China Anti-Cancer Association guideline for diagnosis and treatment of cancer of multiple and unknown primaries (2024 edition)

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Chapter I

Cancer of Unknown Primary

Section 1 General Principles for Diagnosis and Treatment of Cancer of Unknown Primary

1 Overview

Cancer of unknown primary (CUP), also known as a primary unknown tumor, unknown primary tumor, or occult tumor/cancer, refers to metastases that are diagnosed as malignant tumors through pathology, but where the primary site cannot be identified despite detailed medical history, physical examinations, and various tests before treatment. Reasons for CUP may include inadequate diagnostic methods, insufficient tissue sampling, prior removal of the primary lesion, extensive tumor metastasis making identification of the primary lesion difficult, unique patterns of tumor spread, small size of the primary lesion, or spontaneous regression of the primary lesion. Even with autopsy, 20% to 50% of examined patients still cannot locate the primary lesion. Due to its low incidence rate and high heterogeneity, there is relatively limited evidence from evidence-based medicine, and clinical understanding of CUP remains relatively limited. Diagnosis and treatment of CUP continue to face significant challenges. The development and publication of these guidelines aim to provide comprehensive strategies and standardized guidance for the diagnosis and treatment of CUP patients, to improve the efficiency and quality of care for CUP patients.

CUP patients have a poor prognosis. Literature reports indicate a median survival period of only 8 to 12 months, with early dissemination, aggressiveness, and unpredictable patterns of metastasis being characteristic of these tumors. For patients with favorable subtypes of CUP, the median overall survival is approximately 12 to 36 months. Therefore, early and effective diagnosis, along with targeted treatment, are crucial for improving the survival rates of these patients. In terms of diagnosis, any clinical clues that may help track the primary lesion can not be omitted. Based on detailed medical history, comprehensive physical examinations are needed to find diagnostic clues as much as possible. Imaging examination includes ultrasound, X-ray, Computed tomography (CT), Magnetic resonance imaging (MRI), Emission-computed tomography (ECT), and Positron emission tomography (PET)/CT. During the diagnosis and treatment of CUP, endoscopic examinations should not be conducted blindly, but rather selected based on relevant clinical evidence and clues. Other means helpful to find the primary sites include sentinel lymph node theory, isolated or localized bone metastasis histological biopsy, ^{18}F-FES PET/CT (estrogen receptor-targeted molecular imaging), ^{68}Ga-DOTATATE PET/CT (neuroendocrine imaging) and other tumor-specific molecular markers for PET/CT. Tumor markers, especially the spectrum of tumor markers, help to indicate the location or system of the primary tumor. Histopathological examination is the gold standard for diagnosing CUP. The 90-gene expression assay (Canhelp-Origin Assay), as a new generation molecular pathology technique, more accurately identifies the primary site or tissue origin of tumors. If tissue samples cannot be obtained, abnormal/cancerous cells found in pleural/abdominal/cerebrospinal fluid can be embedded in paraffin wax. Subsequent immunohistochemical analysis of these samples can also serve as a crucial diagnostic standard.

Overall, there are two basic principles for clinical diagnosis of CUP: First, consider the possibility that the common malignant tumor in China is the primary cancer; Second, do not misdiagnose or miss the diagnosis of tumors with good prognosis or curability. To formulate individualized precision treatment plans, it is recommended to first perform the 90-gene expression assay followed by Next Generation Sequencing (NGS) to diagnose the tissue origin of the tumor and identify gene mutations corresponding to the tumor type. It is recommended to participate in the Multiple Discipline Team to Holistic Integrative Management (MDT to HIM). It is also strongly recommended to participate in clinical trials or provide site-specific treatment based on the results of the 90-gene expression assay combined with NGS, or to administer empirical therapy. It needs to be emphasized that finding primary lesions is a scientific issue and a very rigorous process. Some primary lesions can appear months or even years later after the first presentation. Once a new lesion is suspected to be a primary lesion, it needs to be confirmed by biopsy again. For most CUP patients, systemic therapy is palliative and does not significantly improve survival. Especially for patients with disseminated disease, the treatment goal is symptom control and optimizing quality of life. Specific pathological studies can identify subtypes of tumors that may respond better to systemic therapy. Treatment choices should be individualized for this specific patient group to achieve optimal response and survival rates. Additionally, during the diagnosis and treatment of CUP, regular follow-up and reexamination should be emphasized to carry out.

2 Epidemiology

CUP accounts for 3%-5% of all malignant tumors and ranks as the sixth to eighth most common cancer worldwide. Its incidence varies by country and ethnicity. Reviewing published CUP registry data suggests a decline in incidence, possibly due to improved identification of primary tumors. In Europe and Australia, CUP incidence initially increased from 1990 to 2000, then transitioned to a subsequent decline. In the United States, CUP incidence peaked in the 1980s and then declined at an annual rate of 3.6% in the following two decades, faster than in other countries. At present, CUP incidence is reported as 4.1 cases/100,000 people in the United States and 5.8-8 cases/100,000 people in Europe, and higher at 14-15 cases/100,000 people in Australia compared to European countries. China's data have not been reported. CUP incidence increases significantly with age, peaking around 80 years old, with a median onset age of 60-75 years. Less than 1% of CUP cases are observed in children associated with solid tumors. CUP incidence in men is slightly higher than that in women in some countries.

Early metastasis involving multiple organs is a primary clinical feature of CUP, with lymph nodes, bones, liver, and lungs being the most commonly affected sites. Less frequently observed sites include the brain, pleura, peritoneum, and skin. Based on histopathological characteristics, CUP is typically classified into several types: well or moderately differentiated adenocarcinoma (approximately 50%), poorly differentiated or undifferentiated adenocarcinoma (approximately 30%), squamous cell carcinoma (approximately 15%), and undifferentiated-tumors (approximately 5%).

3 Writing suggestions for the diagnosis of cancer of unknown primary in case records

3.1 Standardize the disease code of the CUP input

3.2 Suggested CUP diagnostic writing

The sequence is suggested to be as follows: the cancer of unknown primary, the sites or organs of tumor involvement, and the possible primary site. Example: adenocarcinoma of unknown primary lesion, bone and retroperitoneal lymph node metastasis, ovary primary?

(1) If there is a tumor in the ovary and it is not clear whether it is primary, it is needed to write as "possible primary ovarian cancer".

(2) If there is no lesion in the ovary and the ovarian source is clinically and/or pathologically suggestive, it is needed to write as "possible ovarian origin".

3.3 Writing order of metastasis

Metastases are written in order of severity, including brain, liver, lung, bone and lymph nodes. For example, squamous cell carcinoma with unknown primary focus (gland, neuroendocrine, etc.), brain, liver, lung, bone and lymph node metastasis.

3.4 Other contents of diagnostic writing

(1) Should include the concurrent disease that currently receives active treatment.

(2) Should include prior serious diseases, which may affect the choice of drug treatment, such as myocardial infarction and stroke, although fully recovered.

(3) Should include serious conditions including symptoms and laboratory tests needing to be handled in priority, such as pericardial effusion, pathological fracture, grade IV thrombocytopenia, etc.

4 MDT to HIM diagnosis and treatment model of cancer of unknown primary

4.1 MDT to HIM discipline composition

Oncology Department, Surgery Department, Radiotherapy Department, Diagnosis Department (Pathology Department, Imaging Department, Ultrasound Department, Nuclear Medicine Department, etc.), Intervention Department, Endoscopy Department, Nursing Department, psychological experts, nutritional support and social workers.

4.2 MDT to HIM member requirements

It shall at least include: one physician in Oncology Department, Surgery Department, Radiation Diagnosis Department, Nuclear Medicine Department, Histopathology Department and Cell Pathology Department, and several doctors in other specialties. All doctors participating in MDT to HIM discussion shall have the professional title of deputy senior or above and have the ability of independent diagnosis and treatment.

4.3 MDT to HIM discussion content

The possible primary site of the patient and further examinations and treatment needed.

4.4 MDT to HIM daily activities

Fixed experts, fixed time, fixed place, once a week, and submit the medical history and imaging data to relevant experts in advance.

4 MDT to HIM discussion result template of cancer of unknown primary

(1) After the discussion of multidisciplinary experts, it is believed that: according to the patient's medical history, symptoms, physical examination, imaging, endoscopy, pathological examination, etc., the diagnosis is... If the expert's discussion results clarify the source of the tumor, the treatment scheme is recommended according to the current guidelines. If the expert discussion results initially suspect the origin of the tumor, the Pathology Department can supplement the immunohistochemical detection of the corresponding tumor species and can advise the 90-gene expression assay for identifying tumor tissue origin.

(2) It is suggested to detect circulating tumor DNA (ctDNA) or tissue NGS to find possible targeted therapeutic drugs after a full discussion with the patient and his family.

(3) Try to develop a whole course and all-around treatment strategies: ① Evaluate the possibility of major adverse events soon. For example: pathological fracture, spinal cord compression and pericardial tamponade, preventive and therapeutic measures shall be taken. ② If the patient has other basic diseases, it is recommended to see a specialist (such as chronic hepatitis B, tuberculosis, hypertension, diabetes, etc.). ③ Outpatient or oncology outpatient follow-up with an unknown primary site and tumor-specific disease.

Section 2 Diagnostic Principles of Cancer of Unknown Primary

1 Suspected cancer of unknown primary

Diagnosis of Suspected Cancer of Unknown Primary

	General items	Special items
Initial evaluation	① Complete medical history and physical examination: including breast, urogenital tract, pelvic cavity and anal examination for any possible clue, with special attention to: past biopsy history or malignant tumor history, prior resected lesions (re-immunohistochemical detection if necessary), spontaneously retracting lesions, existing imaging examination and tumor family history ② Blood routine, liver and kidney function (including lactate dehydrogenase, LDH), electrolyte; Urine routine, fecal routine + occult blood; Tumor marker detection ③ Chest, abdomen and pelvis enhanced CT, neck enhanced CT or MRI; Or PET/CT	Clinical guided endoscopy, mammography /MRI
Routine and molecular pathological diagnosis	Biopsy: core needle biopsy (preferred) or fine needle aspiration cell mass or pleural and ascites cell mass. Communicate with pathology experts about the sample's meeting requirements and the selection of antibodies for immunohistochemistry	TMB NTRK MSI/MMR detection After initial histological identification, a stepwise approach can be considered, starting with the 90-gene expression assay followed by NGS (or other gene fusion identification techniques) to perform a molecular profiling of the tumor tissue.
Pathological diagnosis results	① Epithelial origin, non-specific site → treat as CUP ② Non-epithelial origin, such as lymphoma, melanoma, sarcoma, germ cell tumor, etc. → treat according to the corresponding guidelines ③ Non-malignancy → further evaluation and appropriate follow-up	

2 Epithelial tumor, non-specific site

(1) If the pathological diagnosis is adenocarcinoma or nonspecific cancer, the tumor location region can be divided into: neck, supraclavicular lymph node, axillary lymph node, mediastinum, chest, liver, retroperitoneum, peritoneum, inguinal, bone and brain. If the tumor lesions are extensive, it can be classified into multiple tumors, including skin.

(2) If the pathological diagnosis is squamous cell carcinoma, the tumor location region can be divided into: head and neck, supraclavicular, axillary, groin and bone. If the tumor lesions are extensive, it can be classified into multiple tumors.

(3) If the pathological diagnosis is neuroendocrine tumor, refer to the corresponding diagnosis and treatment guidelines of neuroendocrine tumor.

