The Royal College of Pathologists
Pathology: the science behind the cure

Standards and datasets for reporting cancers

Dataset for histopathological reporting of cancer of unknown primary (CUP) and malignancy of unknown primary origin (MUO)

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Foreword

The cancer datasets published by The Royal College of Pathologists (RCPath) are a combination of textual guidance, educational information and reporting proformas. The datasets enable pathologists to grade and stage cancers in an accurate, consistent manner in compliance with international standards and provide prognostic information, thereby allowing clinicians to provide a high standard of care for patients and appropriate management for specific clinical circumstances. On rare occasions, it may be necessary or even desirable to depart from the guidelines in the interests of specific patients and special circumstances. The guideline has been developed to cover most common scenarios. However, it is recognised that guidelines cannot accommodate every pathological specimen type and clinical scenario. Deviation from the guidelines may therefore be required occasionally to report the specimen in a way that maximises the benefit to the patient.

Each dataset contains core data items that are mandated for inclusion in the Cancer Outcomes and Services Dataset (COSD – previously the National Cancer Data Set) in England. Core data items are items that are supported by robust published evidence and are required for cancer staging, optimal patient management and prognosis. Core data items meet the requirements of professional standards (as defined by the Information Standards Board for Health and Social Care [ISB]) and it is recommended that at least 90% of reports should record a full set of core data items. All data items should be clearly defined to allow the unambiguous recording of data.

The following stakeholder organisation was consulted during the preparation of the dataset:

- Cancer of Unknown Primary Foundation.[1]

Supporting evidence and recommendations in this dataset are based on:

- PubMed literature searches (up to July 2017)
- National Institute of Health and Care Excellence (NICE) Improving Outcomes Guidance, 2010[2]
- National Cancer Peer Review (NCPR) standards for Cancer of Unknown Primary/Malignancy of Unknown Origin, 2014.[3]

Most of the supporting evidence is level C or D or meets the Good Practice Point criteria (see Appendix F). No major conflicts in the evidence have been identified and any minor discrepancies between evidence have been resolved by expert consensus.

No major organisational changes have been identified that would hinder the implementation of the dataset and there are no new major financial or work implications arising from the implementation. The requirement for a specialist second opinion may have some workforce implications similar to those required for sarcoma and lymphoma diagnosis.

A formal revision cycle for all cancer datasets takes place on a three-yearly basis. However, each year, the College will ask the authors of the dataset, in conjunction with the relevant subspecialty adviser to the College, to consider whether or not the dataset needs to be revised. A full consultation process will be undertaken if major revisions are required, i.e. revisions to core data items (the only exception being changes to international tumour grading and staging schemes that have been approved by the Specialty Advisory Committee on Cellular Pathology and affiliated professional bodies; these changes will be implemented without further consultation). If minor revisions or changes to non-core data items are required, an abridged consultation process will be undertaken, whereby a short note of the proposed changes will be placed on the College website for two weeks for Fellows' attention. If Fellows do not object to the changes, the short notice of change will be incorporated into the dataset and the full revised version (incorporating the changes) will replace the existing version on the College website.

The dataset has been reviewed by the Clinical Effectiveness Department, Working Group on Cancer Services and Lay Governance Group and placed on the College website for consultation with the membership from 8 November to 6 December 2017. All comments received from the above groups and membership were addressed by the authors to the satisfaction of the WGCS Chair and the Clinical Director of Clinical Effectiveness. This dataset was developed without external funding to the writing group.

The College requires the authors of datasets to provide a list of potential conflicts of interest; these are monitored by the Clinical Effectiveness Department and are available on request. Dr Oien has declared that she is and has previously been involved in research collaborations and/or paid consultancies with commercial organisations involved in molecular testing of cancer of unknown primary. She gives her assurances that these potential conflicts of interest have not influenced the content of this dataset.

1 Introduction

The majority of patients with cancer present with a clearly defined primary tumour that manifests with local symptoms. However, about 10–15% of patients present initially with metastatic disease. In many of these patients, the site of origin initially will not be obvious and in about one third of these cases the primary tumour site may never be found.[4] Cancer of unknown primary (CUP)/malignancy of unknown origin (MUO) is thus a common and important clinical problem and represents one of the ten most common cancer diagnoses. As described in recent clinical reviews, 3–5% of new cancer diagnoses are classified as CUP.[5,6] This document is the first edition of the dataset for CUP and MUO. While these tumours are commonly encountered in routine clinical practice, by their nature they provide significant diagnostic challenges to the pathologist. Terminology and definitions vary in different publications[7] and we advise using the NICE agreed terms shown in Table 1, as originally developed for the 2010 NICE guidelines on CUP (metastatic malignant disease of unknown primary origin).[2]

Table 1. NICE guidance on metastatic malignant disease of unknown primary origin

MUO	Metastatic malignancy identified on the basis of a limited number of tests, without an obvious primary site, before comprehensive investigation.
Provisional CUP	Metastatic epithelial or neuroendocrine malignancy identified on the basis of histology or cytology, with no primary site detected despite a selected initial screen of investigations, before specialist review and possible further specialised investigations.
Confirmed CUP	Metastatic epithelial or neuroendocrine malignancy identified on the basis of final histology, with no primary site detected despite a selected initial screen of investigations, specialist review and further specialised investigations as appropriate.

(Based on NICE guidelines on CUP.)[2]

1.1 Epidemiology and clinical context

According to recent Cancer Research UK data, CUP formed 3% of all cancer diagnoses in the UK in 2014 (in keeping with worldwide data above), with 8,930 new cases.[8] CUP incidence increases with age; from 2012 to 2014, more than half (56%) of cases of CUP in the UK were diagnosed in people aged 75 and over.[8] The overall median survival of patients with CUP/MUO within oncology services is widely quoted as 8–11 months;[5,6] however, population wide the figure may be lower, with recent data from Scotland suggesting an overall median survival nearer to 1–3 months.[9]

Conversely, there are subgroups of patients with much longer survival times and/or disease subtypes that respond well to available treatment, especially chemotherapy; the identification of this subgroup of patients is the major goal of the pathological workup.[4] These 'favourable' tumours account for approximately 20–30% of CUP and include lymphoma, germ cell tumour, neuroendocrine carcinoma, squamous carcinoma involving only local lymph nodes and adenocarcinomas for which specific therapy is available.[6,10]

The diagnosis of provisional CUP is usually based on a clinical scenario in which no primary tumour is apparent on initial workup by examination or initial imaging, as described in the CUP guidelines from NICE[2] and from the European Society of Medical Oncology (ESMO),[10] and where biopsy of a presumed metastatic deposit does not show clear evidence of a primary site. As more information becomes available, a significant proportion of these provisional metastatic CUPs will become identified as a specific tumour type and the site of origin will be confirmed clinically; in some cases, the apparent metastasis will be shown to be a primary tumour at that site, often with atypical morphology.

When the pathologist is faced with a provisional CUP biopsy, it is crucial that they obtain all relevant clinical information and check for a past history of malignancy by all possible routes including interrogation of the laboratory information management system.[10] This can be helpful, for example, in the identification of a previously removed melanocytic lesion, which may require histological review. Occupational history should be sought as well as results of appropriate imaging modalities, with CT/PET emerging as the most important of these. Knowledge of serum cancer marker status is also highly valuable.

[Level of evidence – C.]

1.2 Pathological approach

The pathological approach to exclusion or diagnosis of CUP/MUO is stepwise[4] and uses clinical context, morphology, immunohistochemistry and, occasionally, other techniques including molecular analysis.

After optimising the tissue biopsy submitted, which must be embedded entirely to ensure no better differentiated component can be seen on morphology, the specimen needs to be subjected to careful morphological analysis. Sufficient tissue needs to be retained for a detailed evaluation, sometimes involving several rounds of immunohistochemical (IHC) staining and potentially molecular studies. Retention of serial spare sections on coated slides helps maximise the tissue available from small biopsies. Where there are multiple fragments, embedding in separate blocks can also maximise tissue availability. To enable optimal handling of scarce tissue, it is helpful to know in advance that the biopsy is from a provisional CUP case, from the clinical history provided by the referring clinician or from previous multidisciplinary team (MDT) discussions. Should molecular studies be judged likely, and if material is limited, a selective approach to immunohistochemistry may be necessary based on tumour morphology and clinical presentation, to avoid the need for a second biopsy.

[Level of evidence – D.]

The first step is the confirmation of malignancy and then exclusion of carcinoma, melanoma, lymphoma or sarcoma. Once germ cell tumours have been excluded, carcinomas need to be subtyped into squamous, neuroendocrine, solid organ (including liver, renal, thyroid and adrenal) and adenocarcinoma. The final step for metastatic carcinoma diagnosis is the determination of the likely primary tumour site e.g. in adenocarcinoma these may include lung, breast, pancreas, stomach, colon, ovary, kidney and prostate.

