aggressive metastatic spread, poor response to chemotherapy, and dismal prognosis, which has led to the hypothesis of an underlying pro-metastatic signature [3].

2. Definition and Diagnosis of CUP

CUP is defined as a histologically confirmed metastatic cancer for which clinicians are unable to identify a primary tumour source after the diagnostic workup.

Different definitions of CUP have been used by different authors over the years, complicating the identification of a homogeneous entity in literature. BM-CUPs have been variably defined either as BM with no previously known cancer (i.e., BM as the first manifestation of a systemic cancer, with the primary site not always remaining unknown) [4][5][6][7][8][9][10][11][12][13], BM in which the primary site has not been identified within a temporal interval from onset (more often 2–3 months) [14][15][16][17][18], or as BM with no primary tumour identified after standard or extensive work-up [19][20][21][22][23][24][25][26][27][28]. In some studies, the definition of CUP is not clearly stated [29][30][31][32][33][34][35][36][37][38][39][40][41][42][43][44][45][46]. The chance of identifying a primary site through clinical and histopathological evaluation has changed over time due to improvements in imaging and surgical techniques. Thus, it is difficult to provide accurate incidence data and compare studies from different periods. Advances in technology have increased the likelihood of finding the primary tumour, probably contributing to the decrease in the incidence of CUP in general [47][48]; however, in some cases, the primary tumour remains unknown even after autopsy [49]. Overall, CUP seems to account for approximately 10–15% of BMs; however, a range between 1% and 61% is reported throughout the literature, with rates around 1% in more recent studies on large BM cohorts [8][33] as compared to 50–60% in older studies [21][28].

Currently, the diagnostic standard for CUP, as laid down in the European Society of Medical Oncology (ESMO) guidelines [50], includes physical examination, basic blood and biochemistry analyses, as well as computed tomography (CT) or magnetic resonance imaging (MRI) of the chest, abdomen, and pelvis, and tissue for histological characterization and immunohistochemistry (IHC); further tests should be performed based on the clinical picture and the IHC profile. In addition, in case of CUP, the European Association of Neuro-Oncology (EANO) guidelines on BM [51] recommend mammography and/or ultrasound of breast and, if negative, whole-body 18F-fluordesoxyglucose position emission

tomography (FDG-PET). The primary tumour detection rate after FDG-PET in CUP ranges between 22% and 73% [52]. With regard to BM-CUP, a recent study reported a sensitivity of 85–92%; however, the comparison between FDG-PET/CT and chest/abdomen CT did not demonstrate any difference in localising the primary lesion, probably because most primary tumours were located in the lung. However, FDG-PET/CT improved the accuracy in detecting other metastases, thus decreasing the graded prognostic score (GPA) in the prediction of survival [11]. Other studies report a similar detection rate and confirmed the superiority of FDG-PET in identifying additional metastatic lesions [53][54]. Common serum tumour markers currently have no diagnostic, prognostic, or predictive value for CUP due to their unspecific expression [55]. Table 1 summarizes the imaging techniques used in the diagnosis of BM.

Table 1. Imaging techniques for the diagnosis of brain metastases (BM).

Contrast-Enhanced Computed Tomography (CT) Scan	Can be performed in emergency, no contraindications (except pregnancy and allergy to contrast medium). Allows visualization of acute bleeding. Less sensitive than MRI (especially for lesions in the posterior fossa, multiple punctate metastases and for leptomeningeal metastases)
Contrast-enhanced magnetic resonance (MRI)	Higher resolution then CT scan; needs patient's collaboration (not suitable in case of psychomotor agitation, claustrophobia). Contraindicated in patients with medical devices that are not compatible (some types of pacemaker, metallic implants, etc.) and if there are contraindications to the contrast medium (allergy, risk of nephrogenic systemic fibrosis, etc.).
Diffusion-weighted (DW)-MRI	Useful in differential diagnosis with abscesses: diffusion usually restricted in abscesses and unrestricted in BM. Exception: mucinous BM can show restricted diffusion
Gradient echo /other susceptibility-weighted images (SWI)	Useful for the identification of hemosiderin and other blood breakdown products. May improve detection of haemorrhagic BM.
Perfusion MRI - cerebral blood volumes (CBVs)	Peri-tumoral CBV lower in BM than in malignant gliomas, while higher in BM than in abscesses
MRI spectroscopy (MRS)	Lower choline/creatinine ratios in BM compared to high grade gliomas.
18F-fluordesoxyglucose (FDG)-Positron emission tomography (PET)	Lower sensitivity and specificity than MRI in the detection of BMs. Does not provide enough information for differential diagnosis. Whole body FDG-PET useful to identify the primary tumour or other metastasis (see main text).