3 Localized adenocarcinoma or nonspecific carcinoma

Metastatic adenocarcinoma with unknown primary should be examined and evaluated according to the located region of the tumor. The initial diagnostic evaluation should include at least a medical history and physical examination, complete blood cell count, tumor marker analysis, imaging examinations of the neck/chest/abdomen/pelvis, biopsy of the most accessible lesion site with hematoxylin-eosin (H&E) staining and immunohistochemical testing.

(1) For the head and neck, it is recommended to perform CT or MRI scans of the neck, chest, abdomen, and pelvis. If conditions permit, PET/CT or PET/MRI scans can also be considered. Clinical-guided nasopharyngoscope and laryngoscope should be performed. Additionally, biopsy with HE staining and immunohistochemical testing should also be performed. Tumor marker testing should include carcinoembryonic antigen, squamous cell carcinoma antigen (SCC), Epstein-Barr virus, etc.

(2) In the clavicle area, it is recommended to perform CT or MRI scans of the neck, chest, abdomen, and pelvis. If conditions permit, PET/CT or PET/MRI scans can also be considered. Clinical-guided endoscopy should be performed when necessary. For women, it is recommended to undergo breast ultrasound, and if necessary, breast X-ray and breast MRI examinations. Additionally, a

biopsy with H&E staining and immunohistochemical testing should be performed. Tumor marker testing should include carcinoembryonic antigen, carbohydrate antigen (CA) 125, etc. Men over 40 years old should be tested for prostate-specific antigen (PSA).

(3) For the axillary pit, it is recommended to perform CT or MRI scans of the neck, chest, abdomen, and pelvis. If conditions permit, PET/CT or PET/MRI scans can also be considered. For women, it is recommended to undergo breast ultrasound, and if necessary, breast X-ray and breast MRI examinations. Additionally, a biopsy with H&E staining and immunohistochemical testing should be performed. Tumor marker testing should be performed. Men over 40 years old should be tested for PSA.

(4) For the mediastinum, it is recommended to perform CT or MRI scans of the chest, abdomen, and pelvis. If conditions permit, PET/CT or PET/MRI scans can also be considered. Endoscopy should be considered in conjunction with clinical symptoms. For women, it is recommended to undergo breast ultrasound, and if necessary, breast X-ray and breast MRI examinations. Additionally, a biopsy with HE staining and immunohistochemical testing should be performed. Serum tumor marker testing should include alpha-fetoprotein, carcinoembryonic antigen, carbohydrate antigen 199, CYFRA21-1, β -human chorionic gonadotropin (β-HCG), lactate dehydrogenase, etc. If necessary, a testicular ultrasound should be performed. Men over 40 years old are recommended to be tested for PSA.

(5) For the chest, it is recommended to perform CT or MRI scans of the chest, abdomen, and pelvis. If conditions permit, PET/CT or PET/MRI scans can also be considered. Clinical-guided endoscopy should be performed when necessary. Consult the gynecooncologist when necessary if the abnormally increased. For women, it is recommended to undergo breast ultrasound, and if necessary, breast X-ray and breast MRI examinations. Additionally, a biopsy with HE staining and immunohistochemical testing should be performed. Serum tumor marker testing should include carcinoembryonic antigen, carbohydrate antigen 199, carbohydrate antigen 125, CYFRA21-1, etc. Men over 40 years old should be tested for PSA.

(6) For pleural effusion and ascites, it is recommended to perform CT or MRI scans of the chest, abdomen, and pelvis. If conditions permit, PET/CT or PET/MRI scans can also be considered. In general, gastrointestinal endoscopy is necessary. Women should undergo breast ultrasound, and if necessary, breast X-ray and breast MRI. Additionally, tumor markers, exfoliated cells for cytology, sediment of pleural and ascites for embedding, and immunohistochemistry are recommended. If urine cytology is performed, cystoscopy should be performed if necessary. Consult the gynecooncologist when necessary if the abnormally increased. Tumor marker testing should include carcinoembryonic antigen, carbohydrate antigen 199, carbohydrate antigen 125, and HE4. Men over 40 years old should be tested for PSA.

(7) For the retroperitoneum, it is recommended to perform CT or MRI scans of the chest, abdomen, and pelvis. If conditions permit, PET/CT or PET/MRI scans can also be considered. In general, gastrointestinal endoscopy is necessary. Consult the gynecooncologist when necessary if the abnormally increased. Additionally, a biopsy with H&E staining and immunohistochemical testing should be performed. Immunohistochemical markers include those from gastrointestinal and reproductive system origins, or as referenced by clinical indications for selection. For suspected urinary tumors, consider cystoscopy in addition to urine cytology examination. Tumor marker testing; PSA test should be performed for men over 40 years old and β-HCG, AFP, and testicular ultrasonography should be performed for men younger than 65 years old.

(8) For the groin, it is recommended to perform CT or MRI scans of the chest, abdomen, and pelvis. If conditions permit, PET/CT or PET/MRI scans can also be considered. In general, rectal endoscopy is necessary. Women should undergo gynecological physical examination and colposcopy when necessary. Consult the gynecooncologist when necessary if the abnormally increased. Serum tumor marker testing should be conducted. Men over 40 years old should be tested for PSA. Perineal skin examination is conducted to confirm extramammary Paget's disease.

(9) For the liver, it is recommended to perform CT or MRI scans of the chest, abdomen, and pelvis. If conditions permit, PET/CT or PET/MRI scans can also be considered. In general, endoscopy is necessary. For women, if clinical signs or immunohistochemical evidence support breast cancer, it is recommended to perform breast ultrasound, and if necessary, breast X-ray and/or breast MRI. Biopsy with H&E staining and immunohistochemical testing should be performed. Serum tumor marker testing should include AFP, CEA, CA199, CA125, and PSA for men.

(10) For the bone, it is recommended to perform CT or MRI scans of the chest, abdomen, and pelvis, and bone scan. If conditions permit, PET/CT or PET/MRI scans can also be considered. Clinical-guided tracheoscopy should be performed when necessary. Women should be examined by breast ultrasound. If there are clinical signs or immunohistochemical evidence supporting breast cancer, it is needed to perform breast MRI and/or breast X-ray. Biopsy with HE staining and immunohistochemical testing should be performed. Serum tumor marker testing should be conducted. Men should be tested for PSA. For poorly differentiated cancers, protein electrophoresis and bence-jone protein examination are recommended.

(11) For the brain, brain MRI, and CT or MRI scans of the chest, abdomen, and pelvis should be performed. If conditions permit, PET/CT or PET/MRI scans can also be considered. Women are recommended to be examined by breast ultrasound. If there are clinical signs or immunohistochemical evidence to support breast cancer, it is needed to perform a breast MRI and/or breast X-ray. Biopsy with HE staining and immunohistochemical testing should be performed. If there are no contraindications, lumbar puncture for tumor markers in cerebrospinal fluid, exfoliated cells for cytology, sediment embedding, and immunohistochemistry are recommended. Serum tumor marker testing should be conducted.

(12) For multiple parts, it is recommended to perform CT or MRI scans of the neck, chest, abdomen, and pelvis. If conditions permit, PET/CT or PET/MRI scans can also be considered. Endoscopy should be considered in conjunction with clinical symptoms. Women are recommended to be examined by breast ultrasound. If there are clinical signs or immunohistochemical evidence to support breast cancer, it is needed to perform a breast MRI and/or breast X-ray. Biopsy with HE staining and immunohistochemical testing should be performed. Serum tumor marker testing should be conducted. Men should be tested for PSA.

4 Squamous cell carcinoma

Metastatic squamous cell carcinoma with unknown primary should be examined and evaluated according to the location region of the tumor.

(1) For the head and neck, corresponding examinations such as head and neck should be performed. Refer to the corresponding guidelines for head and neck tumors.

(2) For the clavicle area, contrast-enhanced CT scans of the neck, chest, abdomen, and pelvis should be performed. FDG-PET/CT can serve as an alternative method for those who cannot undergo contrast-enhanced CT scans.

(3) For the armpits, contrast-enhanced CT scans of the chest should be performed.

(4) For the groin, contrast-enhanced CT scans of the abdomen and pelvis should be performed. FDG-PET/CT serves as an alternative method for those who cannot undergo contrast-enhanced CT scans. Physical examination of perineum and lower limbs should be performed, male including the penis, scrotum, and other parts, female including the cervix, vulva, etc; Anodigital examination, anoscope/rectoscopy if necessary; Skin examination of buttocks, lower limbs, and feet; Cystoscopy if there are urinary related symptoms.

(5) For the bone, a bone scan should be performed (for example, only chest/abdomen / pelvic CT has been done before); Imaging diagnosis, differential diagnosis, and risk assessment (such as fracture, spinal cord compression, etc.) should be performed on the positive parts of bone scan. If bone scanning cannot be performed, the painful part must be imaged.

Additionally, regardless of the tumor pathology type, PET/CT scans are recommended in the following two situations:

(1) For patients with lymph node metastasis in the head and neck region;

(2) For patients with localized disease that can potentially be cured, with single or few metastases, before local treatment (including surgery and/or radiotherapy/chemotherapy), PET/CT scans are still recommended to rule out potentially overlooked tumors.be imaged.

5 Tumor marker spectrum

Tumor refer to tumor-related substances that can be detected in blood, body fluids, and tissues. When they reach a certain level, they can reflect the existence of some tumors. After surgery, chemotherapy, or radiotherapy, the content of specific tumor markers has a good correlation with the curative effect. These tumor markers can also help to assess the disease condition, monitor the curative effect, recurrence, and

metastasis, and judge the prognosis, to further improve the clinical diagnosis and treatment. Most of them are classified from biochemical properties and tissue sources, and there is no unified and comprehensive standard. For cancer of unknown primary, to further clarify the qualitative and primary-suggestive diagnosis value of tumor markers, the relevant progress is introduced as follows:

According to the high specificity of tumor markers, the following tumor markers are recommended for routine examination

(1) AFP: Alpha-fetoprotein (AFP) is the most sensitive and specific marker of hepatocellular carcinoma recommended for routine clinical use. It is also useful for the diagnosis of germ cell tumors and AFP-producing cancers. AFP is a glycoprotein. Continuous detection of AFP is very important for the diagnosis, curative effect observation, and prognosis of hepatocellular carcinoma. Sometimes it is advocated to use two different imaging methods (such as color Doppler ultrasound, CT, and/or MRI), which can be diagnosed in combination with biopsy.

(2) PSA: Prostate cancer is the most common tumor in men. Prostate-specific antigen (PSA) is the most ideal serum tumor marker of prostate cancer and is also the only recognized organ-specific tumor marker. It is often used in prostate cancer screening, staging and prognosis evaluation, curative effect judgment, and recurrence monitoring. PSA should be routinely examined especially in elderly men.

(3) HCG: HCG (human chorionic gonadotropin) is a glycoprotein hormone secreted by placental syncytiotrophoblast cells. Free-β-HCG is a specific indicator of germ cell tumor, which is closely related to the degree of tumor deterioration. It must be examined when the age is ≤ 40 years old and a germ cell tumor is suspected.

(4) CA125: Cancer antigen 125 (CA125) is a marker of epithelial ovarian cancer and endometrial cancer. It is the most widely used tumor marker for ovarian cancer prediction and efficacy monitoring. The increase in concentration is related to tumor load and stage. The content of CA125 in serous endometrial carcinoma, clear cell carcinoma, fallopian tube carcinoma, and undifferentiated ovarian carcinoma increased significantly. CA125 combined with pelvic examination and transvaginal ultrasonography can benefit women with a family history of ovarian cancer from early intervention.