[Level of evidence – D.]

By definition, microscopic examination of the morphology in a provisional CUP case shows a pattern that is not associated specifically with a single tumour type (and site, if appropriate). For undifferentiated tumours, varied patterns may be seen such as small round blue cell tumour, epithelioid tumour, spindle cell tumour, large cell undifferentiated cell tumour or a combination of these.[4] In this dataset, for each tumour type (and site, if appropriate), potential morphologic features are presented with a description of useful ancillary markers, their staining characteristics and some common diagnostic dilemmas. While immunohistochemistry plays a major role, histochemistry for neutral mucin and glycogen can be helpful in some cases. We recommend combined Alcian Blue PAS stain, with and without diastase (AB/PAS+/-D), for this purpose. The demonstration of neutral mucin can be very valuable in the identification of adenocarcinoma.

[Level of evidence – C.]

1.3 Immunohistochemistry

The process of elimination of primary tumour type in provisional CUP cases requires a careful pathological workup based usually on immunohistochemistry as well as morphology.[4] Different IHC markers will be employed dependent on the morphology of the provisional CUP case and the majority of these tumours turn out to be carcinomas. The exclusion of a 'non-carcinoma' diagnosis is crucial, particularly germ cell tumour, malignant melanoma, lymphoma, leukaemia and various sarcomas.[11] Depending on morphology, a primary panel to exclude these tumours from carcinomas is often employed. If this panel confirms epithelial differentiation, a secondary panel to determine type and likely primary site is employed.[12,13]

[Level of evidence – D.]

Comprehensive review articles have been published in the last few years describing IHC, including newer and emerging markers, for a wide range of tumours of unknown origin[11] and for CUP in particular.[12,13] Our dataset describes the strategic approach and these reviews include more extensive bibliographies for consultation. Other useful sources include IHC online databases, for example, ImmunoQuery,[14] Paul Bishop's Immunohistochemistry Vade Mecum[15] and the work of Rodney Miller.[16] Thus, taken together, the use of cytokeratin profiling, lineage-specific cytoplasmic and membranous markers, and lineage-restricted transcription factors, together with other nuclear markers, allows a definitive diagnosis of a specific tumour in many cases of provisional CUP.

Although generally reproducible and reliable, there are many factors that can contribute to incorrect IHC results, both false positive and false negative.[4,17] These include pre-analytic tissue variables, analytic variables affecting the technical performance of immunohistochemistry and issues around IHC interpretation. Tumour, and thus biomarker, heterogeneity may be marked especially with small samples and can cause diagnostic issues. Thresholds for categorising staining as positive or negative in a binary fashion may vary between biomarkers, corresponding antibodies and previous studies. It is important to be aware of the staining expected in terms of cell and tissue location and tissue type; some antibodies may be relatively unfamiliar and yield unexpected staining patterns, potentially contributing to misinterpretation and misclassification. Overall, the recommendation is to use antibodies in panels, interpret results, especially focal staining or with less familiar biomarkers, with caution and in clinicopathological context, and to have a low threshold for discussion, consultation with colleagues and referral.

[Level of evidence – D.]

1.4 Molecular testing

Molecular testing in CUP is more expensive and less widely available than IHC and is not in widespread use in the UK; such profiling is used more commonly in the USA and

elsewhere.[18–21] Molecular testing in CUP is not currently recommended by NICE for diagnostic purposes, outwith clinical trials and translational studies.[2]

With further technical advances, it is likely that molecular testing will play a role in the future, as highlighted by Greco.[19] Such approaches encompass both molecular profiling for enhanced tumour classification by type and tissue-of-origin, for example gene expression profiling[18–21] and testing for actionable mutations to predict therapeutic benefit.[22–24] Reviews have suggested that expression and genomic profiling may be equally or more relevant in guiding personalised precision cancer therapy in CUP than empiric chemotherapy based on tissue/organ of origin information.[18,25] Ideally, further comparative studies and demonstration of utilities would be needed[26] and are eagerly anticipated to determine which diagnostic approaches could impact the clinical outcome of patients with CUP.[20] However, most oncologists remain keen to explore and support optimisation of existing histopathological and IHC avenues to designate tumour type.[10]

1.5 Use of CUP dataset, worksheet and proforma in practice

Clinical practice varies between hospitals, but this document introduces a standardisation of the pathological approach to the diagnosis of CUP. Completion of the CUP proforma is only required for confirmed CUP. The CUP worksheet is designed to support the evaluation of provisional CUP. If during evaluation, it becomes evident that the tumour can be classified as a specific type or site, for which another dataset exists,[27] that alternative dataset should be completed e.g. colorectal carcinoma (see section 5.4).

Thus, the dataset for confirmed CUP essentially comprises a list of negative investigations undertaken to try to identify a primary site. It should be recognised that confirmed CUP is a relatively rare histological diagnosis when all clinical imaging and pathological parameters have been fully explored; many 'provisional' CUP cases will eventually be considered and treated as a specific tumour type. Because of this, the authors recommend that two consultant-equivalent histopathologists should be involved in the final allocation of the diagnosis of confirmed CUP.

[Level of evidence – GPP.]

In the UK, biopsies taken for provisional CUP diagnosis are reported mainly in general pathology departments that will normally include one or more histopathologists who participate in a CUP MDT. Referral to another pathology department may be necessary to access additional diagnostic techniques that may not be available in all laboratories. Diagnosis of CUP is especially important in patients of good performance status who are likely to be better able to tolerate high intensity therapies.

[Level of evidence – GPP.]

1.6 MDT working and standardised reporting

Since the introduction of peer review standards for CUP[3] and the publication of the NICE guidance on CUP in 2010,[2] hospitals in England and Wales are required to have a multidisciplinary approach to CUP/MUO diagnosis and a CUP/MUO MDT. While some hospitals have established standalone CUP/MUO MDT meetings, many units have arranged combined MDT meetings with lung, upper gastrointestinal cancer or hepatopancreatico-biliary MDTs, for ease of organisation; this in turn means that the pathologists experienced in CUP often practice in one of these subspecialties.

Most diagnoses of CUP/MUO are reported on biopsy specimens rather than excisions and can come from a wide range of sites, requiring a different approach to diagnosis when compared with conventional site-specific datasets. There is a significant challenge in definitively excluding identifiable tumour types or potential sites of origin, which may be

crucial to therapy in this group of patients who have very poor clinical outcome in the majority of cases. However, identification of specific patterns of differentiation or uncovering a 'cryptic' site of origin may enable clinicians to optimise therapy and provide meaningful prognostic information to patients and their relatives and carers. Integration of results with other pathology tests, particularly serum tumour markers, is often vital in making the appropriate diagnosis. One third of advanced malignant tumours present with metastases at the time of diagnosis and the use of improved imaging techniques, including PET/CT, is crucial to identifying primary sites in some cases.

[Level of evidence – D.]

Once the diagnosis of provisional CUP has been reached by the pathologist, it is thus recommended that the case is discussed in a CUP or CUP-related MDT meeting with the treating oncologist to ensure that no additional imaging or tumour marker information has emerged during the diagnostic process in histology.[2,3] Only then should a diagnosis of confirmed CUP be provided by the pathologist. The MDT is particularly important in the diagnosis of CUP, as detailed discussion between pathologists, oncologists, radiologists and oncology nurses is essential to classify these tumours accurately and offer patients the best treatment options.

[Level of evidence – D.]

Standardised cancer reporting and MDT collaborative working help to reduce the risk of histological misdiagnosis or misinterpretation of histopathology reports, and ensure that clinicians have all of the relevant pathological information required for appropriate tumour management and prognosis. Collection of standardised cancer specific data also provides information for healthcare providers and epidemiologists and facilitates international benchmarking and research. Information is often retrieved on the basis of coding and therefore it is important that this is accurate and standardised (see Appendix A).

1.7 Target users of this guideline

The target primary users of the dataset are trainee and consultant cellular pathologists and, on their behalf, the suppliers of IT products to laboratories. The secondary users are surgeons, physicians, oncologists, cancer registries and the National Cancer Registration and Analysis Service.

2 Clinical information required on the specimen request form

In addition to demographic information about the patient and details of destination of the report, several items of clinical information including relevant medical history, particularly previous diagnosis of any malignant disease, family history of malignant disease and occupational exposure to carcinogens, can help the pathologist in the handling and reporting of specimens of presumed metastatic tumour. These should be made available to the pathologist on the specimen request form. It is good practice to include clinical information obtained on the pathology report.

[Level of evidence – D.]