3. Pathogenesis of CUP

The difficulty in identifying the primary tumour might imply that the latter is undetectable either at diagnostic testing or at autopsy, or that CUP constitutes a distinct entity, which challenges classical models of metastasis pathogenesis. Most of the biological steps that are believed to contribute to the pathogenesis of CUP are hallmarks of cancer in general and shared with other neoplastic diseases: chromosomal alterations, self-sufficiency in growth signals, resistance to growth inhibitory signals, reprogramming of energy metabolism, evasion of apoptosis, unlimited replicative potential, bright angiogenesis, tissue invasion, metastasis, and evasion of immune attack [55].

A high degree of chromosome instability (CIN) seems to be related to the characteristics of CUP, including aggressive evolution, early metastatic spread, and resistance to chemotherapies [56]. Moreover, CIN might explain the pathogenesis of CUP: a current hypothesis is that the high degree of CIN in CUP metastatic sites makes CUP metastasis resistant to immune surveillance [57], whereas the corresponding less chromosomally instable primary tumour may have regressed. Therefore, the lack (or regression) of a primary tumour in CUP could be interpreted in some cases as an immune-

mediated event [58]. An alternative or complementary hypothesis considers CUP metastases in a certain organ as an immunologically edited version of a tumour arising from the same organ. Thus, CUP metastases may constitute a phenotypically modified primary tumour lacking tissue identification and resulting from epitope immunoediting. Familial clustering and association of metastatic location with the affected organ system in family members seem to support this hypothesis [59].

4. The Role of Immunohistochemistry

According to EANO guidelines [51], a tissue diagnosis is mandatory in patients with suspected BM on MRI and unknown primary tumour after a systemic workup before any treatment is undertaken. The histological, ultrastructural, and IHC features of BM usually reflect the characteristics of the primary tumour: therefore, these analyses can be helpful in cases of CUP in the attempt of identifying the primary lesion (Table 2). However, in 11% of patients with BM, the primary tumour remains unknown despite extensive IHC and molecular techniques [35]. In studies on BM-CUP, adenocarcinoma was the most common histological type (235/370 lesions, 63.5%), followed by squamous cell carcinoma (SCC) (37/370, 10%) [4][5][6][7][10][14][17][18][23][27][28][44][60][61] (Table 3). Giordana et al. [5] analysed 99 patients with BM as the first manifestation of cancer using antibodies to carcinoembryonic antigen (CEA) for gastrointestinal or lung cancer, carbohydrate antigen (CA)19.9 for gastrointestinal cancer, cytokeratin (CK) 20 for colon cancer, CA125 for ovarian cancer, BCA-225 for breast cancer, PSA for prostatic cancer, and HMB-45 for melanoma. Among BM-CUP, 20/26 were intensely positive for CEA, 2 for CEA and CA125, and 11 for CEA and CA19.9. None were positive for either CEA, CA19.9, CA125, PSA, or HMB-45. Overall, the immunophenotypes of BM-CUP resembled BM from non-small lung cancer (NLSC) or from colon cancer, but no definite conclusion about the origin was drawn. In the study by Drliceck et al. [39], a combination of antibodies against different CK for epithelial markers (AE1/AE3 (a keratin cocktail that detects CK1-8, CK10, CK14–16, and CK19); CK7, CK10/13, CK18, and CK20), vimentin (epithelial cancers), protein S100 (melanoma), TTF-1 (lung), CA 15-3 (breast), CA19.9, CA125, and PSA was used to identify the primary origin of BM. The primary tumour was identified in 37 out of 54 (68.5%) BM, 29/40 (72.5%) in BM associated with clinically known primary, and 8/14 (57.1%) in BM-CUP. The authors established a first-line panel to analyse BM-CUP, consisting of CK AE1/AE3, CK7, CK18, CK20, vimentin, protein S100, TTF-1, and CA 15-3. In case of positivity for CA 15-3, markers, like CA125 and CA19.9 were suggested to confirm the breast as primary tumour; if the first-line investigation is inconclusive, surfactant (lung) and PSA (prostate) should be added.