(5) CA72-4: Cancer antigen 72-4 (CA72-4) is currently one of the best tumor markers for diagnosing gastric cancer. It exhibits high specificity for gastric cancer, with sensitivities ranging from 28% to 80%. When combined with CA199 and CEA in testing, it can detect over 70% of gastric cancers. Its primary advantage lies in its high specificity for distinguishing benign lesions. CA72-4 levels show a significant correlation with the staging of gastric cancer, and the positivity rate of CA72-4 is much higher in gastric cancer patients with metastasis compared to those without metastasis.

In addition to the commonly used tumor markers mentioned above, recommended tumor markers for other types of cancer.

Tumor markers of different tumor types

Suspected tumor type	**Recommended tumor markers**
Pituitary tumor	β-HCG, ACTH, prolactin
Nasopharyngeal tumor	EBV, SCC
Oral cancer	SCC
Thyroid cancer	TG, calcitonin, CEA
Thymic tumor (Type C - Thymic carcinoma)	β-HCG, AFP, SCC
Lung tumor	Squamous cell carcinoma: SCC-Ag, CYFRA21-1, TPA Adenocarcinoma: CEA, CYFRA21-1, TPA Small cell: ProGRP, NSE, TPA
Breast tumor	CA15-3, CEA, HER-2/neu (serum or tissue), CA125
Gastric tumor	CEA, CA72-4, CA199, AFP, CA125, CA50, EGFR, CA242, PG I/II
Hepatocellular cancer	AFP, AFP-L3, DCP

Biliary pancreatic tumor	CA199, CEA, CA242, CA125
Adrenal tumor	ACTH, DHEA-S, cortisol, aldosterone
Colorectal tumor	CEA, CA50, CA199, CA242, Ras (excrement), MSI (excrement)
Ureteral cancer	SCC
Bladder tumor	BTA, BLCA, CYFRA21-1, TPA, NMP22 (urine)
Ovarian tumor	CA125, HE4, CEA, HER-2/neu (serum), TPA
Cervical cancer	HPV, SCC, CEA
Prostate tumor	PSA, F-PSA/ T-PSA
Testicular tumor	AFP, β-HCG
Neuroendocrine tumor	NSE, ProGRP, serotonin, 5-hydroxyindoleacetic acid (urine)
Germ cell tumor	β-HCG, AFP, LDH
Melanoma	S100B, MIA

6 Radiological diagnosis

The purpose of CUP imaging examinations is to detect possible primary tumors. Different imaging techniques have their advantages and limitations, and efforts should be made to conduct thorough examinations whenever possible. In cases where patient conditions do not permit comprehensive imaging, the choice of local examination methods should be based on known sites of metastasis and/or pathological results. Multi-parameter MRI (mp-MRI) is recommended, which includes conventional plain imaging, diffusion-weighted imaging (DWI), dynamic contrast-enhanced MRI (DCE-MRI), or contrast-enhanced CT, and if necessary, three-phase scanning: including plain, arterial phase, and venous phase. Common sites for MRI and CT examinations are recommended as follows:

(1) For the metastatic squamous cell carcinoma of cervical lymph nodes, mp-MRI of the nasopharynx, oropharynx, larynx (pharynx), and oral cavity is recommended.

(2) Detection and differentiation of pulmonary lesions is best achieved by CT scan.

(3) The mp-MRI is superior to mammography and ultrasonography in the diagnosis and differentiation of breast lesions.

(4) CT urography (CTU) is of significant value in detecting small lesions in the urinary system, while for detecting and differentiating renal space-occupying lesions, mp-MRI is recommended.

(5) The mp-MRI is superior to CT in detecting and differentiating hepatic, pancreatic, and biliary tract lesions.

(6) The mp-MRI is of significant value in detecting and differentiating uterine and prostatic lesions.

(7) The mp-MRI is superior to CT in detecting bone and soft tissue lesions.

7 PET/CT

Positron emission tomography (PET) utilizes positron-emitting drugs to image their distribution within the body, enabling diagnosis and evaluation of diseases. PET can be used independently or in conjunction with CT and MRI imaging technologies, combining anatomical clarity and spatial localization to reveal the biological characteristics of tumors. Currently, PET equipment includes PET, PET/CT, and PET/MR, with PET/CT being the most widely used in China.

The indications for PET primarily depend on the selected positron-emitting drug. The most widely used positron imaging agent currently is ^{18}F-Fluorodeoxyglucose (^{18}F-FDG), a glucose analog that reflects the metabolic activity of tumor cells and exhibits high sensitivity in imaging the majority of malignant tumors. In a non-specific context, PET imaging is referred to as ^{18}F-FDG PET/CT whole-body imaging. PET/CT has better diagnostic value for the detection of primary lesions in patients with unknown primary metastases than conventional imaging examination. If possible, ^{18}F-FDG PET/CT examination is recommended as soon as possible. The specific values are reflected in the following aspects:

(1) Finding the primary lesion: The clinical study of small samples shows that the sensitivity and

accuracy of ^{18}F-FDG PET/CT in the detection of primary tumors are significantly higher than those of CT and MRI, but a large-scale randomized study is needed to determine the clinical value of ^{18}F-FDG PET/CT in the routine screening of CUP. At present, recent clinical evidence shows that ^{18}F-FDG PET/CT is shown to be valuable in guiding tracking of primary lesions in CUP patients with squamous cell carcinoma in the head and neck.

(2) Staging: PET/CT improves the accuracy of CUP staging and provides the basis for clinical decision-making. PET/CT can accurately assess the size, location, extent of infiltration, and presence of distant metastases in tumors. This information helps doctors choose the most suitable treatment for patients, improving treatment efficacy and survival rates. For example, when planning local curative treatment for CUP, PET/CT examination is essential

(3) Prognosis: PET/CT is helpful to predict the prognosis of CUP. Through comprehensive analysis of tumor burden, metabolic activity, and metastatic status, PET/CT can predict patient survival prognosis. The survival of localized or oligometastatic CUP is significantly better than that of multi-regional or multi-organ metastatic CUP. This prognostic evaluation helps doctors develop more reasonable treatment and rehabilitation plans, thereby enhancing patients' quality of life and survival.

Positronucleotide-labeled fibroblast activation protein inhibitors (FAPI), such as ^{68}Ga-FAPI and 18-FAPI, are ideal tumor tracers. They specifically bind to fibroblast activation protein on tumor-associated fibroblasts in the tumor microenvironment. Current small-sample studies indicate that FAPI PET is superior to FDG PET in imaging head and neck tumors and gastrointestinal tumors. For patients with unknown primary metastases, it is recommended, where feasible, to consider using FAPI PET imaging.

8 Pathological diagnosis

(1) Pathological diagnosis is the gold standard of CUP diagnosis.

(2) Pathological diagnosis requires sufficient tumor tissue, which can be obtained through surgical resection, incisional biopsy or core needle biopsy (CNB). When conditions are limited, fine needle aspiration (FNA) that can prepare cell blocks or pleural and ascitic cell masses can also be used.

(3) After standard optical microscope evaluation, CUP can generally be divided into five main subtypes, including:

a) Highly or moderately differentiated adenocarcinoma (60%)

b) Poorly differentiated adenocarcinoma (25%)

c) Squamous cell carcinoma (5%)

d) Undifferentiated carcinoma (5%)

e) Neuroendocrine tumor (5%)

(4) Immunohistochemistry and gene expression profiling assay are recommended to analyze the biopsy tissue to determine the origin of tumor tissue.

8.1 Immunohistochemistry

(1) The application of immunohistochemistry in CUP diagnosis is based on the consistency of immunohistochemical markers between primary and metastatic tumors, which can provide information such as tumor lineage, cell type and pathological diagnosis for CUP. See Table 3 for tumor specific markers and their staining patterns.

Table 3 Tumor Specific Markers and Their Staining Patterns

Marker	Tumor type	Staining mode
Arginase-1	Hepatocellular carcinoma	Nucleus
Calretinin	Mesothelioma, sex cord stromal tumor, adrenocortical carcinoma	Cytoplasm
CDX2	Colorectal cancer, gastric cancer, cholangiopancreatic carcinoma	Nucleus
D2-40	Mesothelioma	Cytomembrane
EBV	Nasopharyngeal carcinoma	Nucleus
ER/PR	Breast cancer, ovarian cancer, endometrial cancer	Nucleus
GATA3	Breast cancer, bladder cancer, salivary gland cancer	Nucleus
TRPS1	Breast cancer	Nucleus

PTH	Parathyroid tumor	Nucleus
GCDFP-15	Breast cancer, sweat gland cancer, salivary gland cancer	Cytoplasm
Glypican-3	Hepatocellular carcinoma, germ cell tumor	Cytoplasm
HepPar-1	Hepatocellular carcinoma	Cytoplasm
HPV	Cervical cancer, vulvar cancer, vaginal cancer, penile cancer, anal cancer, oropharyngeal cancer	Nucleus (DNA ISH); Nucleus/Cytoplasm (RNA ISH)
Inhibin	Sex cord stromal tumor, adrenocortical carcinoma	Cytoplasm
Mammaglobin	Breast cancer, salivary gland cancer	Cytoplasm
Melan-A	Adrenocortical carcinoma, melanoma	Nucleus
Napsin A	Lung adenocarcinoma, Ovarian and endometrial clear cell carcinoma	Cytoplasm
DSG3	Lung squamous cell carcinoma	Cytomembrane/ Cytoplasm
NKX3.1	Prostate cancer	Nucleus
P16	Cervical cancer, vulvar cancer, vaginal cancer, penile cancer, anal cancer, oropharyngeal cancer	Nucleus/ Cytoplasm (If positive, HPV ISH should be performed)
PSAP	Prostate cancer	Cytomembrane
PAX8	Thyroid cancer, renal cancer, ovarian cancer, endometrial cancer, cervical cancer, thymic cancer	Nucleus
PSA	Prostate cancer	Cytoplasm
SF-1	Adrenocortical carcinoma, sex cord stromal tumor	Nucleus
SATB2	Colorectal cancer	Nucleus
Thyroglobulin	Thyroid cancer (nipple / follicle)	Cytoplasm
TTF1	Lung adenocarcinoma, thyroid cancer	Nucleus
Uroplakin III	Urothelial carcinoma	Cytomembrane
Villin	Gastric cancer, colorectal cancer	Cytoplasm
WT1	Ovarian cancer, mesothelioma, Wilms tumor	Nucleus
HER-2	Breast cancer	Cytomembrane
MITF	Melanoma	Nucleus
PNL2	Melanoma	Cytoplasm/ Cytomembrane
SOX10	Melanoma, triple-negative breast cancer	Nucleus
DOG1	Gastrointestinal stromal tumor	Cytoplasm/ Cytomembrane
Syn	Neuroendocrine tumor	Cytoplasm
CgA	Neuroendocrine tumor	Cytoplasm
CD56	Neuroendocrine tumor	Cytomembrane
INSM1	Neuroendocrine tumor	Nucleus
SMAD4 (Loss of expression)	Cholangiopancreatic carcinoma	Cytoplasm
ERG	Prostate cancer, vascular tumor	Nucleus
Fli1	Vascular tumor	Nucleus
CD34	Vascular tumor, gastrointestinal stromal tumor	Cytoplasm
PSMA	Prostate cancer	Cytoplasm/ Cytomembrane
SALL4	Germ cell tumor	Nucleus
HMB45	Melanoma	Cytoplasm
OCT3/4	Germ cell tumor	Nucleus
CD138	Plasmacytoma	Cytoplasm
Calcitonin	Medullary thyroid carcinoma	Cytoplasm
S100	Melanoma, fat tumor	Nucleus
CD117	Gastrointestinal stromal tumor	Cytoplasm
CD30	Germ cell tumor	Cytoplasm/ Cytomembrane
NUT	NUT carcinoma of the lung, nasal cavity, and sinuses	Nucleus

(2) A variety of factors can lead to the bias of immunohistochemical results, including insufficient biopsy materials, tissue heterogeneity, factors affecting tissue antigenicity and differences in the interpretation of results by observers.