For all biopsies, the precise anatomical location(s) should be given to help in interpretation. Knowledge of the distribution of disease mainly drawn from CT, MRI or CT/PET imaging is very helpful and should be available. Serum tumour marker status should be made available. In practice, these results are often only available after initial reporting of the case and should be integrated into the report when relevant. This often occurs at or following the MDT meeting at which the patient is discussed in detail.

[Level of evidence – D.]

Details of current and previous therapy can aid morphological interpretation as well as inform the pathologist.

[Level of evidence – D.]

3 Preparation of specimens before dissection

Specimen types from which a diagnosis of CUP/MUO may be made can be submitted from almost any anatomic site and range from small biopsies to large resections. In most cases, patients have evidence of widespread disease at the time of biopsy but in some it will be an incidental finding or an unexpected diagnosis following resection for a presumed primary of known origin. Biopsies from lymph node, liver[28] and lung are most frequently encountered. Other common sites of metastatic disease include brain, bone/bone marrow, pleura or peritoneum, adrenal gland and skin, but any site may be involved and biopsied.

Definitive diagnosis of CUP/MUO on cytological preparations can be difficult because the limited material might not allow the full range of ancillary techniques. The likelihood of CUP/MUO should be communicated to the clinical team managing the patient and a tissue biopsy requested where appropriate.

[Level of evidence – GPP.]

3.1 Request forms

Appropriate labelling of the request form and containers must be observed by the requesting clinical team to avoid delays in the registration (‘booking in’) of specimens.

3.2 Tissue (biopsy and resection) specimens and fixation

The majority of histological specimens are received in 10% buffered formalin. Adequate fixation requires five to ten times the volume of formalin compared to the size of the specimen and the requestor must select a suitable size of container. Adequate fixation is essential for good preservation of morphology, which facilitates morphological diagnosis, immunohistochemistry and other ancillary techniques. However, if fresh tissue is available for research or bio-banking, this should be collected according to agreed protocols and under the guidance of the pathologist. Detailed protocols for research and tissue banking, including ethical and consent issues, are beyond the scope of this document. As a general principle, fresh tissue banking protocols should be designed such that diagnosis is not compromised; if this is likely in a given case, then tissue banking should not occur and the reasons should be recorded.

[Level of evidence – D.]

Once received in the laboratory, large specimens should be incised promptly by a pathologist or trained biomedical scientist (BMS)/advanced practitioner to ensure good formalin penetration. Small specimens that only require tissue transfer may be submitted directly for processing by a BMS.

[Level of evidence – D.]

3.3 Cytology specimens

Cytological specimens are generally direct smears or fluids processed as cell blocks or cytospins and stained with the Papanicolaou (Pap) stain. Pap-stained liquid-based cytology

(LBC) preparations may also be used and unstained LBC slides or sections of cell clots or cell blocks prepared from LBC specimens can be used for immunohistochemistry or fluorescent in situ hybridisation analysis, if required.[29]

[Level of evidence – D.]

4 Specimen handling and block selection

4.1 Biopsies

The number of biopsies and the largest dimension of each piece should be recorded. In cases where there is a likely diagnosis of malignancy, biopsies may be separated into multiple cassettes to maximise tissue available. To enable optimal handling of scarce tissue, it is helpful to know in advance that the biopsy is from a provisional CUP case, from the clinical history provided by the referring clinician or from previous MDT discussions.

[Level of evidence – D.]

Thereafter, alternative approaches can be employed. One approach is to examine a single microscopic level (so-called 'early H&E') with minimum trimming for initial assessment, which would guide the subsequent number of IHC blank/spare sections required. An alternative approach is to examine the tissue at three microscopic levels while retaining all or most of the resulting unstained sections on coated slides for later use. Therefore, the tissue biopsy is not wasted or 'cut through' before all appropriate IHC markers (or other ancillary tests including molecular studies) can be employed. If only necrotic material is seen, then deeper levels must be examined until the block is exhausted before reporting the biopsy as 'non-diagnostic'.

[Level of evidence – GPP.]

4.2 Larger resection specimens

These will be dealt with in accordance with the dissection guidance appropriate to the organ type, as listed in other cancer datasets. As the diagnosis of MUO/CUP is generally only known after examination of tissue slides from the resected organ, optimal fixation is particularly important in these tumours as immunohistochemistry is vital for correct categorisation.

[Level of evidence – D.]

5 Evaluation of potential CUP specimens by morphology and immunohistochemistry (see Appendices C, D and E)

A description of the tumour microscopic appearance is important in the evaluation of any tumour. Following morphological evaluation of potential CUP, immunohistochemistry is required to exclude other diagnoses. As the range of IHC markers that may be necessary runs into the hundreds,[11–13] a checklist of all IHC antibodies currently available would not be helpful, although minimum panels exist in current guidelines.[2,10] The dataset therefore requires the pathologist to declare which techniques they have undertaken without being prescriptive and serves as a synoptic method of providing information to the treating oncologist.

[Level of evidence – D.]

5.1 Workup of CUP/MUO specimens

The diagnostic process on a tissue or cell specimen from a patient with metastasis of (at least initially) unknown origin can be worked through systematically.[4] It will already have been established by the pathologist using standard diagnostic criteria that a lesion is present and that it is a tumour, presumed to be malignant. Thereafter, the first step is to consider the broad tumour type: carcinoma, germ cell tumour, melanoma, lymphoma or sarcoma. Second, if the tumour is carcinoma, then is it squamous or urothelial, neuroendocrine, solid organ or adenocarcinoma; and third, if it is adenocarcinoma, can the site of origin of the tumour be predicted. Each step may be accomplished using morphology, with or without IHC. This approach is summarised as a flowchart in Figure 1 and is reflected in the stepwise structure of the worksheet accompanying the dataset form (see Appendix E).

[Level of evidence – D.]

5.2 Specific approach to diagnosis: broad tumour type

5.2.1 Broad tumour type: morphological description

First, the likely broad tumour type will be considered: carcinoma (including germ cell tumour), melanoma, lymphoma or sarcoma. There are at least four common morphological patterns of tumour type encountered in MUO/CUP:[4]

- epithelioid tumours of cohesive cells lying in sheets or glands, usually in stroma, and with cells that are often round, columnar or cuboidal
- sarcomatoid tumours comprise cohesive cells in sheets and cells are often spindled; some tumours show both patterns and may be called 'biphasic'
- 'small blue cell' tumours comprise sheets and islands of relatively small, often cohesive, cells with dark nuclei and often apoptosis
- undifferentiated and/or pleomorphic tumours lack classic differentiation and may display bizarre cells.

Epithelioid tumours are mostly carcinomas but many melanomas and, rarely, sarcomas (especially gastrointestinal stromal tumours) and lymphomas show epithelioid morphology. Sarcomatoid tumours are mostly sarcomas or melanomas; a few carcinomas, especially breast and renal, and mesotheliomas can show sarcomatoid morphology. Carcinomas, sarcomas and melanomas (and mesotheliomas) can all show a biphasic pattern. Perhaps the most common morphologies encountered in MUO/CUP are classic adenocarcinomas without specific features of primary site and undifferentiated tumours.[4]

1.1 **Is there a lesion present?**

If no, cut in. If still no, check with imaging how definite lesion was. If definite, re-biopsy.

1.2. **Is it malignant?**

If no, then make diagnosis.

↓

2. What is **the broad type of cancer**: **carcinoma (broadly including germ cell tumor), melanoma, lymphoma or sarcoma?**

If not distinguishable on morphology alone, then apply first-line IHC panel:

CD45 (LCA)	**S100**	**AE1/3**	Diagnosis	Action
+	-	-	**Lymphoma**	(Specialist) subtyping and prognostication
-	+	-	Probable **melanoma**	Diagnose, if need be with confirmatory IHC
-	-	+	Almost certain **carcinoma**	Further subtyping
-	-	-	**Sarcoma** or rare tumor	(Specialist) diagnosis, subtyping and prognostication
Multiple +			Rare tumor	Review with further IHC

↓

3. **If carcinoma, what is the subtype: germ cell, squamous, neuroendocrine, solid organ e.g. HCC or adenocarcinoma?**

If not distinguishable on morphology alone, then useful IHC may include any or all of:
(those in bold may be useful representatives of each marker class for a large panel: see also Tables 5&6)