Table 2. Most used immunohistochemical markers for brain metastasis.

Squamous Cell K.	CK5/6, CK7, EMA, GFAP
Small cells K of the lung	CD56, CK7, TTF1, EMA
Lung Adeno-K	CK7, TTF1, Napsin A, EMA, CAM 5.2, CEA, RCC (Variable)
Breast Adeno-K	CK5/6 (Variable), CAM 5.2, CK7, GCDFP15, EMA, S100 (variable), CEA, CA 15-3
Endometrial K	CAM 5.2, CK7, CA 125, ER (variable), CA125, CEA
Colorectal Adeno-K	CAM 5.2, CK20, CDX2, EMA, CEA, CA 19.9
Stomach Adeno-K	CAM 5.2, CK7, CK20, CDX2, EMA, CEA, CA 19.9
Prostate Adeno-K	PSA, EMA
Urothelial K	CK5/6, CK7, CK20, EMA
Renal cell K	RCC, EMA, PAX8, Vimentin
Melanoma	Vimentin, Melan A, S100, HMB 45

K = carcinoma; Adeno-K= adenocarcinoma; CK = cytokeratin; EMA = epithelial membrane antigen; TTF1 = Thyroid transcription factor 1; PSA = prostate-specific antigen; RCC = renal cell carcinoma protein; CEA = Carcinoembryonic antigen; CA = carbohydrate antigen.

Table 3. Histological diagnosis and immunohistochemical (IHC) markers in the diagnosis of brain metastases from cancer of unknown primary (BM-CUP).

Studies	Histological Diagnosis	Immuno-Histochemical Markers Used	Confirmed Primaries at Follow-Up
Matsunaga et al., 2019 [27]	42 Adeno-K, 4 squamous-cells, 2 neuroendocrine	NS	NS
Mavrakis et al., 2005 [6]	7 Adeno-K, 2 poorly differentiated malignant epithelial tumours	NS	2/9 (22%)
Drlicek et al., 2004 [39]	Lung 5, colorectum 1, breast 1, kidney 1	CK (AE1/AE3, 7, 10/13, 18, 20), vimentin, protein S100, TTF-1, and CA 15-3, 19.9, 125, PSA	0
Bartelt and Lutterbach, 2003 [14]	15 Lung Adeno-K,4 Squamous-cells, 4 Large-cells, 4 Small-cells, 20 Other	NS	NS
Klee et al., 2002 [60]	14 Adeno-K (12 CK 7 +, none CK 20), 1 carcinoma (no CK 7, CK 20 and CK), 1 melanoma	CK7, CK20, PSA, HCG, CA125 and “antibodies indicating breast or pulmonary primary”	7/14 (50%)
Rudà et al., 2001 [61]	4 Lung Adeno-K, 1 Squamous-cells, 3 Colon Adeno-K, 1 Pancreatic Adeno-K, 18 no diagnosis	NS	27/33 (81%)
Maesawa et al., 2000 [17]	10 Adeno-K, 2 Squamous-cells, 1 Clear-cells, 2 Undifferentiated	NS	4/15 (26.7%)
Nguyen et al., 1998 [18]	31 Adeno-K, 2 small-cells,1 squamous-cells, 4 other, 1 missing	NS	12/39 (31%)
Salvati et al., 1995 [10]	65 Adeno-K, 10 Squamous-cells, 10 Melanoma, 7 Undifferentiated, 7 other small-cells	NS	64/100 (64%)
Debevec, 1990 [44]	Anaplastic K and adeno-K were most frequent	NS	47/75 (63%)
Merchut, 1989 [7]	8 Adeno-K + 1 squamous-cells	NS	47/56 (84%)
Chee and Byrnes, 1988 [4]	5 Adeno-K, 3 anaplastic, 4 squamous-cells, 1 sarcoma, 1 transitional-cells	NS	35/51 (68%)