(3) Multiple rounds of immunohistochemical tests are recommended to determine the origin of tumor tissue step by step:

a) In the first round, lineage specific markers are used to determine the tumor lineage (such as cancer, sarcoma, lymphoma, melanoma, etc.) (Table 4).

b) In the second round, organ specific markers are used to indicate the presumed primary site (Table 4).

Table 4 Marker Combinations of Undifferentiated Tumors

Marker	Highly possible tumor cell lineage
Pan-keratin (AE1/AE3 & CAM5.2)	Cancer
CK7, CK19, CK20	Adenocarcinoma
CK5/6, p63, p40	Squamous cell carcinoma
HMB45, SOX10	Melanoma
LCA, CD20, CD3	Lymphoma
SALL4, OCT3/4	Germ cell tumor
Calretinin, WT1, D2-40	Mesothelioma
Vimentin	Sarcoma

Table 5 Tumor Specific Immunohistochemical Marker Combination

CK7 and CK20	Tumor location or type	Tumor specific marker
CK7+; CK20-	Breast cancer	ER+/PR+, GATA3+, GCDFP15+, Mammagloblin+, SOX10+, TRPS1+
	Serous carcinoma of ovary	PAX8+, ER+, WT1+, FSH+
	Clear cell carcinoma of ovary	PAX8+, HNF-1β+, Napsin A+
	Endometrial carcinoma	ER+, PAX8+, Vimentin+
	Cervical adenocarcinoma	p16+, HPV+, CEA+
	Lung adenocarcinoma	TTF1+, Napsin A+
	Lung squamous cell carcinoma	DSG3
	Thyroid cancer (follicular or papillary)	TTF1+, Thyroglobulin+, PAX8+
	Thyroid carcinoma (medullary carcinoma)	TTF1+, Calcitonin+, CEA+, Syn+, CgA+
	Gastric cancer	CEA+, CDX2+, CK19+, villin+
	Pancreaticobiliary carcinoma (pancreatic cancer, cholangiocarcinoma and gallbladder carcinoma)	CK19+, SMAD4-
	Thymic carcinoma	CD5+, p63+, PAX8+, CD117+
	Salivary adenocarcinoma	GATA3+, AR+, GCDFP-15+
	Renal cell carcinoma (chromophobe renal cell carcinoma or partial papillary renal cell carcinoma)	PAX8+, Vimentin+, CA9+
	Bladder cancer	GATA3+, p63+, CK5/6+, p40+, Uroplakin III+
	Mesothelioma	Calretinin+, WT1+, CK5/6+, MOC31-
CK7+; CK20+	Pancreaticobiliary carcinoma (pancreatic cancer, cholangiocarcinoma and gallbladder carcinoma)	CK19+, SMAD4-
	Gastric cancer	CEA+, CDX2+, CK19+
	Bladder cancer	GATA3+, p63+, CK5/6+, p40+, Uroplakin III+
	Colorectal cancer	CDX2+, Villin+, SATB2+
	Carcinoma of small intestine	CDX2+, Villin+
	Adenocarcinoma of appendix	CDX2+, Villin+, SATB2+
CK7-; CK20+	Colorectal cancer	CDX2+, Villin+, SATB2+
	Adenocarcinoma of appendix	CDX2+, Villin+, SATB2+
	Carcinoma of small intestine	CDX2+, Villin+
	Skin Merkel cell carcinoma	CgA+, Syn+, CD5/6+, INSM1+
CK7-; CK20-	Squamous cell carcinoma	CK5/6+, p63+, p40+, P16+
	Prostate cancer	PSA+, NKX3.1+, PSAP+, PSMA+, P504S+, ERG+, AR+
	Renal cell carcinoma (clear cell renal cell carcinoma or partial papillary renal cell carcinoma)	PAX8+, Vimentin+, CA9+
	Hepatocellular cancer	HepPar1+, AFP+, Glypican-3+, Arginase-1+
	Adrenocortical carcinoma	Melan A+, Inhibin +, Synaptophysin+, SF1+, CYP17A1, CYP11B1, CYP11B2
	Adrenal medullary tumor	CgA, GATA3, PHOX2B
	Germ cell tumor	SALL4+, OCT3/4+, CD30+, Glypican-3+, PLAP+
	Melanoma	MITF+, PNL2+, SOX10+, HMB45+, S100+, Melan A+

(4) Tumor types with coexpression of epithelial marker Cytokeratin and mesenchymal marker Vimentin

a) Cytokeratin and Vimentin are frequently co-expressed in endometrial carcinoma, mesothelioma, myoepithelial carcinoma, renal cell carcinoma, sarcomatoid carcinoma and thyroid carcinoma.

b) Cytokeratin and Vimentin are rare co-expressed in breast cancer, gastrointestinal cancer, non-small cell lung cancer, ovarian cancer, prostate cancer and small cell carcinoma.

c) Stromal tumors with frequent co-expression of Cytokeratin and Vimentin: chordoma, desmoplastic small round cell tumor, epithelioid angiosarcoma/endothelial tumor, epithelioid sarcoma, leiomyosarcoma, malignant rhabdomyoma and synovial sarcoma.

9 Gene expression profiling assay

9.1 Rationale of gene expression profiling assay

(1) Tumors of different tissue origins have specific gene expression profiles similar to those of their counterpart normal tissue. By analyzing the gene expression profiles of tumor tissues, their tumor types or origin can be extrapolated.

(2) The gene expression profiling assay mainly uses real-time PCR or gene microarray technology to analyze the gene expression profile in tissue samples fixed in 4% neutral buffered formalin-fixed and paraffin-embedded. By comparing the gene expression profile of the test sample with the gene expression profile of different tumor types in the database, the similarity between the detected samples and different tumor types was calculated and the tissue of origin was given based on the similarity score.

(3) The 90-gene expression assay based on the expression level of 90 genes has been developed in China. In a multicenter clinical trial involving 1417 samples across 21 types of tumors, the overall accuracy rate was 94.4%. Furthermore, the 90-gene expression assay has shown clinical utility in primary site identification of multiple primary tumors, triple-negative breast cancer, brain metastases, and liver metastases, with an overall accuracy range of 92.0% to 97.4%. Based on these study results, the 90-gene expression assay (Canhelp-Origin Assay, Canhelp Genomics Co., Ltd.) was approved by China National Medical Products Administration for tissue origin identification in patients with poorly differentiated tumors or suspected metastases.

9.2 Clinical practice of the 90-gene expression assay

(1) Clinical use: the 90-gene expression assay includes Canhelp-Origin Assay (Approved No. 20223400901) and Canhelp-Origin Software (Approved No. 20223210928).

a) Canhelp-Origin Assay is used to detect the expression patterns of 90 tissue-specific genes in tumor tissue samples and compare gene expression patterns with the reference database in the Canhelp-Origin Software to qualitatively identify the tumor type and tissue origin.

b) The database of Canhelp-Origin Software covers 21 common tumor types, including adrenal tumor, brain tumor, breast cancer, cervical cancer, colorectal cancer, endometrial cancer, gastroesophageal cancer, head and neck squamous cell carcinoma, renal cancer, hepatobiliary tumor, lung cancer, melanoma, mesothelioma, neuroendocrine tumor, ovarian cancer, pancreatic cancer, prostate cancer, sarcoma, germ cell tumor, thyroid cancer and urothelial cancer.

(2) Clinical value: The team specializing in multiple primary and unknown primary tumors at Fudan University Shanghai Cancer Center led a prospective, randomized phase III clinical trial (Fudan CUP-001). The study results confirmed that utilizing the 90-gene expression assay to predict the tissue of origin in patients with CUP and administering site-specific therapy significantly improved progression-free survival (PFS) and overall survival (OS) compared to empirical chemotherapy. The PFS for patients in the site-specific therapy group was 9.6 months, compared to 6.6 months in the empirical therapy group, representing a 32% reduction in the risk of disease progression ($P = 0.017$). The incidence of grade 3 or higher adverse events was lower in the site-specific therapy group compared to the empirical chemotherapy group (56% vs. 61%). This study provides high-level evidence for guiding site-specific therapy in patients with CUP.

Section 3 Treatment Principle of Cancer of Unknown Primary

1 Localized adenocarcinoma or nonspecific carcinoma

Localized or nonspecific cancer with unknown primary should be treated according to the status of a definitive primary lesion. Emphasis should be placed on obtaining high-quality tissue specimens for histological and immunohistochemical testing to aid in locating the primary site.

1.1 If the primary tumor is found and definitive, refer to the specific disease guidelines for treatment.

1.2 If there is no primary lesion found and all tumor lesions are limited to local or regional areas, such as head and neck, supraclavicle, axillary, mediastinum, multiple pulmonary nodules, pleural and peritoneal effusion, abdomen, retroperitoneum, inguinal, bone, brain, and liver, please refer to the treatment strategy of specific tumors:

(1) For the head and neck, refer to the treatment guidelines for head and neck tumors.

(2) For the clavicle area, refer to the treatment guidelines for head and neck tumors/lung cancer/abdominal tumors.

(3) For the axillary pit, refer to the guidelines for breast cancer treatment for women. Axillary lymph node dissection should be performed for men. If there are clinical indications, radiotherapy or chemotherapy should be considered.

(4) For the mediastinum, it can be specially discussed with the pathologist, which can help with the following treatments. Less than 40 years old: Refer to the guidelines for germ cell tumors with poor prognosis; 40-50 years old: Refer to the guidelines for treatment of germ cell tumors or non-small cell lung cancer with poor prognosis; 50 years old and above: Refer to the guidelines for treatment of non-small cell lung cancer.

(5) For the pulmonary nodules, surgical resection should be considered for patients who can operate. Non-operative patients should consider chemotherapy, stereotactic body radiation therapy (SBRT), symptom control, supportive treatment or participate in clinical trials.

(6) For the pleural effusion, refer to tumor markers. If breast markers are positive, refer to the treatment principle of breast cancer. If breast markers are negative, consider chemotherapy, symptom control, and supportive treatment or participate in clinical trials.

(7) For the peritoneum/ascites, refer to the histopathological morphology. If the histological morphology conforms to be the source of the ovary, refer to the treatment principle of ovarian cancer. If the histological morphology does not conform to the ovarian origin, consider chemotherapy, symptom control, supportive treatment, or participate in clinical trials.

(8) For the retroperitoneal tumors, if the histological morphology is consistent with germ cell tumors, refer to the treatment principles of germ cell tumors. If it does not meet the diagnosis criteria of germ cell tumor, consider chemotherapy, surgical treatment or radiotherapy, symptom control, supportive treatment, or participate in clinical trials.

(9) For the inguinal lymph nodes, unilateral lesions, lymph node resection is recommended. If there are clinical indications, radiotherapy ± chemotherapy should be considered. Bilateral lymphadenectomy is recommended for bilateral lesions. If there are clinical indications, radiotherapy ± chemotherapy should be considered.

(10) For the liver lesions, if resectable, surgical resection is recommended, and postoperative chemotherapy is considered. If it cannot be surgically removed, systemic treatment and local interventional therapy can be considered.