Differential diagnosis	Useful positive markers
Germ cell tumor	**OCT4, SALL4,** PLAP, AFP, HCG (for diagnosis then subtyping required)
Squamous carcinoma	**CK5/6**, **p63**, (CK7/20, uroplakin for urothelial carcinoma)
Neuroendocrine carcinoma	**Chromogranin**, synaptophysin, PGP9.5, **CD56**, TTF1, (CDX2)
Hepatocellular carcinoma	**Hepar1**, canalicular pCEA/CD10/CD13
Renal cell carcinoma	**RCC**, CD10, PAX8, Napsin A
Thyroid carcinoma	**TTF1**, thyroglobulin, PAX8
Adrenocortical carcinoma	**Melan-A**, inhibin
Adenocarcinoma	Diagnosed on morphology and lack of markers above plus positivity for markers in table below especially **CK7/20, PSA**

↓

4. **If adenocarcinoma, then can we predict the primary site e.g. prostate, lung, breast, colon, ovary or pancreas, biliary tract or stomach?**

Morphology may provide clues. IHC is helpful particularly through the more specific markers (those commonly used in bold) but should be undertaken as a panel to avoid errors (see later Tables 5 & 6):

Useful markers	Differential diagnosis
PSA, PAP, NKX3.1	Prostate
TTF1, Napsin A	Lung
GCDFP-15, mammaglobin, GATA3	Breast
CDX2+and/orCK20+ but CK7-	Colon; less commonly stomach
CDX2+and/orCK20+ and **CK7+**	Pancreas, biliary tract or stomach; less commonly colon
ER+ but CA125-/mesothelin-	Breast
ER+ and **CA125**+/**mesothelin**+	Ovary
WT1, PAX8	Ovary (providing mesothelioma excluded)
Other results e.g. CK7+ but few other markers+	Interpret using wider information e.g. diagnostic Tables 5 & 6

Figure 1: Flowchart for the pathological approach to CUP/MUO (based on Oien K. Pathological evaluation of unknown primary cancer. *Semin Oncol* 2009;36:8–37 [with permission from Elsevier][4] and updated from recent literature[11–13]).

5.2.2 Broad tumour type: IHC studies

If the type of tumour cannot be definitely diagnosed on morphology alone, then a first-line IHC panel can be applied, such as that shown in Table 2.

Table 2: Basic initial IHC panel for broad cancer types

Tumour type	Epithelial marker e.g. pan-cytokeratin AE1/3	Melanocytic marker e.g. S100	Lymphoid marker e.g. CD45 (LCA)
Carcinoma	Positive	Negative usually	Negative
Melanoma	Negative	Positive	Negative
Lymphoma	Negative	Negative	Positive
Sarcoma	Negative usually	Negative in most but positive in nerve sheath tumours, etc.	Negative

(Based on literature.)[4,11]

A first-line IHC panel would generally include:

- an epithelial marker, demonstrated alone or in combination with others e.g. broad-spectrum anti-cytokeratin reagents such as AE1/3, MNF116, CAM5.2, EMA and CK7/20
- melanocytic markers e.g. S100, Melan A and HMB45
- a lymphoid marker e.g. CD45 (LCA).[4,11]

[Level of evidence – C.]

An extended first-line panel (especially for large cell undifferentiated tumours and/or where initial markers are negative) could also include:

- multiple broad-spectrum anti-cytokeratins or other epithelial markers, for example AE1/3 plus CAM5.2, since some carcinomas (especially hepatocellular) may be negative with AE1/3
- CD138 for plasmablastic tumours and CD30 and CD246 (ALK) for anaplastic large cell lymphoma, which are often negative with CD45 (LCA)
- antibodies reactive with OCT3/4 and SALL4 for germ cell tumours, where OCT3/4 is now standard for seminoma and embryonal carcinoma and SALL4 is a newer 'pan-germ cell marker' that unlike OCT3/4 is also positive in yolk sac tumours.[11]

[Level of evidence – D.]

Because sarcoma rarely presents as metastatic CUP, sarcoma markers are generally not used in a first-line IHC panel unless morphology is suggestive, i.e. a spindle cell tumour (or minority of small round blue cell tumours), when vimentin, desmin, smooth muscle actin, caldesmon, CD34, CD31, S100 and EMA may be useful markers.[4,11] It should be noted that vimentin positivity is relatively non-specific and can be seen in a wide variety of non-sarcomatous malignancies.

[Level of evidence – D.]

If the tumour is convincingly negative with the first-line markers for carcinoma, melanoma and lymphoma, then the diagnosis of sarcoma may also be considered (see Table 3) along with rarer CUP tumours including the CD45 (LCA)-negative haematolymphoid tumours, germ cell tumours (which may be CK negative, with the markers described above) and poorly differentiated carcinomas (considered later in the carcinoma section).

Table 3: Supplementary IHC markers for use in lymphoma, sarcoma and small round blue cell tumour

Lymphoma*	
CD246 (ALK) and CD30	To exclude anaplastic large cell lymphoma
CD15, CD43, CD68 and myeloperoxidase	To exclude myeloid sarcomas Please see lymphoma dataset[30] for specific information about lymphoma work-up
CD138	To exclude plasmablastic tumours
Sarcoma	
Vimentin, alpha-smooth muscle actin, desmin, myoD1, myogenin, S100, CD31, CD34, CD30, bcl2, MNF116, EMA, c-kit and CD99	To exclude sarcoma Some sarcomas will also stain with S100 or focally with epithelial markers Please see sarcoma dataset[27] for specific information about sarcoma workup
Small round blue cell tumour	
Cytokeratins (e.g. antibody CAM5.2) and CD56	To exclude small cell carcinoma
CD45 (LCA)	To exclude lymphomas and leukaemias
Desmin, myoD1 and myogenin	To exclude rhabdomyosarcoma
CD99, FLI1 and Pax5	To exclude Ewing's sarcoma and primitive neuroectodermal tumour
EMA and cytokeratins (e.g. antibody MNF116)	To exclude synovial sarcoma
Chromogranin, synaptophysin, GFAP and S100 protein	To exclude endocrine and neurogenic tumours including olfactory neuroblastoma

*The subtyping of lymphomas should be undertaken within a designated regional Haematological Malignancy Diagnostic Service, in line with long-standing NICE guidance; once lymphoma is indicated, e.g. by demonstration of CD45 expression, the tissue should be referred to such a service. The further work-up at such a centre might include: B-cell markers CD20 and CD79a; a T-cell marker CD2 or CD3; further lymphocyte subset markers, CD4, CD5, CD7, CD8 and CD10; activation marker CD30; CD246 (ALK); B-cell lymphoma proteins bcl-2 and bcl-6; TdT. (Based on literature.)[11] Full guidance is provided in the College's '*Standards for specialist laboratory integration and Dataset for the histopathological reporting of lymphomas*' [30].

[Level of evidence – D.]

Sarcoma

Sarcomas have a wide range of histological appearances but generally their cells are cohesive and lie in sheets, with an elongated spindled shape. The College's sarcoma dataset provides detailed information.[27] As for lymphomas, NICE guidance anticipates that all suspected soft tissue sarcomas will undergo diagnostic review within a specialist sarcoma service. In terms of CUP and sarcoma, there are three main diagnostic issues.

First, a subset of carcinomas and melanomas may take on a sarcomatoid morphology. Metastatic sarcomatoid carcinoma or melanoma is much more common in CUP than metastatic sarcoma. Such tumours need to be treated as carcinoma or melanoma, and

therefore their correct identification is important. Sarcomatoid differentiation is particularly common in squamous tumours, in carcinomas from the breast and genitourinary system (especially kidney and bladder),[31] and in germ cell tumours. It is not uncommon in a presumed CUP biopsy to find a prior history of nephrectomy, mastectomy or even orchidectomy ten or more years previously so a full past medical history is vital.

[Level of evidence – D.]

Second, metastasis of carcinoma, melanoma or lymphoma to soft tissue and first presenting there, mimicking a primary soft tissue sarcoma, is increasingly common.[32] Third, we have the relatively rare first presentation of sarcoma as a metastatic deposit.

The first and second scenarios above should be dealt with by the first-line IHC panel already described. Carcinomas will generally be widely positive with the pan-cytokeratin AE1/3, in the spindled cells as well as any epithelioid cells; this differs from sarcomas in which cytokeratin staining, if any, is generally limited to epithelioid cells. Melanomas will generally be widely positive with S100. Some sarcomas, particularly of the peripheral nerve, are also S100 positive but the staining is usually more focal and weaker. Lymphomas will generally be CD45 (LCA) positive. If there is any doubt, then the second IHC panel can be undertaken, for sarcoma, carcinoma or both as appropriate.

[Level of evidence – D.]