Eapen et al., 1988 [23]	9 No diagnosis, 19 Adeno-K, 7 Squamous K, 5 Anaplastic K, 1 large-cells, 1 small-cells, 1 transitional-cells	NS	11/43 (25%)
Zimm et al., 1981 [28]	14 Adeno-K, 2 squamous	NS	10/16 (37%)

NS = not specified, IHC = immunohistochemistry, K = carcinoma, Adeno-K = adenocarcinoma.

5. Gene Expression Profiling: A New Frontier

In recent years, gene expression profiling (GEP) has become an additional tool for diagnostic, prognostic and predictive purposes. Second-generation microRNA-based assays, GEP-based microarray tests, or quantitative-PCR (qRT-PCR) low-density arrays have reached a sensitivity of 77–94% in identifying CUP [48][55]. Results from a blinded series of high-grade metastatic cases demonstrated the superior accuracy of a 92-gene assay versus standard-of-care IHC, supporting the diagnostic utility of a molecular investigation in difficult-to-diagnose metastatic cancer [62]. Nevertheless, scepticism persists regarding the use of GEP in CUP, as frequent mutations in CUP include TP53, RAS, CDKN2A, MYC, ARID1A, PIK3CA, and BRAF, which are not tissue specific. Moreover, significant discrepancy has emerged between the suspected primary tumour based on molecular profiling and autoptic confirmation. Finally, tumour cells may undergo extensive immunoediting (see the pathogenesis section), which raises doubts over the accuracy of a diagnosis based on GEP positivity [55][58]. Applications of these techniques to BM-CUP have been limited. Mueller et al. described a microRNA-based test (48 microRNAs) that classified 84% (75 of 89) of BM of known primary. When applied to central nervous system (CNS) metastasis from CUP (CNSM-CUP), the test prediction was in agreement with the diagnosis, either clinically or pathologically confirmed, in 80% of cases [35]. An updated version of the assay (64 microRNAs) reached an overall sensitivity of 85%, measured blindly on a validation set of 509 independent samples. Moreover, a clinical validation study on 52 CNSM-CUP patients showed an 88% concordance [34]. A method based on DNA methylation profiles has recently been used to distinguish gliomas from BM, and has been tested in the identification of the origin of BM with known primary, correctly classifying 95% of the samples. Data on BM-CUP are not available [63]. Liquid biopsies collect and analyse circulating tumour cells (CTCs) and cell-free tumour (ctDNA) in body fluids (such as cerebrospinal fluid (CSF) and plasma); these can be a surrogate of tumour tissue in the management of both primary and secondary brain tumours [64]. For example, CSF ctDNA analysis has been successfully used in a case of multiple leptomeningeal and spinal cord metastases from CUP [65] (this case is discussed below).

Indeed, a promising application of GEP in BM-CUP (and CUP in general) relies on the possibility of identifying targetable molecular markers, rather than identifying a suspected primary site. This is motivated not only by a recent hypothesis in the pathogenesis of CUP but more importantly by recent advances in target therapies.

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