(11) For the bone lesions, such as solitary lesions, pain, and fracture risk, radiotherapy, bone cement, bisphosphonate, or desumumab treatment shall be considered. For those with good PS scores, surgical treatment shall be considered. In other cases, systemic treatment should be considered.

(12) Brain lesions are treated for brain metastasis of primary-known cancers.

1.3 If the primary lesion is not found and all tumor lesions are spreading beyond the locoregional boundary, symptom control will be carried out. Clinical trials are the first choice, and empirical chemotherapy and specific treatment will be considered.

2 Squamous cell carcinoma

Squamous cell carcinoma with unknown primary should be treated according to the status of a definitive primary lesion.

2.1 If the primary lesion is found and definitive, refer to the specific disease guidelines for treatment.

2.2 If no primary lesion is found and all tumor legions are limited to the locoregional areas, such as head and neck, supraclavicle, axillary, mediastinum, multiple pulmonary nodules, pleural effusion, groin, bone and brain, refer to the treatment strategy of specific tumors:

(1) For the head and neck, refer to the guidelines for head and neck cancer.

(2) For the clavicle area, refer to the guidelines for head and neck cancer / non-small cell lung squamous cell carcinoma / esophageal cancer.

(3) For the axillary, axillary lymph node resection should be performed. If there are clinical indications, radiotherapy ± chemotherapy should be considered.

(4) For the mediastinum, refer to the guidelines for non-small cell lung squamous cell carcinoma / esophageal squamous cell carcinoma.

(5) For the multiple pulmonary nodules, it is recommended to participate in clinical trials; Chemotherapy; and Symptomatic treatment.

(6) For the inguinal (unilateral), lymph node resection should be performed. If there are clinical indications, radiotherapy ± chemotherapy should be considered.

(7) For the groin (bilateral), bilateral lymph node resection should be performed. If there are clinical indications, radiotherapy ± chemotherapy should be considered.

(8) For the bone (isolated metastases; pain metastases; bone scan positive and bearing bones at risk of fracture), surgery (generally good patients) and/or radiotherapy for possible fracture parts is suggested to be carried out.

(9) For the bone (multiple metastases), symptoms can be controlled; Recommended for clinical trials; Individualized chemotherapy.

(10) For the brain, refer to the treatment of metastatic tumors in the guidelines for the treatment of central nervous system tumors.

2.3 If the primary lesion is not found and all tumor lesions are spreading beyond the locoregional boundary, symptom control is performed. Clinical trials are preferred and empirical chemotherapy is considered.

3 Principle of chemotherapy for cancer of unknown primary

(1) Chemotherapy can be recommended in patients with invasive lesions and symptoms (ECOG PS 1-2) and asymptomatic patients (ECOG PS 0). Chemotherapy for patients with PS 3 should be careful and balanced between benefits and possible harms.

(2) Different chemotherapy regimens are selected according to different tissue types.

(3) For neuroendocrine tumors, such as poorly differentiated (high-grade or degenerative) or small cell subtypes, refer to the guidelines for small cell lung cancer. For highly differentiated neuroendocrine tumors, refer to the guidelines for neuroendocrine tumors.

(4) As immunotherapy and targeted therapy continue to advance, an increasing number of CUP cases in clinical practice are attempting combinations of chemotherapy with immunotherapy and/or targeted therapy; however, such combinations still lack high-level evidence.

4 Chemotherapy of adenocarcinoma with unknown primary

(1) Common chemotherapy regimens: Paclitaxel and carboplatin/cisplatin; Gemcitabine and cisplatin; Oxaliplatin and capecitabine; mFOLFOX6, FOLFIRI.

(2) Other options: Docetaxel and carboplatin; Docetaxel and cisplatin; Gemcitabine and docetaxel; Irinotecan and carboplatin; Gemcitabine and carboplatin; Capecitabine; 5-Fluorouracil (5-FU).

(3) Options under special circumstances: Paclitaxel, carboplatin, and etoposide or FOLFIRINOX (ECOG PS 0-1); Irinotecan and gemcitabine (for patients who are not eligible for platinum-based treatments.).

4.1 Common regimens

(1) Paclitaxel and carboplatin / cisplatin

Paclitaxel 175-200mg/m^2 intravenous infusion D1

Carboplatin AUC 5-6 intravenous infusion D1 or cisplatin 75mg/m^2 intravenous infusion D1

Repeat every 3 weeks

(2) Gemcitabine and cisplatin

Cisplatin 75mg/m^2 intravenous infusion D1

Gemcitabine 1,000-1,250mg/m^2 intravenous infusion D1, 8

Repeat every 3 weeks

(3) Oxaliplatin and capecitabine

Oxaliplatin 130mg/m^2 intravenous infusion D1

Capecitabine 850-1,000mg/m^2 oral, twice a day, D1-14

Repeat every 3 weeks

(4) mFOLFOX6

Oxaliplatin 85mg/m^2 intravenous infusion D1

Formyl tetrahydrofolate 400mg/m^2 intravenous infusion D1

5-FU 400mg/m^2 intravenous injection D1, then 5-FU 1,200mg/m^2 /day continuous intravenous drip×2days (Total 2,400mg/$m^2$46-48 hours maintenance)

Repeat every 2 weeks

(5) mFOLFOX6 + radiotherapy

Oxaliplatin 85mg/m^2 intravenous infusion D1

Formyl tetrahydrofolate 400mg/m^2 intravenous infusion D1

5-FU 400mg/m^2 intravenous injection D1

5-FU 800mg/m^2 continuous intravenous drip for 24 hours D1-2

Once every 2 weeks, combined with radiotherapy after 3 cycles

(6) FOLFIRI

Irinotecan 180mg/m^2 intravenous infusion D1

Formyl tetrahydrofolate 400mg/m^2 intravenous infusion D1

5-FU 400mg/m^2 intravenous injection D1, then 5-FU 1,200mg/m^2 /day continuous intravenous drip×2days (Total 2,400mg/$m^2$46-48 hours maintenance)

Repeat every 2 weeks

4.2 Other options

(1) Docetaxel and carboplatin

Docetaxel 65mg/m^2 intravenous infusion D1

Carboplatin AUC 5-6 intravenous infusion D1

Repeat every 3 weeks

(2) Gemcitabine and docetaxel

Gemcitabine 1000-1250mg/m^2 intravenous infusion D1, 8

Docetaxel 75mg/m^2 intravenous infusion D8

Repeat every 3 weeks

(3) Docetaxel and cisplatin

Docetaxel 60-75mg/m^2 intravenous infusion D1

Cisplatin 75mg/m^2 intravenous infusion D1

Repeat every 3 weeks

(4) Irinotecan and carboplatin

Irinotecan 60mg/m^2 intravenous infusion D1, 8, 15

Carboplatin AUC 5-6 intravenous infusion D1

Repeat every 4 weeks

(5) Capecitabine

Capecitabine 850-1250mg/m^2 oral, twice a day, D1-14

Repeat every 3 weeks

(6) Capecitabine + radiotherapy

Capecitabine 625-825mg/m^2 oral, twice a day, D1-5 or D1-7

Once a week for 5 weeks, combined with radiotherapy

(7) 5-FU + radiotherapy

5-FU 200-250mg/m^2 intravenous infusion continuous drip for 24 hours, once a day, D1-5 or D1-7

Once a week for 5 weeks, combined with radiotherapy

(8) Gemcitabine + Carboplatin

Gemcitabine 1000mg/m^2 intravenous infusion D1, 8

Carboplatin AUC 5 intravenous infusion D8

Repeat every 3 weeks

4.3 Options under special circumstances

(1) Paclitaxel, carboplatin and etoposide

Paclitaxel 175-200mg/m^2, intravenous infusion D1

Carboplatin AUC 5-6, intravenous infusion D1

Etoposide 50mg/d oral and 100mg/d oral alternately, D1-10

Repeat every 3 weeks

(2) Irinotecan and gemcitabine

Irinotecan 100mg/m^2, intravenous infusion D1, 8

Gemcitabine 1,000mg/m^2, intravenous infusion D1, 8

Repeat every 3 weeks

(3) FOLFIRINOX

Oxaliplatin 85mg/m^2, intravenous infusion D1

Irinotecan 180mg/m^2, intravenous infusion D1

Calcium folinate 400mg/m^2, intravenous infusion D1

5-FU 400mg/m^2, intravenous infusion D1

5-FU 1,200mg/m^2, Continuous intravenous infusion for 24 hours × 2d (starting from D1, total 2,400mg/m^2, infusion for 46-48 hours)

Repeat every 2 weeks

5 Primary chemotherapy of unknown squamous cell carcinoma

(1) Common chemotherapy regimens: Paclitaxel and carboplatin/cisplatin; mFOLFOX6.

(2) Other options: Gemcitabine and cisplatin; Gemcitabine and carboplatin; Capecitabine; 5-FU; Docetaxel and carboplatin; Docetaxel and cisplatin; Cisplatin and 5-FU.

(3) Options under special circumstances (ECOG PS 0-1): Docetaxel, cisplatin, and 5-FU.

5.1 Common regimens

(1) Paclitaxel and carboplatin / cisplatin

Paclitaxel 175-200mg/m^2 intravenous infusion D1

Carboplatin AUC 5-6 intravenous infusion D1 or cisplatin 75mg/m^2 intravenous infusion D1

Repeat every 3 weeks

(2) mFOLFOX6

Oxaliplatin 85 mg/m^2 intravenous infusion D1

Formyl tetrahydrofolate 400 mg/m^2 intravenous infusion D1

5-FU 400 mg/m^2 intravenous injection D1, then

5-FU 1,200mg/m^2 /day continuous intravenous drip×2 days (Total 2,400mg/m^2 for 46-48 hours maintenance)

Repeat every 2 weeks

5.2 Options

(1) Gemcitabine and cisplatin

Cisplatin 75 mg/m^2 intravenous infusion D1

Gemcitabine 1,000-1,250 mg/m^2 intravenous infusion, D1 and D8;

Repeat every 3 weeks

(2) mFOLFOX6 + radiotherapy

Oxaliplatin 85 mg/m^2 intravenous infusion D1

Formyl tetrahydrofolate 400 mg/m^2 intravenous infusion D1

5-FU 400 mg/m^2 intravenous injection D1

5-FU 800 mg/m^2 continuous intravenous drip for 24 hours D1-2

Once every 2 weeks, combined with radiotherapy after 3 cycles

(3) Capecitabine

Capecitabine 850-1,250 mg/m^2 oral twice a day, D1-14

Repeat every 3 weeks

(4) Capecitabine + radiotherapy

Capecitabine 625-825 mg/m^2 oral twice a day D1–5 or D1–7

Once a week for 5 weeks

(5) 5-FU + radiotherapy

5-FU 200-250 mg/m^2 intravenous infusion

Continuous intravenous infusion for 24 hours once a day D1–5 or D1–7

Once a week for 5 weeks combined synchronous radiotherapy

(6) Docetaxel and carboplatin

Docetaxel 75 mg/m^2 intravenous infusion D1

Carboplatin AUC 5-6 intravenous infusion D1

Repeat every 3 weeks

(7) Docetaxel and cisplatin

Docetaxel 60-75 mg/m^2 intravenous infusion D1

Cisplatin 75 mg/m^2 intravenous infusion D1

Repeat every 3 weeks

(8) Cisplatin and 5-FU

Cisplatin 20mg/m^2 intravenous infusion D1-5

5-FU 700mg/m^2/d intravenous infusion continuous injection D1-5

Repeat every four weeks

(9) 5-FU and cisplatin + radiotherapy

Cisplatin 75-100 mg/m^2 intravenous infusion D1 D29

5-FU 750-1,000 mg/m^2 intravenous infusion; continuous injection for 24 hours every day D1-4; D29-32

Combined 35-day radiotherapy

Cisplatin 15 mg/m^2 intravenous infusion D1-5

5-FU 800 mg/m^2 intravenous infusion, continuous injection for 24 hours every day, D1-5; repeat every 21 days;

Two cycles of chemotherapy combined with radiotherapy

5.3 Options under special circumstances (ECOG PS 0-1)

(1) Docetaxel, cisplatin and 5-FU

Docetaxel 75mg/m^2 intravenous infusion D1

Cisplatin 75 mg/m^2 intravenous infusion D1

5-FU 750 mg/m^2/d continuous intravenous infusion D1-5

Repeat every 3 weeks

6 Specific treatment of cancer of unknown primary

Compared with the non-specificity of traditional chemotherapy, the specific treatment of CUP can be divided into primary site-directed treatment (also called organ specific treatment), molecular target-directed treatment and a combination of both. In recent years, with the rapid advancement of tumor diagnostic technologies, site-specific therapy based on the 90-gene expression assay and target-specific therapy combined with NGS detection have gradually been applied in the clinical management of CUP. Currently, a prospective randomized phase III clinical trial support that site-specific therapy based on the 90-gene expression assay can significantly improve the prognosis of CUP patients.