Lymphoma

Lymphoma is often easily identified on the basis of morphology and IHC. The lymphoma dataset provides detailed information.[30] In the MUO/CUP setting, the most likely to be considered are anaplastic large cell lymphoma (ALCL) or anaplastic forms of plasma cell tumours or immunocytoma. Relevant IHC is listed in Tables 2 and 3. The other occasional problematic diagnosis is tissue-based acute myeloid leukaemia/granulocytic sarcoma/chloroma. With regard to the CUP diagnostic dilemmas, CD45 (LCA) will stain positively for both low-grade lymphomas and diffuse large B-cell lymphomas, as well as almost all other non-Hodgkin lymphomas. However, the other two haematolymphoid tumours that may resemble carcinoma are generally negative for CD45 (LCA), as well as for cytokeratins and S100. ALCL is sometimes positive for CD246 (ALK), which is a specific marker when present, and universally positive for CD30. The latter, as a marker of activated lymphocytes, is expressed in many lymphoid lineages.[33] It is also expressed in some non-lymphoid malignancies. Myeloid sarcomas, which include granulocytic sarcoma, are positive with a range of myeloid markers including CD15, CD33, CD43, CD68 and myeloperoxidase.[34]

[Level of evidence – D.]

This discussion has largely excluded Hodgkin lymphoma, which presents rarely as CUP but may enter the differential diagnosis of lymph node biopsies. The morphology of non-Hodgkin lymphoma is generally characteristically lymphoid. Diagnostic difficulty is usually between Hodgkin lymphoma and other types of lymphoma or benign processes, not other types of cancer. If necessary, IHC for CD30, CD15, MUM1, PAX5 and EBV-LMP can be helpful in diagnosis.

[Level of evidence – GPP.]

Small round blue cell tumour

In adult CUP, the common differential diagnoses of small round blue cell tumours include leukaemia/lymphoma, small cell neuroendocrine carcinoma and (basaloid) squamous carcinoma. Other rarer possibilities include Merkel cell tumour and sarcomas including desmoplastic small round cell tumours, etc. Relevant IHC is listed in Table 3.[11] Although small round blue cell tumours may be lymphomas, most low-grade lymphomas are

diagnosable as probable lymphoma on morphology. While the same may be true of high-grade lymphomas, some may appear epithelioid. Undifferentiated and/or pleomorphic tumours can arise from any of the broad tumour types.

Melanoma
At this step, always consider melanoma, especially if the tumour contains brown granular pigment. If much pigment is present, consider an alternative (non-DAB i.e. non-brown) chromogen for IHC. Relevant IHC is listed in Table 2.

[Level of evidence – D.]

It is worth being aware of more unusual clinical scenarios or metastatic sites that may suggest specific entities e.g. germ cell tumours in young males and/or in midline mediastinal or abdominal tumours.

[Level of evidence – C.]

5.3 Specific approach to diagnosis: carcinoma type

5.3.1 Carcinoma type: morphological description

Once it has been decided that the specimen contains a carcinoma then the next question is what is the broad carcinoma subtype: squamous tumours, which for our broad purposes may include basal tumours, plus urothelial carcinomas; adenocarcinomas; carcinomas of solid organs, which are sometimes grouped with adenocarcinomas, arising from liver, kidney, thyroid and adrenal glands; neuroendocrine carcinomas, both well differentiated and poorly differentiated; and germ cell tumours, which are distinct from carcinomas but which they morphologically may resemble.[4]

Squamous carcinomas comprise cohesive cells lying in sheets or islands; the cells are usually large and often round. Adenocarcinomas comprise cohesive cells lying mainly as glands, ducts or islands, usually within stroma; the cells are usually columnar or cuboidal. The solid organ carcinoma pattern comprises cohesive cells in sheets, cords and/or acini, often without much stroma; the cells are often round. A similar pattern may be seen in well-differentiated endocrine carcinoma, with cohesive cells lying in sheets or islands; its cells are usually round and uniform and the tumour is often highly vascular. Small blue cell tumours comprise sheets and islands of relatively small, often cohesive, cells with dark nuclei and often apoptosis. Undifferentiated and/or pleomorphic epithelioid tumours lack classic differentiation and may display bizarre cells. Some tumours show more than one epithelioid morphological pattern and some may be both epithelioid and sarcomatoid ('biphasic').

These morphologies relate to the carcinoma subtypes as follows. Squamoid morphology is seen in squamous carcinomas but also in urothelial/transitional carcinomas, some basal cell carcinomas and some adenocarcinomas. Obviously, more differentiated squamous tumours may show keratin 'pearls' and intercellular 'prickles'. Adenocarcinomas show their classic glandular pattern, but similar morphology may be found in some solid organ carcinomas, germ cell tumours and mesotheliomas. The solid organ morphology is seen in hepatocellular, renal, thyroid and adrenal carcinomas, as well as in some well-differentiated endocrine tumours. Solid organ carcinomas may resemble the corresponding normal organ e.g. abundant pale 'clear' cytoplasm in renal cancer, follicular structures and secretions in thyroid carcinoma. The undifferentiated and/or pleomorphic morphology may be seen with any carcinoma subtype; it is worth considering and excluding germ cell tumour in particular.

[Level of evidence – D.]

5.3.2 Carcinoma type: IHC studies

If the carcinoma subtype cannot be definitely diagnosed on morphology alone, then IHC panels may be applied, such as those shown in Tables 4 and 5.[4,11–13] The markers used would be tailored to the morphological pattern.

For probable adenocarcinomas, classified according to their morphology, proceed to the next section of considering its likely primary site.[4,11–13]

For squamoid tumours, CK5/6, p63 and p40 are usually positive in squamous and urothelial carcinomas.[4,12,13] Urothelial carcinomas are usually also positive with CK7 and CK20 and urothelial markers e.g. uroplakin and GATA3. Squamous carcinomas are usually CK20 negative but CK7 staining is variable. CK5/6 and p63 are absent from almost all solid organ carcinomas and from most adenocarcinomas; exceptions include basaloid breast carcinoma. CK5/6 may be positive in other tumour types e.g. mesothelioma. For the primary site of squamous carcinomas, immunohistochemistry is not specific but EBVLMP may be positive in nasopharyngeal carcinoma and HPV and p16 may be positive in oropharyngeal and genitourinary tumours.[35]

[Level of evidence – D.]

For possible solid organ (liver, kidney, thyroid and adrenal) carcinomas, many useful IHC markers relate to organ of origin.[4,11–13] Hep Par-1 is often, but not always, positive in hepatocellular carcinoma; a small proportion of adenocarcinomas (especially so-called 'hepatoid') may also stain with Hep Par-1. Demonstration of a canalicular rather than luminal pattern of staining with CD10 and polyclonal CEA, for example, may help in the diagnosis of hepatocellular carcinoma. Renal cell carcinoma (RCC) marker is often, but not always, positive in RCC; staining in other tumour types is rare. RCCs are often also positive with PAX8. Adrenocortical carcinoma is usually negative for cytokeratins and positive with Melan-A, synaptophysin and inhibin; obviously Melan-A is also positive in melanomas. TTF1 and thyroglobulin are usually positive in thyroid carcinomas; obviously TTF1 and Napsin A are also positive in lung and renal adenocarcinomas, and TTF1 is frequently positive in small cell carcinomas from any site. Thyroid carcinomas are often also positive with PAX8. Solid organ carcinomas may or may not show CK7 positivity, but are usually negative for CK20 and for CK5.

[Level of evidence – C.]

Table 4: Expression of CK7 and CK20 in carcinomas and related tumours

	CK7 positive	**CK7 negative**
CK20 positive	**Gastrointestinal adenocarcinomas and transitional cell carcinoma** Pancreas and biliary tract (one third) Stomach (one quarter) Ovary (mucinous: but many of these likely to be metastatic from gut) Urothelial carcinoma (two thirds)	**Gastrointestinal adenocarcinomas** Colorectum Stomach (one third) Neuroendocrine tumour of Merkel cell type (poorly differentiated)
CK20 negative	**Many adenocarcinomas** Breast Lung (adenocarcinoma) Ovary (serous and endometrioid) Pancreas and biliary tract (two thirds) Stomach (one sixth) Endometrium	**Prostatic and other adenocarcinomas plus solid organ, squamous and most neuroendocrine carcinomas** Prostate Stomach (one sixth) Squamous carcinoma Germ cell tumour

	Salivary tumours Thyroid tumours Urothelial carcinoma (one third) Neuroendocrine, poorly differentiated: small cell carcinoma (one quarter) Malignant mesothelioma (two thirds)	Hepatocellular carcinoma Renal (clear) cell carcinoma Adrenocortical carcinoma Neuroendocrine, poorly differentiated: small cell carcinoma (three quarters) Malignant mesothelioma (one third)

(Based on literature.)[4,11–13]

[Level of evidence – C.]

For possible well-differentiated neuroendocrine carcinomas, useful general IHC markers include synaptophysin and chromogranin; strong positivity with these markers is usually seen only in endocrine tumours.[4,13] Other neuroendocrine markers including CD56 and NSE are generally less specific and may stain other tumour types. Well-differentiated neuroendocrine carcinomas may also show staining with markers specific to their site of origin e.g. TTF1 for lung, CDX2 for gastrointestinal and SATB2 for lower gastrointestinal.[36] In poorly differentiated endocrine carcinomas (small cell carcinomas), TTF1 staining is not site specific. Many neuroendocrine tumours (including undifferentiated small cell carcinoma) will exhibit paranuclear dot-like cytokeratin staining.

[Level of evidence – D.]

For small blue cell tumours in adults, which are positive with epithelial markers on IHC, the differential diagnosis includes the following: basaloid squamous cancer, which stains positively with CK5 and p63 whereas undifferentiated small cell carcinoma may stain with endocrine markers including synaptophysin, chromogranin and CD56, as well as TTF1; and Merkel cell carcinoma, which typically stains positively with CK20 rather than CK7, in contrast to other small cell neuroendocrine tumours.

[Level of evidence – C.]

For undifferentiated and/or pleomorphic carcinoma, it is worth considering whether germ cell tumour is a possibility.[4,13] If so, potential markers include OCT3/4, which is positive in seminoma and embryonal carcinoma. SALL4 is a newer marker for multiple germ cell tumours, which unlike OCT3/4, is also positive in yolk sac tumours, as well as PLAP, AFP and HCG;[11] other carcinomas would only rarely be positive with these markers e.g. AFP in hepatocellular carcinoma.

[Level of evidence – D.]

Table 5: IHC markers commonly used for subtyping of carcinomas

	Marker often used	**Comments on sensitivity and specificity**
Adenocarcinoma	**CK7, CK20, PSA,** TTF1, Napsin A and other adenocarcinoma markers	
Squamous carcinoma	**CK5, CK5/6, p63,** p40, CK34 beta E12 (CK903)	80–90% sensitive for squamous and basal carcinomas and for urothelial carcinomas (p63); also seen in a minority of adenocarcinomas especially breast (basal phenotype), thus moderately specific

Urothelial carcinoma	p63, CK7, CK20, GATA3, uroplakin	
Neuroendocrine carcinoma	**Chromogranin, CD56**, synaptophysin; TTF1 in some; paranuclear dot-like cytokeratin staining	TTF1 expressed in most poorly differentiated neuroendocrine carcinomas (small cell) and in some well-differentiated neuroendocrine tumours of lung origin (c.f. CDX2 in those of intestinal origin)
Solid carcinoma: renal	**RCC**, PAX8, Napsin A, luminal membranous CD10	RCC 55–86% sensitive
Solid carcinoma: liver	**Hep Par-1**, canalicular CD10, glypican-3	Hep Par-1: 55–99% sensitive; moderately specific (may stain some adenocarcinomas)
Solid carcinoma: thyroid	**TTF1**, thyroglobulin, PAX8	
Solid carcinoma: adrenal	**Melan-A**, inhibin	50–100% sensitive
Germ cell tumour	**OCT4, SALL4,** PLAP, HCG, AFP, glypican-3	OCT4 nearly 100% sensitive and 100% specific for embryonal carcinoma and seminoma; SALL4; PLAP highly sensitive and moderately specific; AFP yolk sac tumour; HCG choriocarcinoma
Mesothelioma	**Calretinin, CK5,** CK7, D2-40, WT1	BerEP4 and ERA negative

(Based on literature.)[4,11–13]

[Level of evidence – D.]

If a germ cell tumour has been excluded or is unlikely, then a broad panel to establish carcinoma subtype may be useful. Again, this can be tailored to the morphological pattern but a set of markers covering the most common tumours could include CK5, CK7, CK20, synaptophysin, Hep Par-1, RCC, PAX8 and/or TTF1 (Table 5). It is worth also considering and excluding mesothelioma.[11]

[Level of evidence – D.]

If the carcinoma subtype is found to be a specific solid organ (liver, kidney, thyroid and adrenal) carcinoma, well-differentiated endocrine carcinoma, squamous carcinoma with likely primary site (e.g. head and neck) or urothelial carcinoma, for further reporting guidance please move on to and complete the relevant tumour specific dataset. Relevant common scenarios include the following:

- metastatic squamous cell carcinoma in cervical lymph nodes is generally managed as being of head and neck origin, and metastatic squamous cell carcinoma in inguinal lymph nodes is generally treated as of anal/lower gynaecological tract/urological origin[5,6]
- adenocarcinoma with a specific IHC profile is now often managed (and thus classified) as originating from the relevant site e.g. CK20-positive CDX2-positive metastatic adenocarcinoma is generally treated as of colorectal origin.

[Level of evidence – D.]

If markers for squamous, solid organ and neuroendocrine carcinomas and germ cell tumours are negative, then consider using additional markers if the morphology remains suggestive of

a specific carcinoma subtype. If these remain negative, or if the morphology is not differentiated, then proceed to the next step of considering primary site of probable adenocarcinoma.

5.4 Specific approach to diagnosis: predicted primary site of adenocarcinoma

5.4.1 Primary site of adenocarcinoma: morphological description

If the tumour is an obvious adenocarcinoma, or alternatively if the tumour shows no other specific carcinoma subtype differentiation on morphology or immunohistochemistry, but is presumed to be an adenocarcinoma, then the next question is what is the primary site of the adenocarcinoma?

Adenocarcinomas may show morphological features characteristic or suggestive of primary site. Such features include:

- glands with columnar epithelium, apoptosis and luminal 'dirty' necrosis in colorectal and some other gastrointestinal adenocarcinomas
- papillary epithelium and/or calcispherites in ovarian adenocarcinoma
- diffuse morphology and 'signet ring' cells in gastric and occasionally colorectal and lobular breast adenocarcinoma.

Certain sites of metastasis are more common with particular primary sites, as previously described.[4] Bone metastases are commonly from breast and prostate; axillary lymph node metastases are commonly from breast (or melanoma); inguinal lymph node metastases are commonly from prostate, urological or gynaecological tracts or gastrointestinal tract; cervical lymph node metastases are commonly from head and neck or lung; peritoneal spread is commonly from ovary or gastrointestinal tract; and pleural spread is more commonly from lung or breast as well as other sites. Liver,[28] lung and brain metastases may arise from a wide range of primary sites.

5.4.2 Primary site of adenocarcinoma: IHC studies

If the primary site of adenocarcinoma cannot be diagnosed on morphology alone, then IHC panels may be applied,[4,11–13] such as those shown in Tables 4, 5 and 6, and tailored to clinical scenario and morphology.

Specific IHC markers include PSA and NKX3.1 for prostate and TTF1 for lung. PSA can be positive in other tumours e.g. salivary gland and some breast carcinomas. TTF1, depending on the antibody clone, may show cross-reaction in other tumours, especially colorectal carcinoma, but usually the morphology is helpful. PAP (PSAP) is an additional specific prostate marker and Napsin A is a useful lung marker, although it is also often present in renal carcinoma (especially papillary), adrenocortical carcinoma and ovarian clear cell carcinomas.[13,37] ER is positive in many breast and gynaecological adenocarcinomas. GCDFP-15 is positive in some breast carcinomas[38] and GATA3 may be more sensitive.[12]

[Level of evidence – D.]

CA125 and mesothelin are often positive in gynaecological adenocarcinomas, but may also be positive in mesothelial tumours and pancreaticobiliary and lung adenocarcinomas. WT1 is a more specific marker of gynaecological (especially primary serous ovarian) carcinomas and mesothelial tumours as well as Wilms tumour. PAX8 stains most primary renal carcinomas, most thyroid and thymic tumours and most primary ovarian serous, endometrioid and clear cell adenocarcinomas.[13] CK7 is positive in many adenocarcinomas; almost all breast, lung, ovary and pancreaticobiliary adenocarcinomas are CK7 positive. CK20 is positive in gastrointestinal adenocarcinomas, especially colorectal and other intestinal adenocarcinomas; CK20 is also positive in urothelial and Merkel cell carcinomas and in well-differentiated endocrine carcinomas from the gastrointestinal tract. CDX2 is

positive in gastrointestinal adenocarcinomas, especially colorectal, and in gastrointestinal endocrine carcinomas.[39]

[Level of evidence – C.]