6.1 Primary site-directed treatment

(1) Detection method: gene detection of tumor tissue origin.

(2) Current evidence:

a) The results of the prospective single-arm Phase II clinical study in America showed that site-specific treatment concerning the detection of tumor tissue origin gene can prolong the survival compared with historical control;

b) The CUP001 study, as the world's first prospective randomized controlled phase III clinical trial for CUP initiated by Chinese researchers, has demonstrated for the first time internationally that predicting the tissue origin of tumors in CUP patients based on the 90-gene expression assay and implementing site-specific therapy can significantly improve progression-free survival compared to empirical chemotherapy. This study potentially provides high-level evidence supporting the use of the 90-gene expression assay to guide site-specific therapy in CUP patients.

6.2 Molecular target-directed treatment

(1) Detection method: NGS detection.

(2) Current evidence:

a) PD1 monoclonal antibody pertuzumab is used to treat unresectable or metastatic solid tumors with MSI-H and dMMR, and inoperable or metastatic solid tumors of adults and children who progressed after previous treatment and had tTMB-h (tissue TMB ≥10 mutation/Mb) and no satisfactory alternative treatment;

b) Larotinib and entrectinib is used to treat NTRK gene fusion-positive locally advanced or metastatic solid tumors.

c) Dabrafenib combined with trametinib is used for the treatment of BRAF V600E-mutated locally advanced or metastatic solid tumors.

d) Selpercatinib is used for RET gene fusion-positive locally advanced or metastatic solid tumors.

e) The CUPISCO trial compared the efficacy of targeted therapy or immunotherapy guided by comprehensive genomic analysis to platinum-based chemotherapy in newly diagnosed CUP patients with poor prognosis, non-squamous histology, and a response to induction platinum chemotherapy. The results showed an improvement in progression-free survival (6.1 months vs. 4.4 months) and a 28% reduction in the risk of disease progression (P = 0.0079).

6.3 Specific treatment of combination of the two approaches

(1) Detection methods: tumor tissue origin + NGS detection.

(2) Current evidence: According to the results of prospective single arm Phase II study, the one-year survival rate was 53% and the median OS was 13. 7 months. The median PFS was 5.2 months and the ORR was 39%.

7 Targeted biologics and immunotherapy of cancer of unknown primary

(1) Pembrolizumab (dMMR/MSI-H tumor or TMB-H [≥10 mut/Mb] tumor)

200 mg intravenous infusion, D1, every 3 weeks in a cycle or

400 mg intravenous infusion, D1, every 6 weeks in a cycle

(2) Larotrectinib (NTRK gene fusion-positive)

100mg oral twice a day

(3) Entrectinib (NTRK gene fusion-positive)

600mg oral once a day

(4) Dabrafenib combined with trametinib (BRAF 600E gene fusion positive)

Dabrafenib 150mg oral twice a day

Trametinib 2mg oral once a day

(5) Selpercatinib (RET gene fusion-positive)

Under 50kg: 120mg oral twice a day

Over 50kg: 160mg oral twice a day

(6) Trastuzumab deruxtecan (T-DXd) (HER-2 3+)

T-DXd 5.4mg/kg, intravenous infusion, D1, every 3 weeks in a cycle

8 Principle of radiotherapy for cancer of unknown primary

8.1 Localized lesions

Radical radiotherapy can be considered for localized lesions or oligometastatic lesions (1-3), including stereotactic radiosurgery (SRS), SBRT, hypofractionated radiotherapy, or conventional fractionated Radiotherapy.

According to the location of oligometastasis, different dose segmentation methods can be selected as appropriate. For example, 48- 60GY/4-5F can be considered for pulmonary oligometastasis. 16-24GY/1F or 30-36GY/3F can be considered for brain oligometastasis, and 16-18GY/1F, 24GY/2F, 30GY/3F, 35-40GY/5F can be considered for bone oligometastasis, 60GY/8F can be considered for adrenal oligometastasis, etc. For cases where SRS or SBRT cannot be performed due to proximity to critical organs, hypofractionated radiotherapy or conventional fractionated radiotherapy can be administered. For instance, in cases of a

retroperitoneal lymph node metastatic tumor with an unknown primary, conventional fractionated radiotherapy can be given and the dose limits (54-60 GY/27-30F) will depend on the surrounding critical organs.

8.2 Adjuvant radiotherapy

(1) For single lymph node dissection with extracapsular extension (ECE) or multiple lymph node metastases with limited lymph node dissection, adjuvant radiotherapy can be considered.

(2) For localized supraclavicular, axillary or inguinal lymph node metastasis, it is recommended to give the lymph node drainage area 45GY prophylactic dose +/- boost radiation 5-9GY/1.8-2GY.

8.3 Palliation therapy

Palliative radiotherapy can be considered for symptomatic patients.

For uncontrollable pain, impending pathological fracture or spinal cord compression, hypofractionated radiotherapy can be considered. A variety of hypofractionated radiotherapy methods can be considered, and the most commonly used ones are 8GY/1F, 20GY/4-5F or 30GY/10F.

Section 4 Follow-up Principle of Cancer of Unknown Primary

1 Prognosis of cancer of unknown primary

80% of patients have poor prognosis. The median OS is 3-10 months. The prognosis of squamous cell carcinoma is generally better than that of adenocarcinoma and undifferentiated tumors, with 3-year overall survival (OS) rates of 41.6% and 3.5%, respectively.

1.1 Factors of poor prognosis

Male, ≥ 65 years old, high PS score, many complications, multiple organ metastasis (liver, lung, bone), malignant peritoneal effusion caused by non-papillary adenocarcinoma, peritoneal metastasis, multiple brain metastasis, multiple lung / pleural adenocarcinoma, multiple bone metastasis adenocarcinoma.

1.2 Prognostic factors

A single lesion, small lesion, potentially resectable lesion, poorly differentiated carcinoma with nodular distribution in the midline, cervical lymph node metastatic squamous cell carcinoma, isolated inguinal lymph node metastatic squamous cell carcinoma, poorly differentiated neuroendocrine carcinoma, female peritoneal papillary adenocarcinoma, female simple axillary lymph node metastatic adenocarcinoma, male osteoblastic bone metastasis with elevated PSA.

2 Follow-up of unknown primary

(1) For patients with no active lesions or local remission, the follow-up frequency should be determined according to clinical needs. Follow-up included: medical history, physical examination (H&P), and relevant examination based on symptoms;

(2) For those who have active lesions and cannot be cured, symptomatic treatment, psychological support, enhancing nursing intervention and hospice care should be considered;

(3) A few CUPs show potential primary lesions during the follow-up, which should be checked regularly to find the primary lesions and treated accordingly.

Chapter II

Cancer of Multiple Primaries

Section 1 General Principles for Diagnosis and Treatment of Cancer of Multiple Primaries

1 Overview

Cancer of multiple primaries (CMP), as defined in this guideline, refers to two or more primary tumors occurring simultaneously or successively in the same individual. CMP can be derived from different sites, different tissue types, or different pathological types in the same site. Pathological diagnosis is the only gold standard for the diagnosis of CMP, but the abnormalities of clinical data, such as symptoms, signs, laboratory examination, and auxiliary examination, can help in obtaining appropriate tissue materials and then making a correct pathological diagnosis. The diagnosis of CMP is written in the order of onset time with the most recent diagnosis written in the front. For the staging of CMP, TNM staging should be carried out according to each primary tumor as far as possible. In the treatment of synchronous/metachronous CMP, it is needed to first consider the primary tumor with the higher life-threatening degree to formulate the treatment strategy, but it is also needed to take into account the treatment of all primary tumors. The prognosis of CMP is generally better than that of disease recurrence and metastasis, but it is also related to the biological behavior of the tumor itself.

2 Epidemiology of cancer of multiple primaries

With the gradual rise in overall cancer incidence and the increasing number of cancer survivors due to advancements in early screening and treatment, the long-term effects of early antitumor therapy, enhanced diagnostic sensitivity, and the ongoing impact of genetic and environmental risk factors, the occurrence of CMP has become increasingly common.

2.1 Incidence rate

At present, the literature reports that the incidence of CMP varies greatly, ranging from 1% to 17% abroad. The main reason is that the definition, follow-up time and data acquisition methods of CMP are different, resulting in inconsistent statistical methods. The two most common definitions currently used are provided by the US Surveillance, Epidemiology and Results Database (SEER) project and the International Association for cancer registration and the International Agency for Research on Cancer (IACR/IARC). There are still differences in site sub-classification and the interval used to define metachronous multiple primary cancers between the two definition systems. A SEER-based cancer cohort reported that about 8.1% had a second CMP. Retrospective studies based on autopsy reported that CMP accounted for 0.8% - 1.1% of all autopsies and 3.6% - 5.0% of all cancer autopsies. According to IACR standards, CMP is found in about 6.3% of a study of about 3 million cancer patients in 22 European countries. The Italian cancer registry followed more than 1.6 million cancer patients for a median of 14 years and observed that CMP occurs in 6.3%. At present, there are only several hospital-based single-center data in China, and the overall incidence is 0.4% - 2.0%, lower than that reported abroad. CMP cases are mainly double primaries, accounting for about 90%, and three primaries, four primaries, five primaries, and above account for about 5%, 3%, and 1%, respectively. Over time, the occurrence of CMP tends to increase gradually.

2.2 Age and interval of onset

There are different reports on the age of onset of CMP. The average age of initial cancer onset is generally about 50-60 years old. The average interval between the first and second primary cancers reported varied from 1 to 7 years. The shorter the interval, the worse the prognosis. The younger the first primary tumor was diagnosed, the greater the relative risk of CMP. The relative risk of the first diagnosed cancer between the ages of 0-17 is six times that of those over the age of 70.

2.3 Sex ratio

There is no significant difference between men and women in the occurrence of CMP, ranging from 2.34:1 to 1:1.3. The sex ratio is different in different age groups, cancer types or race.

2.4 Predilection site

The predilection sites of CMP are the same organ, paired organ and organ of the same system. The risk of CMP varies in different cancer sites, and the cancer spectrum varies greatly in different countries and

regions. The head and neck, mammary gland, urinary system and digestive system are the predilection sites of CMP in China. The first primary cancer with the highest risk of second CMP in the United States is primary bladder cancer, and the lowest is primary liver cancer. Breast, colorectal cancer and prostate are not only the sites with the largest number of primary malignant tumors, but also one of the sites with the largest number of CMP.