Table 6: IHC markers commonly used for prediction of primary site in adenocarcinomas

	PSA, PAP or NKX3.1	TTF1 or Napsin A	GCDFP-15, mamma-globin or GATA3	WT1 or PAX8	ER	CA125	Meso-thelin	CK 7	CDX2 and/or CK20
Prostate	+	-	-	-	-	-	-	-	-
Lung	-	+	-	-	-	-/+	-/+	+	-
Breast	-	-	+/-	-	+/-	-/+	-	+	-
Ovary serous	-	-	-	+	+/-	+	+	+	-
Ovary mucinous	-	-	-	-	-/+	-/+	-/+	-/+	-/+
Pancreas	-	-	-	-	-	+/-	+/-	+	-/+
Stomach	-	-	-	-	-	-	-/+	+/-	-/+
Colon	-	-	-	-	-	-	-	-/+	+

+ = 90% or more +/- = 50–90% -/+ = 10–50% - = 10% or less

(Based on Dennis and colleagues[40] and updated from literature.[4,11–13])

For dataset purposes, if the adenocarcinoma subtype is found to be a specific adenocarcinoma, then please move on to and complete the relevant tumour specific dataset. If the tumour remains unclassified or is an adenocarcinoma without an obvious primary site, then please complete the CUP dataset.

6 Core data items

6.1 When to complete the CUP dataset

If the diagnosis of provisional CUP is overturned in favour of a definitive diagnosis, the biopsy will be subject to the requirements of the dataset of the particular tumour type. In an ideal world, there would be no dataset requirement for CUP as all cases of provisional CUP would be allocated to a particular primary site. The CUP dataset is therefore different to other College cancer datasets as it details which techniques have been undertaken in the failed attempt to determine the primary site. This means that the dataset is normally completed only after the CUP MDT discussion. Completion of the CUP dataset is not required if a primary site is identified during workup.

6.2 Descriptions of morphology and immunohistochemistry

These aspects of analysis are crucial in any potential CUP workup and so form the main content of the dataset.

[Level of evidence – C.]

6.3 Outcome of discussion in CUP MDT

All confirmed CUP cases should have been discussed in a CUP MDT, or related MDT, and this should be recorded on the dataset. This ensures that the reporting pathologist has access to all clinical data prior to making the diagnosis of confirmed CUP. The range of

additional information included in CUP reports is very wide and should be relevant for the individual case. No specific guidance is therefore feasible that would be applicable to all cases.

[Level of evidence – C.]

7 Diagnostic coding and staging

There are numerous possible tumour sites and therefore the coding should be assigned as appropriate to the individual case. Relevant codes are listed in Appendix A.

Most staging systems require identification of the primary tumour for allocation of a staging system, but the *TNM Classification of Malignant Tumours (8th edition)* from the Union for International Cancer Control[41] includes staging systems for squamous cell CUP involving cervical lymph nodes; different classifications are applied if the tumour is known to be HPV/p16 positive or EBV positive (see Appendix B).

8 Reporting of small biopsy specimens

We recommend that the pathologist seek additional biopsy material if they believe that there is any possibility that this would lead to a specific diagnosis other than confirmed CUP. The minimum size of biopsy cannot be stipulated, but adequate tissue for the wide range of immunohistochemistry testing must be made available before a diagnosis of confirmed CUP is made.

[Level of evidence – GPP.]

9 Reporting of frozen sections

We do not recommend that a diagnosis of CUP is rendered using frozen section as the full range of ancillary tests required to make a diagnosis of CUP is not available.

[Level of evidence – GPP.]

10 Criteria for audit

The following are suggested criteria for audit of the dataset:

- proportion of confirmed CUP cases reviewed in the CUP MDT meeting and which have the process of review recorded
  - standard: 90% of cases.

As recommended by the RCPath as key performance indicators (see *Key Performance Indicators – Proposals for implementation,* July 2013*,* www.rcpath.org/clinical-effectiveness/kpi/KPI):

- histopathology cases should be reported and authorised within seven and ten calendar days of the procedure.
- owing to the complexity of CUP cases, a provisional report is often required to meet these targets and definitive diagnosis may take longer
  - standard: 80% of cases must be reported within seven calendar days and 90% within ten calendar days.

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Appendix A SNOMED codes

Topographical codes (T) and morphological codes (M)

Topographical codes are used in SNOMED 2 and SNOMED 3 to indicate the site of lesions and morphological codes (M) are used to indicate the morphological diagnosis. Common topography and morphology codes are given in the second table below, although the list is not exhaustive.

Tumour site	SNOMED 2/3 CODE	SNOMED CT terminology	SNOMED CT code
Liver	T-56000/T-62000	Entire liver (body structure)	181268008
Brain	T-X2000/T-A0100	Entire brain (body structure)	258335003
Lung	T-28000/T-28000	Entire lung (body structure)	181216001
Lymph node (NOS)	T-08000/T-C4000	Entire lymph node (body structure)	181756000
Axillary lymph node	T-08710/T-C4710	Axillary lymph node structure (body structure)	68171009
Cervical lymph node	T-08200/T-C4200	Cervical lymph node structure (body structure)	81105003
Inguinal lymph node	T-08810/T-C4810	Inguinal lymph node structure (body structure)	8928004
Para-aortic lymph node	T-08480/T-C4480	Para-aortic node (body structure)	181761003
Mesenteric lymph node	T-08400/T-C4400	Structure of lymph node of mesentery (body structure)	279795009
Mediastinal lymph node	T-08360/T-C4360	Mediastinal lymph node structure (body structure)	62683002
Bone (NOS)	T-1X500/T-11000	Bone (tissue) structure (body structure)	3138006
Pleura	T-29000/T-29000	Pleural membrane structure (body structure)	3120008
Peritoneum	T-Y4400/T-D4400	Peritoneum (serous membrane) structure (body structure)	15425007

Morphological codes	SNOMED 2/3/ICD-O CODE	SNOMED CT terminology	SNOMED CT code
Metastatic malignant neoplasm, NOS	M-80006	Neoplasm, metastatic (morphologic abnormality)	14799000
Metastatic carcinoma, NOS	M-80106	Carcinoma, metastatic (morphologic abnormality)	79282002
Metastatic adenocarcinoma, NOS	M-81406	Adenocarcinoma, metastatic (morphologic abnormality)	4590003
Metastatic squamous cell carcinoma	M-80706	Squamous cell carcinoma, metastatic (morphologic abnormality)	64204000

SNOMED versions

Different versions of SNOMED are in use. For the sites and disease entities applicable to the current dataset, the older coding systems known as SNOMED 2 and SNOMED 3 (including version 3.5, its most recent update released in 1998) use the same codes (shown in the two left-hand columns of the table). SNOMED CT, also known as SNOMED International, is a newer SNOMED system, first introduced in 2002 with multiple updates (it is shown in the two right-hand columns) and uses different codes from SNOMED 2 and SNOMED 3 (numerical code only is used for SNOMED CT, rather than T and M codes followed by a number).

Please note that SNOMED 2 and SNOMED 3 are no longer licensed for use.

Procedure codes (P)

These are used in SNOMED 2 and SNOMED 3 to distinguish biopsies, partial resections and radical resections to indicate the nature of the procedure.

Local P codes should be recorded. At present, P codes vary according to the SNOMED system in use in different institutions.

Appendix B TNM staging for squamous cell carcinoma of unknown primary involving cervical lymph nodes[41]

T category

pT0 – No evidence of primary tumour

N category

	EBV or HPV/p16 negative or unknown	*HPV/p16 positive*	*EBV positive*
pN1	Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension without extranodal extension	Unilateral metastasis, in cervical lymph node(s), all 6 cm or less in greatest dimension	Unilateral metastasis, in cervical lymph node(s), and/or unilateral or bilateral metastasis in retropharyngeal lymph nodes, 6 cm or less in greatest dimension, above the caudal border of cricoid cartilage
pN2		Contralateral or bilateral metastasis in cervical lymph node(s), all 6 cm or less in greatest dimension	Bilateral metastasis in cervical lymph node(s), 6 cm or less in greatest dimension, above the caudal border of cricoid cartilage
pN2a	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension without extranodal extension		
pN2b	Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension, without extranodal extension		
pN2c	Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension, without extranodal extension		
pN3		Metastasis in cervical lymph node(s) greater than 6 cm in dimension	Metastasis in cervical lymph node(s) greater than 6 cm in dimension and/or extension below the caudal border of cricoid cartilage
pN3a	Metastasis in a lymph node more than 6 cm in greatest dimension without extranodal extension		
pN3b	Metastasis in a single or multiple lymph nodes with clinical extranodal extension		

M category

M0 – No distant metastases
M1 – Distant metastasis

Appendix C Reporting proforma for cancer of unknown primary (CUP)

Surname..... Forenames..... Date of birth..... Sex.....
Hospital..... Hospital no.....NHS/CHI no.....Date of receipt.....
Date of reporting..... Report no..... Pathologist..... Surgeon.....

Site of sample* – tick box:

Liver ☐ Lung ☐ Brain ☐ Lymph node ☐ Skin ☐ (specify site ……)

Bone ☐ (specify site…….....) Other ☐ (specify site ……..)