3 Writing suggestions for the diagnosis of cancer of multiple primaries in case records

3.1 Standardize the CMP disease code

3.2 CMP diagnostic writing

For multiple primary cancers, involving the site, it is needed to write the primary site in chronological order, and the most recent one is written in the front. The term, post-operation or radiation is used to indicate that the primary lesion has been locally treated and there may be a chance of adjuvant treatment.

(1) If the source of metastasis is unknown, it can be written as: For example, multiple primary cancers, retroperitoneal lymph node and pelvic metastasis, postoperative ovarian cancer, postoperative lung squamous cell carcinoma.

(2) If the source of metastasis is clearly diagnosed, it can be written as: For example, multiple primary cancers, right lung adenocarcinoma, liver and bone metastasis; post-oprational left breast cancer.

(3) If one primary lesion is clear and another primary lesion is not clear, it can be written as: For example, multiple primary cancers, metastatic squamous cell carcinoma of right supraclavicular lymph node; post-operational left breast cancer.

3.3 Writing order of metastatic lesions

Metastatic lesions are written in order of the degree of adverse effect on prognosis, including brain, liver, lung, bone and lymph nodes.

3.4 Other contents of diagnostic writing

(1) Should include the disease which receives active treatment.

(2) Should include serious diseases, although recovered, which may affect the choice of drug treatment, such as myocardial infarction and stroke.

(3) Should include serious symptoms and laboratory tests needed to be handled in priority, such as pericardial effusion, pathological fracture, grade IV thrombocytopenia, etc.

Section 2 Principles of Diagnosis of Cancer of Multiple Primaries

1 Physical examination, laboratory test and molecular test

1.1 Initial evaluation

Carefully inquire about past tumor history (including gene test results), corresponding treatment history, family history, infection history (HBV, HPV, EBV), and other personal history (smoking, drinking, lifestyle, occupational environment, psychological emotions, etc.). The clinical characteristics of multiple primary tumors should be suspected:

(1) Atypical metastatic spread of the primary tumor (e.g., radiographic findings of lytic bone metastases in prostate cancer patients);

(2) The value of tumor markers is not consistent with the tumor burden (such as prostate cancer with extensive liver metastasis of low level of prostate-specific antigen).

(3) New metastatic spread (e.g., liver, lung) occurs several years (usually > 5 years) after diagnosis of the primary cancer;

(4) A single new metastasis after primary cancer diagnosis (e.g., a single lung nodule in a previous primary head and neck cancer patient);

(5) Chronologically atypical metastatic spread (e.g., primary small cell lung cancer recurred after 5 years);

(6) Relapse in patients exposed to environmental carcinogens (such as smokers);

(7) Hematological malignancy is suspected after previous chemotherapy (such as etoposide and anthracycline);

(8) Secondary malignancies are suspected in patients who have previously received radiation therapy for malignant tumors, especially in patients who have recurred in previous radiation areas;

(9) Suspected lesions (such as PET/CT) were found during staging or follow-up of the primary tumor;

(10) There was a significant difference in the standard uptake value (SUV) of PET/CT suspected lesions (e.g., lesions with a high SUV value and lesions with a low SUV value).

1.2 Physical examination

Complete physical examination: special focus on targeted physical examination of superficial lymph nodes, previous tumor affected sites, previous radiotherapy fields and new suspected second tumor sites.

1.3 Laboratory examination

It is needed to improve routine examination in combination with medical history: blood routine, urine routine, fecal routine + occult blood, liver and kidney function, electrolyte. If the patient has previously used cardiotoxic cytotoxic drugs (anthracyclines, alkylating agents, anti-microtubules and anti-metabolites, and novel targeted anti-tumor agents: anti-HER-2, anti-VEGF, multi-target VEGF-TKI, and immune checkpoint inhibitors, etc.), it is necessary to monitor with cardiac ultrasound and ECG. It is needed to improve the detection of tumor markers (including all relevant markers for different primary tumors) and refer to the results of previous tumor marker alterations. According to the clinical guidance, it is required to order other examinations, such as clinical-guided endoscopy and ultrasound. For those who have received combined chemotherapy in the past, if there are obvious abnormalities in the new blood routine, it is required to order bone marrow puncture in order to exclude the possibility of secondary primary blood system diseases.

A recent study showed that approximately 21% of patients with CMP have a detectable causative mutation, 44% of which are high penetrance genes, so genetic testing should be considered if the patient is unusually young, or if multiple first - and second-degree relatives in the family history have or have had two or more primary cancers with markedly different histological features. Because these features are indicative of hereditary cancer syndromes, second-generation sequencing is conditionally recommended.

Common hereditary cancer syndromes and corresponding mutated genes

(1) Hereditary masto-ovarian cancer syndrome: mutations of breast cancer susceptibility gene 1 (BRCA1) or breast cancer susceptibility gene 2 (BRCA2);

(2) Li-Fraumeni syndrome: p53 mutation;

(3) Lynch syndrome: Caused by inherited mutations in at least five different mismatch repair (MMR)

genes, including MLH1, MSH2, MSH6 and PMS2.

(4) Familial adenomatous polyposis: mutation of APC regulator of WNT signaling pathway (APC);

(5) Familial atypical multiple mole melanoma syndrome: cyclin-dependent kinase inhibitor 2A (CDKN2A) mutation;

(6) Multiple hamartoma syndrome: detection of phosphatase and tensin homolog (PTEN) mutations;

(7) Checkpoint kinase 2 (CHEK2) syndrome: mutations at sites 51 and 52 of CHEK2;

(8) MutY DNA glycosylase (MUTYH) -associated polyposis: germline biallelic mutation of MUTYH;

(9) Hereditary diffuse gastric cancer: cadherin 1 (CDH1) mutation encoding E-cadherin;

(10) von Hippel-Lindau tumor suppressor (VHL) syndrome: germline mutation of the VHL tumor suppressor gene on the short arm of chromosome 3;

(11) MEN1/MEN2 syndrome: embryonal mutations in the MEN1 gene that predispose to parathyroidoma, pituitary adenoma, islet cell tumor, gastrinoma, alveoloma, or insulinoma. Defects in the MEN2 gene lead to thyroid cancer and pheochromocytoma, and nearly 90% of patients have mutations in the Ret proto-oncogene.

Furthermore, if the histology of the newly biopsied site is identical to the previous tumor but there is a strong clinical suspicion of a second primary tumor, it is recommended to use the 90-gene expression assay to determine the tissue origin of the tumor. If it is confirmed to be a second primary tumor, further NGS testing specific to the tumor type should be conducted. The combination of the 90-gene expression assay and NGS testing provides a basis for precision diagnosis and treatment.

2 Pathological examination

2.1 Cytopathological examination

2.1.1 Fine-needle aspiration biopsy

(1) Fine-needle aspiration (FNA) is used for the initial qualitative diagnosis of CMP or when histopathological examination cannot be performed.

(2) Immunohistochemical testing can be attempted on cell block samples prepared by FNA, but there is usually no guatantee of sufficient specimens for histopathological examination.

2.1.2 Exfoliative cytologic examination

Exfoliative cytologic examination can occasionally be used to find the second primary lesion, such as:

(1) If lung tumor is suspected, sputum exfoliative cell examination can be performed.

(2) If esophageal tumor is suspected, esophageal exfoliated cell examination can be performed.

(3) If the tumors of urinary system are suspected, the exfoliated cells in the urine can be examined.

2.2 Histopathological examination

(1) Histopathological diagnosis of tumor biopsy or surgical specimens is the gold standard for the diagnosis of CMP.

(2) Pathological diagnosis needs sufficient tumor tissue. The optimal way to obtain specimens is tissue resection/incisional biopsy or core needle biopsy. If conditions are limited, FNA that can prepare cell mass can also be selected, or a pleural fluid cell mass pathology examination can also be performed.

2.2.1 Classification under light microscope

Under the light microscope, CMP can appear as the same or different histological types:

(1) Observe the cancer components in situ, supporting CMP.

(2) Different histological types can be easily distinguished into CMP, such as cancer and sarcoma, squamous cell carcinoma, and adenocarcinoma.

(3) The same histological type is difficult to distinguish into CMP, and further immunohistochemical or molecular detection is required for identification.

2.2.2 Immunohistochemical examination

(1) Immunohistochemical examination is usually carried out in paraffin-embedded tissue samples fixed with 4% formaldehyde solution. Further immunohistochemical examination is required for tumors that cannot be diagnosed under the light microscope.

(2) Immunohistochemical examination can determine the tumor lineage (cancer, sarcoma, lymphoma, malignant melanoma, etc.) and identify the tissue of origin in the same histological tumor type.

2.2.3 Specific immunohistochemical markers of tumors

Specific Immunohistochemical Markers of Tumors

Immunohistochemical results	Tumor type
GCDFPl5, Mammaglobin, GATA3, TRPS1	Breast cancer
TTF1 (when CK7+, CK20-)	Lung cancer
HepPar1, Arginase-1	Liver cancer
RCC	Renal carcinoma
Thyrobolulin (TG), TTF1, PAX8	Thyroid tumor
PLAP/OCT4, SALL4	Germ cell tumor
CDX2 (when CK7+, CK20-), SATB2	Colorectal cancer
WT1, PAX8	Oophoroma
Chromogranin A (CgA), Synaptophysin (syn), INSM1	Neuroendocrine tumor
Leukocyte common antigen (LCA)	Lymphoma or leukemia
Differential expression of p53, p16, p27 and HER-2 in lung cancer lesions	Differential diagnosis between CMP and intrapulmonary metastasis
EBER	Nasopharyngeal carcinoma or Epstein Barr virus associated lymphoma
P16	Oropharyngeal tumor or cervical cancer

3 Molecular testing

3.1 Principle of molecular testing

(1) The genetic characteristics of metastatic and recurrent tumors are similar to those of primary tumors.

(2) The genetic characteristics of the second primary tumor and the first primary tumor may be different.

3.2 Clinical evidence

(1) The 90-gene expression assay is based on real-time PCR platform, which can be used to identify the tissue origin of CMP by analyzing the gene expression profiling, and the consistency with pathological diagnosis is 93.2% in CMP.

(2) Different mutational profiles of oncogenic driver gene mutations indicate different clonal origins. For example, the detection of EGFR gene mutation and ALK gene rearrangement can distinguish multiple primary lung cancer and intrapulmonary metastatic cancer. However, the presence of the same driver gene mutation does not necessarily indicate the same clonal origin of the tumor. A common genetic background and environmental exposure may result in multiple independent primary lung cancers with the same KRAS or EGFR mutation. Using large-scale NGS testing can often better distinguish between metastatic cancer and CMP.

(3) Polymorphic microsatellite marker analysis shows an inconsistent trend in multiple primary lung cancers, but shows a consistent trend between metastatic tumors and primary tumors.

(4) By analyzing the gene copy number changes by microarray comparative genomic hybridization, it was found that the concordance rates of metastatic cancer and CMP are 55.5% and 19.6%, respectively, and the concordance with pathological diagnosis is 83%.

(5) Using targeted genome sequencing to screen for germline mutations, If Lynch syndrome is suspected, MSI detection should be improved, APC gene mutation detection should be improved for those suspected of familial colorectal adenomatous polyposis, TP53 gene mutation detection should be improved for those suspected of Li-Fraumeni familial cancer syndrome, BRCA1/2 gene mutation detection should be improved for those with a family history of breast/ovarian cancer, etc. BRCA1 185delAG, BRCA1 T300G, BRCA1 2080delA, BRCA1 4153delA, BRCA1 5382insC, BRCA2 6174delT, CHEK2 1100delC, and BLM C1642T are responsible for the development of most inherited breast and ovarian cancer syndromes.

4 Radiological diagnoses

The purpose of imaging diagnosis of CMP is to detect and differentiate different sites or the same site occupation at the same time or at different times, and to determine whether there is a correlation. CT is the main examination, it is recommended to enhance, if necessary, three stages of scanning: including plain scan, arterial phase, and venous phase. In recent years, multi-parameter magnetic resonance imaging (mp-MRI), including conventional plain scan images, diffusion-weighted imaging (DWI), and dynamic contrast-enhanced MRI (DCE-MRI), has been more and more widely used in the diagnosis, differentiation, and efficacy evaluation of tumors, especially in the detection and differentiation of some tissue and organ tumors:

(1) mp-MRI is the best method for the diagnosis and differentiation of central nervous system, head, neck, and soft tissue tumors.

(2) A CT scan is the best way to diagnose and distinguish pulmonary metastases and second primary tumors.

(3) The mp-MRI is superior to mammography and ultrasonography in the diagnosis and differentiation of breast lesions.

(4) The mp-MRI has important supplementary diagnostic value in the diagnosis and differential diagnosis of liver and adrenal tumors.

(5) CT urography (CTU) is of great value in the detection of small lesions in the urinary system. The mp-MRI is recommended for the diagnosis and differentiation of renal space-occupying lesions.

(6) mp-MRI is superior to CT in the diagnosis and differentiation of uterine and prostatic diseases.

(7) mp-MRI is superior to CT in the detection of bone tumors, but thin-slice CT bone window images are still needed for differentiation.

5 Nuclear medicines

5.1 CMP diagnosis

PET/CT is more sensitive in detecting systemic tumor foci, which is helpful for CMP diagnosis or guiding selection of biopsy sites.

5.2 Tumor load and staging

PET/CT is used to evaluate the whole-body tumor burden and stage each tumor respectively, so as to provide tumor treatment decision-making information.

5.3 Radical treatment decision

In some cases, ^{18}F-FDG PET/CT examination or specific tumor PET/CT before treatment is necessary for local radical treatment of CMP.

5.4 Tumor-specific PET/CT examination

According to the pathological immunohistochemical markers, if possible, the hospitals can use the relevant tumor specific PET/CT molecular imaging to help determine the source of metastatic tumors, such as: For breast cancer with estrogen receptor positive, ^{18}F-FES PET/CT (estrogen receptor imaging) can be used. In CMP patients with one primary cancer being neuroendocrine tumor (net), ^{68}Ga-DOTATATE or ^{68}Ga-DOTANOC PET/CT (somatostatin receptor imaging) can be considered. In CMP patients with one primary cancer being prostate cancer, ^{18}F/^{68}Ga-PSMA PET/CT (prostate specific membrane antigen imaging) can be used. In CMP patients with one primary cancer being HER-2 positive breast cancer or gastric cancer, ^{68}Ga-HER-2 PET/CT (HER-2 receptor imaging) can be used.

Section 3 Treatment Principles of Cancer of Multiple Primaries

1 Treatment principles of cancer of multiple primaries

Multiple primary solid tumors, multiple primary blood tumors, and multiple primary blood tumors combined with multiple primary solid tumors should be considered in the treatment of CMP. The principles of multidisciplinary cooperation, individual evaluation, attention to drug interactions and patient quality of life should be followed. In the treatment of CMP, the priority of each primary tumor should be defined and the treatment principle of each primary tumor should be integrated. The priority of treatment should be determined according to the biological behavior and stage of each primary tumor. The tumors with high malignant degree and late stage should be treated first, and the treatment plan should be formulated after fully evaluating the patient's age and organ function tolerance.

1.1 CMP of solid tumors

For multiple primary solid tumors, the first principle of treatment is to independently evaluate each primary tumor and determine the treatment strategy according to its stage, pathological type, and biological behavior. The treatment should give priority to the treatment of tumors with high malignant degree and late stage while taking into account the holistic treatment of multiple primary tumors. Procedures such as surgery, radiotherapy, and chemotherapy need to be tailored to the specific situation.

1.2 CMP of hematologic tumors

Multiple primary hematologic tumors usually involve the coexistence of different types of blood diseases. The therapeutic principles focus on the overall control of the disease, considering chemotherapy, targeted therapy, hematopoietic stem cell transplantation, cellular immunotherapy, and other options.

1.3 CMP of hematologic tumors combined with solid tumors

Such multi-primary tumors require interdisciplinary collaboration and the development of individualized treatment strategies. The treatment plan should take into account the treatment needs of both, avoid the interaction between the treatment plans, and focus on toxic and side effects monitoring to ensure that patients can obtain the best treatment results and quality of life.

2 Surgical treatments of CMP

For synchronous CMP, staging two or more primary tumors should be evaluated first. If they are early and there are no surgical contraindications, it can be evaluated whether they can tolerate simultaneous or successive surgery. If two or more primary tumors cannot be surgically removed, both should be considered as much as possible, and the one with higher malignancy should be the top priority. Similarly, if there are surgical contraindications or intolerable surgery, the primary cancer with higher malignancy should also be first considered when formulating the treatment plan.

For metachronous CMP, the staging of the primary tumor should be fully evaluated first. If the second primary tumor is early, and the first primary tumor has no recurrence or metastasis and no surgical contraindications, surgery should be performed first under tolerable conditions. If the second primary tumor is unresectable, or the first primary tumor has recurrence or metastasis at the same time, or there are surgical contraindications, both should be considered, and the one with higher malignancy should be the top priority.

3 Medical treatment of CMP

For synchronous CMP, if two or more primary tumors are early, they can be surgically removed. It is recommended to take medical treatment according to the principle of adjuvant treatment of each primary tumor. If two or more primary tumors cannot be removed, or there are surgical contraindications, it is recommended to give consideration to both, and the primary cancer with higher malignancy is the top priority. The medical treatment scheme should give consideration to multiple primary tumors as much as possible, and there is no evidence of adverse drug interaction between used drugs. The adverse effects of previous radiotherapy and chemotherapy must be considered in the treatment plan. For example, if the local second primary cancer after the prior nasopharyngeal carcinoma is highly malignant, the possibility of bleeding and cerebrospinal fluid leakage with new anti-cancer treatment should be considered. In addition, the curative effect evaluation of different primary tumors should be described separately, if possible. For example, the curative effect evaluation of lung cancer and breast cancer should be carried out separately for patients with dual primary lung cancer and breast cancer. If there is obvious inconsistency in tumor regression in different parts in a clinic, it is recommended to make a re-biopsy to clarify the nature and origin of the lesion.

For metachronous CMP, if the second primary tumor is early, it can be removed surgically. If the first

primary tumor has no recurrence or metastasis, it is recommended to give medical treatment according to the principle of adjuvant treatment of the second primary tumor. If the second primary tumor is unresectable, or the first primary tumor has recurrence and metastasis at the same time, or there are surgical contraindications, it is recommended to give consideration to both and choose the medical treatment scheme with the higher degree of malignancy being top priority.

4 Radiotherapy for CMP

For synchronous CMP, if one primary cancer can be cured by radiotherapy, radiotherapy will be performed whereas another primary tumor will be fully evaluated. If the latter is early, surgical resection will be performed; if the disease is local but cannot be surgically removed, or if there are surgical contraindications, medical treatment shall be carried out. If two or more primary tumors are early and all are surgically removed, radiotherapy is recommended according to the principle of adjuvant radiotherapy for each primary tumor. If two or more primary tumors are limited-stage but unresectable; or surgical contraindications, taking into account both and with a higher degree of malignancy, radiotherapy is selected.

For metachronous CMP, if the second primary tumor is early and has been surgically cured, and the first primary tumor has no recurrence or metastasis, radiotherapy shall be carried out according to the principle of adjuvant radiotherapy for the second primary tumor. If the second primary tumor cannot be surgically removed and is locally advanced, or the first primary tumor has recurrence and metastasis at the same time, or there are surgical contraindications, it is recommended to give consideration to both and give priority to the one with higher malignancy, and the radiotherapy scheme can be selected according to the pathological type of the tumor.

Section 4 Follow-up and Rehabilitation Principles for Patients with Cancer of Multiple Primaries

The attending clinician should conduct rigorous follow-ups for each patient with multiple primary tumors based on their clinical characteristics, clinical staging, histopathological features, pathological staging, molecular pathology, and classification, as well as their treatment status. The principles of follow-up are as follows:

(1) After completing treatment (including local and systemic therapies) for second, third, and subsequent primary tumors, the attending physician should implement a comprehensive management plan, closely monitor the patient's condition, and conduct follow-up visits.

(2) The attending physician should develop a personalized follow-up plan and implementation schedule for patients with second, third, or subsequent primary tumors, based on factors such as the histological type, degree of differentiation, clinical or pathological staging of the tumors, as well as the patient's age, gender, occupation, lifestyle, and family history of genetic diseases. A follow-up archive should also be established for each patient.

(3) The content of follow-up records mainly includes name, gender, age, occupation, living habits, family history, histopathological characteristics, immunohistochemistry, molecular pathology, and/or genetic testing of primary tumors such as the first, second, and third tumors, clinical or pathological staging, specific treatment methods, efficacy evaluation, the occurrence of adverse events and their treatment, etc.

(4) The main contents of follow-up examinations include general condition assessment (ECOG-PS score), imaging examinations (such as Ultrasound, CT, MRI, Whole-Body Bone Scan, with PET-CT not routinely recommended), endoscopy, and routine detection of tumor, etc.

Section 5 The Key Points for Rehabilitation of Patients with Cancer of Multiple Primaries

The Key Points for Rehabilitation of Patients with Cancer of Multiple Primaries

For patients with cancer of multiple primaries, after completing treatment (including local and systemic therapies), it is essential to not only strictly adhere to the follow-up instructions from healthcare providers but also enhance their own physical and mental rehabilitation. This approach will contribute to prolonging survival and improving the quality of life. The key points and principles of rehabilitation include:

(1) Maintain an objective and correct attitude towards your illness, maintain a good mindset, and foster a firm belief in overcoming the tumors.

(2) Strictly adhere to the follow-up plan and schedule formulated by your attending physician, and adhere to correct, scientific, and standardized rehabilitation methods.

(3) Encourage regular participation in cancer-related science popularization and health education lectures organized by professional associations and societies.

(4) Do not fall for superstitions, false advertisements (including various online, TV advertisements, flyers, and promotional slogans), ancestral secret recipes, or treatments from unlicensed healers or itinerant doctors.

(5) Correct unhealthy lifestyle habits, such as quitting smoking and alcohol, adopting a low-fat diet, increasing intake of vegetables and soy products, and avoiding the use of various health supplements as much as possible.

(6) Engage in appropriate exercise (including fitness and travel) reasonably, ensuring adequate rest and sleep.

(7) Actively participate in relevant social and public welfare activities.

(8) Cultivate at least one hobby that benefits physical and mental health, such as music, calligraphy, painting, fishing, bird-keeping, gardening, traveling, crafts, etc., to alleviate various psychological pressures.

(9) Reduce conflicts within the family, create a harmonious family atmosphere, and actively seek understanding and support from family members.

(10) Consider resuming relatively light work if physical and psychological conditions permit.

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