Type of sample* – tick all boxes which apply:

Small biopsy e.g. needle core ☐ Small excision biopsy ☐ Effusion cytology ☐ FNA ☐

Other ☐ (specify……..)

Morphology – tick box:

Epithelioid ☐ Sarcomatoid or spindle ☐ Small round blue cell ☐ Undifferentiated/pleomorphic ☐

Other ☐ (specify……)

Immunohistochemistry – list markers employed:

Positive……

Equivocal……..

Negative……

Have you excluded: Lymphoma? YES ☐ /NO ☐ Germ cell tumour? YES ☐ /NO ☐

Melanoma? YES ☐ /NO Sarcoma? YES ☐ /NO ☐

Broad morphological diagnosis*:

Malignant neoplasm, NOS ☐ Carcinoma, NOS ☐ Squamous cell carcinoma ☐

Adenocarcinoma, NOS ☐ Neuroendocrine carcinoma ☐

Has the case been discussed at CUP MDT: YES ☐ /NO ☐

Date of discussion at CUP MDT……

TNM staging if squamous cell carcinoma with lymph node metastases involving cervical lymph nodes*:

TNM edition:

EBV positive: YES ☐ / NO ☐ / NOT KNOWN ☐

HPV/p16 positive: YES ☐ / NO ☐ / NOT KNOWN ☐

pT….. pN…… pM……

Comment:.....

Pathologist …… Date……/……/……..

SNOMED codes* T..... M……

*Data items that are currently part of the Cancer Outcomes and Services Dataset (COSD) v7.

Appendix D Reporting proforma for cancer of unknown primary (CUP) in list format

Element name	Values	Implementation comments
Site of sample	Single selection value list: • Liver • Lung • Brain • Lymph node • Skin • Bone • Other	
Site of sample, specify	Free text	Only applicable if 'Site of sample, Skin', 'Site of sample, Bone' or 'Site of sample, Other' is selected.
Type of sample	Multiple selection value list: • Small biopsy e.g. needle core • Small excision biopsy • Effusion cytology • FNA • Other	Only applicable if 'Specimen laterality, Other' is selected.
Type of sample, specify	Free text	Only applicable if 'Type of sample, Other' is selected.
Morphology	Single selection value list: • Epithelioid • Sarcomatoid or spindle • Small round blue cell • Undifferentiated/pleomorphic • Other	
Morphology, specify	Free text	Only applicable if 'Morphology, Other' is selected.
Immunohistochemistry, positive	Free text	
Immunohistochemistry, equivocal	Free text	
Immunohistochemistry, negative	Free text	
Lymphoma excluded	Single selection value list: • Yes • No	

Germ cell tumour excluded	Single selection value list: • Yes • No	
Melanoma excluded	Single selection value list: • Yes • No	
Sarcoma excluded	Single selection value list: • Yes • No	
Broad morphological diagnosis	Single selection value list: • Malignant neoplasm, NOS • Carcinoma, NOS • Squamous cell carcinoma • Adenocarcinoma, NOS • Neuroendocrine carcinoma	
Confirmation of discussion at CUP MDT	Single selection value list: • Yes • No	
Date of discussion at CUP MDT	Date	
TNM edition	Single selection value list: • UICC 8 • Not applicable	
EBV positive	Single selection value list: • Yes • No • Not known	
HPV/p16 positive	Single selection value list: • Yes • No • Not known	
pT	Single selection value list: • Not applicable • pT0	
pN	Single selection value list: • Not applicable	

	• pN1 • pN2 • pN2a • pN2b • pN2c • pN3 • pN3a • pN3b	
pM	Single selection value list: • pM1 • Not applicable	
Comment	Free text	
SNOMED Topography code	May have multiple codes. Look up from SNOMED tables.	
SNOMED Morphology code	May have multiple codes. Look up from SNOMED tables.	

Appendix E Histopathology worksheet for metastatic carcinoma of uncertain primary site

Surname..... Forenames..... Date of birth..... Sex.....
Hospital..... Hospital no.....
NHS/CHI no.....
Date of receipt..... Date of reporting..... Report no.....

Carcinoma subtype: immunohistochemistry

Panel	Specific immunohistochemical markers used	Positive	Negative	Equivocal
Adenocarcinoma				
Squamous carcinoma				
Transitional carcinoma				
Neuroendocrine carcinoma				
Solid carcinoma: renal				
Solid carcinoma: liver				
Solid carcinoma: thyroid				
Solid carcinoma: adrenal				
Germ cell tumour				
Mesothelioma				

Result for CK7…… Result for CK20……..
Any other relevant IHC markers employed:……
……..
Diagnosis (specific carcinoma subtype):……..

Adenocarcinoma subtyping: morphology

Morphological pattern	Present? (tick more than one if necessary)
Poorly differentiated carcinoma	
Adenocarcinoma NOS	
Papillary adenocarcinoma	
Signet ring cell/diffuse adenocarcinoma	
Other specific morphology (describe)	

Adenocarcinoma subtyping: immunohistochemistry

Panel	Specific immunohistochemical markers used	Positive	Negative	Equivocal
Prostate				
Lung				
Breast				
Ovary and other gynaecological				
Colorectum				
Gastro-oesophageal				
Pancreatico-biliary				
Other (specify)				

Adenocarcinoma subtype diagnosis: ……..

Any further comments especially for assessment of poorly differentiated malignancy:
……

Appendix F Summary table – Explanation of levels of evidence

(modified from Palmer K *et al*. *BMJ* 2008;337:1832)

Level of evidence	Nature of evidence
Level A	At least one high-quality meta-analysis, systematic review of randomised controlled trials or a randomised controlled trial with a very low risk of bias and directly attributable to the target cancer type or A body of evidence demonstrating consistency of results and comprising mainly well-conducted meta-analyses, systematic reviews of randomised controlled trials or randomised controlled trials with a low risk of bias, directly applicable to the target cancer type.
Level B	A body of evidence demonstrating consistency of results and comprising mainly high-quality systematic reviews of case-control or cohort studies and high-quality case-control or cohort studies with a very low risk of confounding or bias and a high probability that the relation is causal and which are directly applicable to the target cancer type or Extrapolation evidence from studies described in A.
Level C	A body of evidence demonstrating consistency of results and including well-conducted case-control or cohort studies and high quality case-control or cohort studies with a low risk of confounding or bias and a moderate probability that the relation is causal and which are directly applicable to the target cancer type or Extrapolation evidence from studies described in B.
Level D	Non-analytic studies such as case reports, case series or expert opinion or Extrapolation evidence from studies described in C.
Good practice point (GPP)	Recommended best practice based on the clinical experience of the authors of the writing group.

Appendix G AGREE compliance monitoring sheet

The datasets of The Royal College of Pathologists comply with the AGREE II standards for good quality clinical guidelines. The sections of this datatset that indicate compliance with each of the AGREE II standards are indicated in the table.

AGREE standard	Section of guideline
Scope and purpose	
1 The overall objective(s) of the guideline is (are) specifically described	Foreword, Introduction
2 The health question(s) covered by the guideline is (are) specifically described	Foreword, Introduction
3 The population (patients, public, etc.) to whom the guideline is meant to apply is specifically described	Foreword
Stakeholder involvement	
4 The guideline development group includes individuals from all the relevant professional groups	Foreword
5 The views and preferences of the target population (patients, public, etc.) have been sought	Foreword
6 The target users of the guideline are clearly defined	Introduction
Rigour of development	
7 Systematic methods were used to search for evidence	Foreword
8 The criteria for selecting the evidence are clearly described	Foreword
9 The strengths and limitations of the body of evidence are clearly described	Foreword
10 The methods for formulating the recommendations are clearly described	Foreword
11 The health benefits, side effects and risks have been considered in formulating the recommendations	Foreword and Introduction
12 There is an explicit link between the recommendations and the supporting evidence	5–7
13 The guideline has been externally reviewed by experts prior to its publication	Foreword
14 A procedure for updating the guideline is provided	Foreword
Clarity of presentation	
15 The recommendations are specific and unambiguous	1–9
16 The different options for management of the condition or health issue are clearly presented	1–9
17 Key recommendations are easily identifiable	1–9
Applicability	
18 The guideline describes facilitators and barriers to its application	Foreword
19 The guideline provides advice and/or tools on how the recommendations can be put into practice	Appendices A–E
20 The potential resource implications of applying the recommendations have been considered	Foreword
21 The guideline presents monitoring and/or auditing criteria	10
Editorial independence	
22 The views of the funding body have not influenced the content of the guideline	Foreword
23 Competing interest of guideline development group members have been recorded and addressed	Foreword